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Review

Mechanistic insights into bone destruction in multiple myeloma: Cellular and molecular perspectives☆

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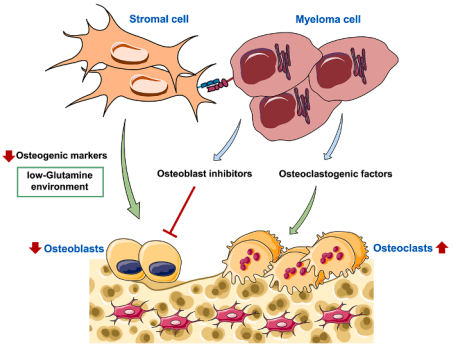
HIGHLIGHTS

- Bone disease is a defining feature of multiple myeloma (MM), affecting 80–90 % of patients.
- The development of MM-related bone disease is driven by complex interactions between myeloma cells and the bone microenvironment.
- Myeloma cells release factors such as CCL3, and IL-6, which promote osteoclast (OC) activation and bone resorption.
- Malignant plasma cells (PCs) also produce osteoblast (OB) inhibitors, further exacerbating bone disease.

GRAPHICAL ABSTRACT



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ABSTRACT

Multiple myeloma (MM) is a hematological malignancy that leads to significant bone destruction, resulting in debilitating pain and skeletal-related events. The pathophysiology of osteolytic bone destruction in MM involves complex interactions between malignant plasma cells (PCs) and the bone marrow (BM) microenvironment. This review aims to provide a comprehensive synthesis of the cellular and molecular pathways underlying MM-associated bone disease. We discuss the role of osteoclast (OC), osteoblast (OB), osteocytes, along with the complex interactions between immune cells and the BM microenvironment in shaping disease progression. Additionally, we explore the molecular signaling pathways involved in bone disease as well as the influence of inflammatory cytokines, and the role of the metabolic alterations that characterize the MM BM. We also explore novel therapeutic strategies targeting these pathways to improve clinical outcomes. Understanding these mechanisms is crucial for the development of more effective treatments to prevent bone damage in MM patients.

1. Introduction

Multiple myeloma (MM) is a hematologic malignancy characterized by the clonal proliferation of malignant plasma cells (PCs) within the bone marrow (BM). MM cells exert profound effects on the surrounding microenvironment, giving rise to a wide range of clinical manifestations.

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Among these, one of the most debilitating and defining features of MM is its propensity to induce severe and progressive bone destruction [1]. This bone damage, which is a hallmark of the disease, manifests as osteolytic lesions visible on imaging, pathological fractures, spinal compression, and diffuse skeletal weakening. These complications significantly impair patients' quality of life and are major contributors to morbidity and mortality [1].

The mechanisms underlying MM-associated bone disease are multifactorial and complex, mainly involving local effects. Direct interactions between malignant PCs and BM stromal cells (BMSCs) play a pivotal role, as do the dysregulation of cellular pathways governing bone remodeling. Specifically, the dynamic balance between osteoclast (OC)-mediated bone resorption and osteoblast (OB)-mediated bone formation is disrupted, favoring bone loss [2]. The secretion of cytokines, growth factors, and chemokines by malignant PCs and their BM microenvironment further amplifies this imbalance by enhancing OC activity and inhibiting OB differentiation and function. Key molecular pathways, such as the RANK-RANKL-OPG axis for OC formation and activity, the Wnt/β-catenin signaling pathway for OB differentiation, and the influence of pro-inflammatory mediators, have emerged as critical regulators of this process [3]. More recently evidence has also shed light on the role of altered glutamine (Gln) metabolism in MM progression and associated bone disease. Gln serves as a critical nutrient for myeloma cells, supporting their rapid proliferation and survival. Moreover, Gln metabolism appears to influence the tumor-bone microenvironment, modulating interactions between malignant PCs and BMSCs [4]. These metabolic alterations may further exacerbate bone destruction by promoting OC activity and impairing OB function. Understanding the contributions of Gln metabolism to MM pathophysiology opens new avenues for therapeutic intervention, particularly in targeting metabolic vulnerabilities of myeloma cells and their effects on the bone microenvironment.

This review aims to provide a comprehensive synthesis of the current understanding of the cellular and molecular mechanisms underlying bone destruction in MM. Particular attention will be given to recent advances in research, including the role of metabolic dysregulation such as altered Gln metabolism, and their implications for therapeutic intervention.

2. Cellular mechanisms underlying bone destruction in multiple myeloma

Throughout life, bones undergo continuous modeling and remodeling processes to support growth, maintain structural integrity, and adapt their shape to changing mechanical and physiological demands [5]. The primary cells responsible for bone remodeling are OBs and OCs. These cells work in a coordinated manner, with OCs destroying old or damaged bone tissue and OBs forming new bone in its place. Other important contributors to the remodeling process include bone lining cells and osteocytes, which function as mechanosensors and endocrine cells derived from OBs [5]. In MM, the process of bone remodeling results to be uncoupled and unbalanced, with a dominant increase in OC activity [6] (Fig. 1). Osteolytic lesions occur in almost all MM patients and are associated with skeletal-related events (SREs), including severe bone pain, pathological fractures, hypercalcemia, and spinal cord compression [7]. In the next sections, we will discuss the role of bone remodeling cells in the development and progression of bone disease in MM. The topic will focus on how the imbalance between OC-mediated bone resorption and OB-mediated bone formation contributes to the characteristic skeletal complications of myeloma. Additionally, the impact of MM cells on the BM microenvironment and their interactions with bone remodeling cells will be highlighted, offering insights into the mechanisms driving bone disease.

2.1. Osteoclasts

OCs are specialized multinucleated cells responsible for bone resorption, a crucial process in skeletal remodeling, repair, and calcium homeostasis [8,9]. OC differentiation, also known as osteoclastogenesis, originates from hematopoietic progenitors of the monocyte/macrophage lineage under tightly regulated conditions [10]. Two critical cytokines govern this process: macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL) [9]. M-CSF, secreted by OBs and stromal cells, binds to its receptor c-Fms on OC precursors, promoting their proliferation, survival, and initial commitment to the osteoclastic lineage. Simultaneously, RANKL, produced primarily by OBs, osteocytes, and activated T cells, binds to its receptor RANK on the surface of precursor cells [11]. This interaction triggers a cascade of intracellular signaling pathways, including the activation of tumor necrosis factor receptor-associated factor 6 (TRAF6), which subsequently activates the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways. The signaling induced by RANKL culminates in the activation of the transcription factor nuclear factor of activated T-cells cytoplasmic 1 (NFATc1), considered the master regulator of osteoclastogenesis. NFATc1 regulates the expression of various OC-specific genes, including those encoding tartrate-resistant acid phosphatase (TRAP), cathepsin K, and the αvβ3 integrin complex, which are critical for OC function [12]. Additionally, c-Fos, a component of the activator protein-1 (AP-1) complex, works synergistically with NFATc1 to drive the differentiation program [13]. The maturation of OCs involves fusion of mononuclear precursors, a process mediated by proteins such as dendritic cell-specific transmembrane protein (DC-STAMP) and OC-stimulatory transmembrane protein (OC-STAMP) [14,15]. During their active phase, mature OCs attach to the bone matrix via integrins, particularly αvβ3 integrins, forming a specialized structure known as the sealing zone [16]. Within

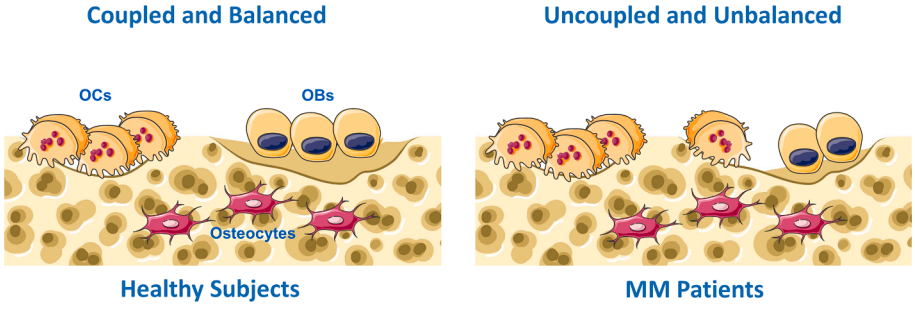

Fig. 1. Bone remodeling in healthy subjects versus MM patients. In healthy individuals, the process of bone remodeling is "coupled and balanced," with a coordinated activity between osteoclasts (OCs, orange cells) responsible for bone resorption and osteoblasts (OBs, yellow cells) responsible for bone formation, maintaining bone health. In MM patients, the process becomes "uncoupled and unbalanced," with increased OC activity and insufficient OB activity, leading to bone degradation and structural damage. Osteocytes (pink cells) are embedded in the bone matrix and play a regulatory role in bone metabolism. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

this zone, the OC creates a resorption lacuna, where it secretes hydrogen ions and proteolytic enzymes like cathepsin K to dissolve mineralized bone and degrade organic matrix components, respectively [17]. The cytoskeletal reorganization required for this activity is regulated by small GTPases, including Rho and Rac, which modulate the actin ring structure [18]. Moreover, OC differentiation and activity are influenced by systemic factors such as parathyroid hormone (PTH), vitamin D, and calcitonin, as well as local cytokines and signaling molecules such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) [19]. Emerging evidence also highlights the role of microRNAs [20] and epigenetic modifications in fine-tuning osteoclastogenesis [21].

OCs play a central role in the pathogenesis of bone disease associated with MM. The imbalance between osteoclastic bone resorption and osteoblastic bone formation is heavily skewed toward increased OC activity due to the interaction of MM cells with the BM microenvironment. Malignant PCs secrete OC activating factors as IL-6 and TNF-α, and chemoattractant molecules as macrophage inflammatory protein-1 alpha (MIP-1α), also known as C-C motif chemokine ligand 3 (CCL3), which directly or indirectly promote osteoclastogenesis [22]. However, the main mechanism involved in MM-induced OC formation is mediated by the BM microenvironment that supports osteoclastogenesis due to the recruitment of BMSCs and other supportive cells able to produce the main pro-osteoclastogenic factor RANKL and other pro-resorptive cytokines through the tight cell contact with MM cells. Concurrently, myeloma cells inhibit the production of osteoprotegerin (OPG), a decoy receptor for RANKL, further enhancing OC activation. Additionally, elevated levels of MIP-1α act on OC precursors to enhance their sensitivity to RANKL and promote their migration to bone resorption sites [23]. Activated OCs resorb bone, releasing growth factors such as transforming growth factor-beta (TGF-β) and insulin-like growth factor 1 (IGF-1) from the bone matrix. These factors, in turn, create a feedback loop that promotes the proliferation and survival of MM cells, intensifying both the bone disease and tumor burden [24,25]. Adhesive interactions between PCs and BMSCs and cytokines like IL-6 upregulates the proto-oncoprotein growth factor independence 1 (Gfi1) in MM cells which, in turn, promotes MM cells survival and growth. In mice, the inoculation of MM cells overexpressing Gfi1 promotes bone destruction by enhancing the number and size of OCs [26]. Beyond the interaction between PCs and BMSCs and the release of various osteoclastogenic factors, the proinflammatory profile of OC precursors also plays a role in osteoclastogenesis in MM patients. Interleukin-21 (IL-21), the ligand for IL-21 receptor (IL-21R), acts as a growth factor for MM cells and is predominantly produced by T cells. When IL-21 binds to its receptor, it activates the Jak-STAT signaling pathway, specifically involving Jak1, Jak3, STAT1, and STAT3. It has been shown that CD14 monocytes in MM patients exhibit increased expression of IL21R as compared to monoclonal gammopathy of unknown significance (MGUS) subjects and that the IL-21/IL21R axis promotes osteoclastogenesis [27] being a possible new target. In addition, IL-21 is involved in the stimulation of several metalloproteinases (MMPs) including MMP-13 [28]. Interestingly, recent study identified MMP-13 as a myeloma-derived factor that induces OC activation independently of its proteolytic activity. PD-1H (programmed death-1 homolog) has been identified as the MMP-13 receptor on OCs. Silencing PD-1H or using Pd-1 $h^{-}/^{-}$ BM cells prevent MMP-13-enhanced OC fusion and bone resorptive activity. Additionally, PD-1H interacts with the actin cytoskeleton, supporting c-Src activation and sealing zone formation, which are essential for OC function [29].

2.2. Osteoblasts

OBs are crucial bone-forming cells that originate from mesenchymal stem cells (MSCs) through a tightly regulated differentiation process involving multiple signaling pathways and transcriptional regulators [30]. MSCs, found in the BM and other tissues, commit to the osteogenic lineage under the influence of specific extracellular signals, such as Bone Morphogenetic Proteins (BMPs), which activate the SMAD signaling pathway, and Wnt/β-catenin signaling, which enhances the transcription of key differentiation markers. Hedgehog signaling also plays a pivotal role in early osteogenic differentiation [31,32]. The progression of differentiation involves the activation of critical transcription factors, notably runt-related transcription factor 2 (Runx2), distal-less homeobox 5 (Dlx5) and osterix (Osx) [33]. Runx2, is indispensable for the transition from MSCs to pre-OBs and regulates genes associated with bone matrix production, including type I collagen (COL1A1), osteocalcin (OCN) and osteopontin (OPN). Two isoforms of OPN, secretory OPN (sOPN) and intracellular OPN (iOPN), have been shown to have opposing effects on the osteogenic differentiation of OBs. Therefore, it is plausible that the sOPN/iOPN expression ratio in OBs may regulate the degree of their osteogenic differentiation [34]. Osx, a downstream target of Runx2, is crucial for the maturation of pre-OBs into functional OBs capable of matrix mineralization [35]. As pre-OBs mature, they proliferate and begin synthesizing extracellular matrix components, primarily COL1A1. In their final stages, mature OBs upregulate the production of mineralization-associated proteins such as OCN and alkaline phosphatase (ALP). They then deposit hydroxyapatite, a crystalline form of calcium and phosphate, into the organic matrix to form mineralized bone [36]. Following their active bone-forming phase, OBs adopt one of three fates: they may become osteocytes, embedded within the bone matrix, where they contribute to mechanosensation and bone remodeling; they may differentiate into bone-lining cells, which regulate mineral homeostasis; or they may undergo apoptosis, a necessary process for maintaining bone tissue balance [37]. The differentiation of OBs is further modulated by growth factors including IGF-1 and vascular endothelial growth factor (VEGF), which support their proliferation and maturation [38]. Additionally, epigenetic regulation, including histone modifications [39] and microRNA-mediated post-transcriptional control, plays a critical role in fine-tuning the expression of OB-specific genes [40,41].

In the context of MM, the balance between bone resorption and formation is severely disrupted [2,42]. The interplay among OBs and myeloma cells promotes the proliferation of MM cells, creating a favorable niche for the survival of dormant malignant PCs [43]. On the other hand, inhibits OB differentiation through complex molecular and cellular interactions that involve direct cell–cell contact and the secretion of soluble factors [44,45]. MM cells adhere to BMSCs via adhesion molecules like integrins and cadherins. The very late antigen-4 (VLA-4) integrin is crucial for facilitating the adhesion of MM cells to the BMSCs through its interaction with the vascular cell adhesion molecule-1 (VCAM-1) ligand. This interaction activates signaling pathways that suppress OB differentiation and function, favoring an environment conducive to MM cell survival and proliferation [46]. Another mechanism involved in OB suppression is the alteration of the transcriptional profile that MM cells trigger in BMSCs. D'Souza et al. demonstrated that MM cells upregulate Gfi1 expression and facilitate its nuclear localization in BMSCs, thereby suppressing Runx2 expression under osteogenic conditions [47]. Gfi1 acts as a transcriptional repressor of Runx2 in BMSCs by directly binding to the Runx2-P1 promoter and recruiting a chromatin co-repressor complex that includes HDAC1 and EZH2 [48]. This process inhibits osteoblastogenesis and impairs new bone formation in MM bone disease. In addition, malignant PCs release factors that directly inhibit OB differentiation and activity, as Dickkopf-1 (DKK1) and sclerostin (SOST), that suppress the Wnt/β-catenin signaling pathway, TGF-β, OPN and OCN: these proteins, produced in excess in MM, inhibit OB function and enhance MM cell adhesion to the BM niche [49,50]. Cytokines such as interleukin-3 (IL-3), interleukin-7 (IL-7), and TNF-α, secreted by MM cells or BMSCs, intensify the suppression of OBs. These cytokines modulate the BM microenvironment to favor osteoclastogenesis while hindering OB activity [3].

The role of OBs in myeloma progression remains a complex and ongoing area of research, with various studies shedding light on their involvement. While the exact mechanisms remain unclear, several studies have demonstrated that targeting OB activity can influence the

progression of MM within the bone microenvironment. In MM animal models, treatment with bone-anabolic agents, such as anti-DKK1 antibodies, lithium chloride (a GSK-3β inhibitor), and TGF-β inhibitors, not only helps restore the bone's structural integrity, but also led to significant tumor regression in the bone [51–53]. These findings suggest that promoting OBs activity or restoring bone formation could potentially have therapeutic effects in MM. However, it is important to note that these agents alone did not show significant inhibition of MM cell growth *in vitro*, indicating that the effect may be more context-dependent or related to the bone microenvironment rather than a direct impact on the tumor cells. On the other hand, it has been reported that bortezomib and other Proteasome Inhibitors (PIs) may exert an anabolic effect both *in vitro* and *in vivo* in MM patients [54–56], potentially contributing to their anti-tumor activity. More recently, it has been observed that OB-derived extracellular vesicles (EVs), particularly those containing the microRNA miR-125b, have the potential to induce myeloma cell death [57]. This suggests that OBs might not only participate in bone remodeling but also directly influence myeloma cell survival and apoptosis through the release of bioactive molecules like miRNAs. Furthermore, OBs have been implicated in creating a non-permissive niche for MM cell survival, modifying the BM microenvironment in a way that prevents myeloma cells from thriving [57]. This is particularly important as the BM microenvironment plays a critical role in MM progression and resistance to treatment.

2.3. Osteocytes

Osteocytes are the most abundant and long-lived cells in bone, constituting over 90 % of all bone cells, that serve as the central regulators of bone remodeling and mineral homeostasis. These specialized cells originate from OBs that become embedded in the mineralized bone matrix, where they reside in small spaces called lacunae [58]. From these lacunae, osteocytes extend dendritic processes through an interconnected network of canaliculi, enabling communication with other osteocytes, OBs, and OCs, as well as with the bone surface and vasculature. This unique arrangement allows osteocytes to function as mechanosensors, detecting mechanical strain or changes in the mechanical environment of the bone [59]. Through their dendritic processes within the canalicular network, osteocytes perceive fluid flow generated by mechanical loading [59]. This mechanical signal is converted into biochemical signals, such as prostaglandins, nitric oxide, and Wnt proteins, which influence OB and OC activity, promoting bone formation or resorption to adapt to mechanical demands [60]. Central to their regulatory role, osteocytes produce a range of signaling molecules, including SOST, RANKL and OPG. SOST, secreted predominantly by osteocytes, acts as a potent inhibitor of the Wnt signaling pathway by binding to low-density lipoprotein receptor-related proteins 5 and 6 (Lrp5/6), thereby suppressing OB differentiation and activity [61]. Mechanical loading reduces SOST expression, thus enhancing bone formation, while unloading or conditions such as aging and osteoporosis are associated with increased SOST levels, leading to bone loss [62]. Osteocyte-derived RANKL plays a key role in osteoclastogenesis by binding to its receptor RANK on OC precursors, stimulating their differentiation and promoting bone resorption. This activity is balanced by OPG which osteocytes also regulate to fine-tune bone resorption processes [63].

Beyond their role in bone remodeling, osteocytes contribute to systemic mineral homeostasis. They control calcium [64] and phosphate levels through a process known as *peri*-lacunar remodeling, wherein they secrete enzymes like MMPs and cathepsin K to resorb the mineralized matrix surrounding their lacunae, releasing calcium and phosphate into the bloodstream [65,66]. Osteocytes also produce fibroblast growth factor 23 (FGF23), an endocrine hormone that acts on the kidneys to decrease phosphate reabsorption and suppress vitamin D activation, thus playing a critical role in maintaining mineral balance [67]. Osteocyte dysfunction or death, often due to aging, mechanical unloading, or metabolic disorders, has profound consequences for bone health [68]. In essence, these cells are the orchestrators of bone homeostasis, integrating mechanical, hormonal, and metabolic signals to maintain skeletal integrity and systemic mineral equilibrium. Their dysfunction not only undermines bone health but also contributes to systemic metabolic imbalances, highlighting their central role in physiological processes.

In the context of MM, the regulatory function of osteocytes is profoundly disrupted due to the interaction with myeloma cells and the surrounding bone microenvironment [69]. One of the major alterations in MM is the increased expression of RANKL by osteocytes and concurrently, a decreased production of OPG [70,71]. Additionally, osteocytes in MM show elevated secretion of SOST that negatively regulates the Wnt signaling pathway [72]. Furthermore, the osteocyte network itself becomes dysregulated in MM, with evidence suggesting that myeloma cells induce osteocyte apoptosis [73]. Toscani et al. highlighted the role of autophagy in MM-induced osteocyte death and associated bone remodeling alterations. They also emphasized the potential of PIs in preserving osteocyte viability and enhancing bone integrity in MM patients [74]. Advanced myeloma bone disease (MBD) has also been reported to significantly alter the integrity of the osteocyte lacuna-canalicular network, showing decreased canalicular length, reduced network coverage and altered structural organization [75,76]. To conclude, osteocytes are pivotal in the pathogenesis of MM-associated bone disease, as they enhance OC activity, inhibit OB function, and contribute to a supportive microenvironment that fosters MM progression. Given their central role in bone health, osteocytes are targets for therapeutic interventions. Anti-SOST antibodies (e.g., romosozumab) are being developed to promote bone formation and treat conditions like osteoporosis. Additionally, strategies to enhance osteocyte viability and function are being explored to combat age-related bone loss and improve bone quality [72]. Targeting osteocyte-mediated pathways, such as inhibiting SOST or RANKL, represents a promising therapeutic strategy to mitigate bone destruction and improve skeletal outcomes in MM patients.

2.4. Interplay between myeloma cells, the immune system, and bone cells

The relationship between myeloma cells, immune cells, and bone cells is a complex process that significantly contributes to the pathophysiology of MM. This interaction not only promotes the survival and proliferation of myeloma cells but also drives the bone disease, including osteolytic lesions and BM suppression. The immune system plays a dual role in MM. On one hand, immune cells such as T lymphocytes, natural killer (NK) cells, and dendritic cells attempt to control the growth and spread of myeloma cells. On the other hand, myeloma cells evade immune surveillance by creating an immunosuppressive microenvironment. They can inhibit immune cell function through the secretion of cytokines such as IL-6, IL-10, and TGF-β, which suppress immune responses and promote tumor survival. Myeloma cells can also interact with regulatory T cells (Tregs), further dampening anti-tumor immunity [77]. The cytokine network of the BM microenvironment (such as IL-6, TNF-α, RANKL) not only contribute to myeloma cell survival but also mediate interactions between immune cells and bone [78]. OCs play a crucial role in myeloma-induced immunosuppressive microenvironment via upregulating various inhibitory checkpoint molecules and immunesuppressive cytokines [79]. It was demonstrated that T cell in co-culture with OC increased inhibitory checkpoints (PD-1, TIGIT) through direct contact while reducing co-stimulatory checkpoints (OX40, CD137) in $CD3^+$ T cells. Additionally, PD-L1 expression on MM cells significantly increased in the presence of both T cells and OCs but not with OCs alone. This suggest that OCs stimulate T cells to produce IFN-γ, contributing to PD-L1 upregulation on MM cells, a process partially reversed by IFN-γ neutralizing antibody. It was also observed an enhanced differentiation of naïve $CD4^+$ T cells into Th17 lineage and increased Th17 expansion in co-culture with OCs, along with similar dysregulation of immune

checkpoints [80]. In addition, among the possible molecules implicated in the link between the immune-microenvironment and osteoclastogenesis was hypothesized the role of CD38 expressed by myeloma cells, monocytes and early OC progenitors. Indeed, the therapeutic anti-CD38 antibody (daratumumab) significantly suppresses OC formation by targeting OC progenitors, offering potential clinical relevance [81]. On the other hand, *in vitro* and *in vivo* experiments revealed that OB-Runx2 deficiency induces a cytokine-rich and immunosuppressive microenvironment in MM through upregulation of myeloid-derived suppressor cells (MDSCs), downregulation of cytotoxic T cells, and activation of TGFβ1 [82]. Thus, targeting OBs and OCs or key cytokines which are involved in both immune evasion and bone resorption, may not only help to prevent lytic bone lesions, but also restore impaired immune surveillance in MM.

3. Molecular signaling pathways in multiple myeloma-associated bone disease

MM-associated bone disease is driven by intricate molecular signaling pathways involving dynamic interactions between malignant PCs and the bone microenvironment (Fig. 2). Key pathways include the RANK/RANKL/OPG axis, which regulates OC activation, and the Wnt/β-catenin pathway, crucial for OB function. Additionally, cytokines such as IL-6, TNF-α, and TGF-β play pivotal roles in promoting osteoclastogenesis and suppressing bone formation [22]. Emerging evidence also highlights the role of altered Gln metabolism in myeloma progression and bone disease, as Gln serves as a critical nutrient for MM cells and influences the tumor-bone microenvironment [4]. Understanding these molecular mechanisms provides valuable insights into the progression of MM bone disease and highlights potential targets for therapeutic intervention. In the following section, we will examine the role of molecular pathways in MM bone disease, current treatment approaches, and promising new therapeutic strategies.

3.1. RANK/RANKL/OPG axis

The RANK/RANKL/OPG pathway plays a pivotal role in bone remodeling and is critically dysregulated in MM, contributing to the characteristic bone disease and tumor progression. Terpos et al. showed that the RANKL/OPG ratio is significantly increased in MM patients and its alteration is associated with markers of bone resorption, osteolytic lesions, and disease activity. This study provides the first evidence in humans highlighting the critical role of the RANKL/OPG balance in the progression of bone disease [83]. RANK, expressed on OC precursors and mature OCs, is activated by its ligand RANKL, which is produced by OBs, BMSCs, and immune cells. This interaction promotes OC differentiation, activation, and survival, leading to bone resorption. RANKL is a 217-amino-acid polypeptide that functions biologically in two forms: a transmembrane form weighing approximately 40–45 kDa and a soluble form of 31 kDa. The *TNFSF11* gene, which encodes the RANKL protein, is situated on human chromosome 13q14. The cells of BM microenvironment produce RANKL in response to systemic factors like PTH, dexamethasone, and vitamin D3, as well as to local osteoclastogenic cytokines, including IL-1, TNF, and IL-11 [84,85]. RANK interacts with TRAFs 1, 2, 3, 5, and TRAF6, triggering the activation of MAPKs such as p38 and JNK, as well as the canonical NF-κB pathway in response to RANK signaling [86]. A distinctive feature of RANK, compared to other TNFR superfamily members, is its ability to activate both the canonical and non-canonical NF-κB pathways. At the gene regulation level, RANK signaling plays a central role in inducing the transcription factors c-Fos and NFATc1/NFAT2 [87,88].

OPG, a soluble decoy receptor encoded by *TNFRSF11B* gene, competes with RANK for RANKL binding, thereby inhibiting osteoclastogenesis and maintaining bone homeostasis. OPG is predominantly expressed by BMSCs and pre-OBs but can also be induced in B lymphocytes, dendritic cells (DCs), and follicular dendritic cells (FDCs) [89]. In MM, the balance of this pathway is disrupted: MM cells and the

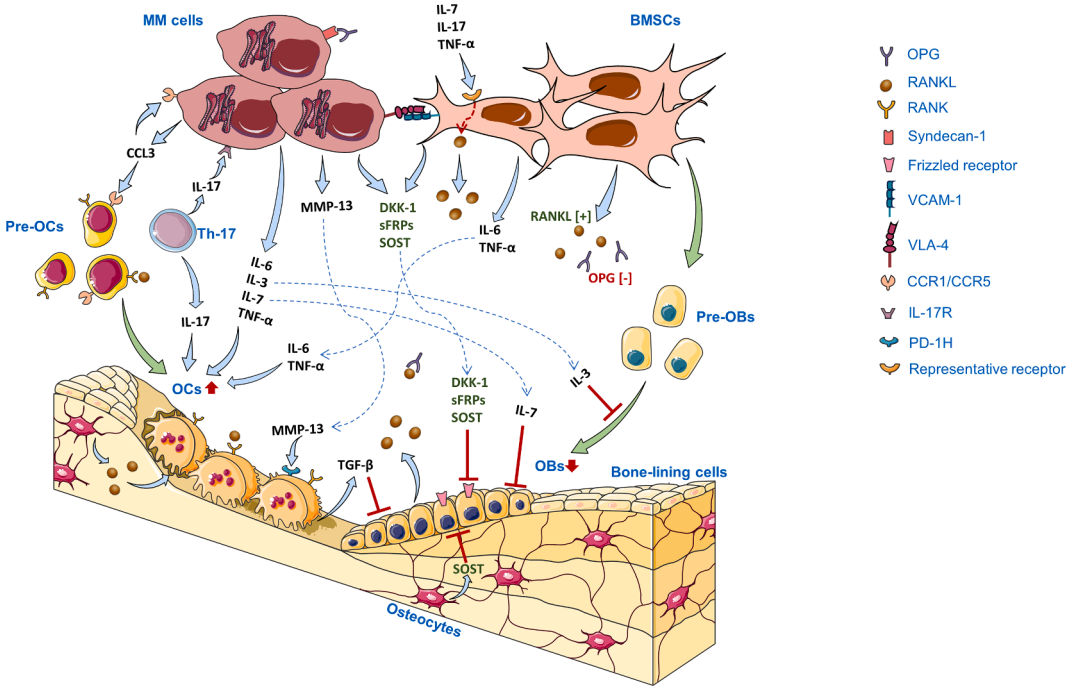

Fig. 2. An overview of the key molecular pathways involved in the pathophysiology of myeloma bone disease (MBD). Abbreviations: BMSCs: Bone Marrow Stromal cells; CCL3: Chemokine (C-C motif) ligand 3; CCR1/CCR5: C-C chemokine receptor type 1/5; DDK1: Dickkopf-1; sFRPs: secreted Frizzled-related proteins; IL-3: Interleukin-3; IL-6: Interleukin-6; IL-7: Interleukin-7; IL-17: Interleukin-17; IL-17R: Interleukin-17 receptor; MM cells: Multiple Myeloma cells; MMP-13: Matrix Metalloproteinases-13; OBs: Osteoblasts; OCs: Osteoclasts; OPG: Osteoprotegerin; PD-1H: Programmed Death-1 Homolog, MMP-13 Receptor; RANK: Receptor Activator of Nuclear Factor-kappa B; RANKL: Receptor Activator of Nuclear Factor-kappa B Ligand; SOST: Sclerostin; TGF-β: Transforming Growth Factor beta; Th-17: T helper 17; TNF-α: Tumor Necrosis Factor-alpha; VCAM-1: Vascular Cell Adhesion Molecule 1; VLA-4: Very Late Antigen-4.

surrounding BM microenvironment increase RANKL production while simultaneously reducing OPG expression, favoring excessive OC activity [90]. The high expression of Syndecan-1 (CD138) on MM cells binds OPG, leading to its internalization and subsequent degradation within the lysosomal compartment. This process significantly lowers OPG levels, further impairing its ability to neutralize RANKL [91]. This dysregulation drives bone resorption, leading to lytic lesions, pathological fractures, and severe skeletal morbidity, while also suppressing OB activity, resulting in defective bone formation. Furthermore, enhanced OC activity facilitates the release of growth factors, including TGF-β, from the bone matrix, which in turn promote MM cell proliferation and survival, establishing a vicious cycle of bone destruction and tumor growth [92,93]. In addition, MM-derived exosomes have been found to influence the RANK/RANKL/OPG axis by enhancing RANKL expression and osteoclastogenesis, emphasizing the complex interplay between MM cells and the bone microenvironment [94].

Recent discoveries have expanded our understanding of this pathway and its therapeutic implications. denosumab, a monoclonal antibody that neutralizes RANKL, has emerged as a key therapeutic agent for MM-related bone disease, offering advantages over bisphosphonates, as zoledronic acid, particularly in patients with renal impairment [95]. Ongoing research also explores dual inhibitors and multi-targeted agents capable of simultaneously suppressing OC activity and enhancing OB function. These advances underscore the centrality of the RANK/RANKL/OPG pathway in MM pathophysiology, offering a dual opportunity to mitigate skeletal-related events and disrupt tumor-bone interactions, thus improving patient outcomes and quality of life.

3.2. *Wnt/β-catenin pathway*

The Wnt/β-catenin pathway is a highly conserved signaling cascade that plays a central role in the regulation of bone homeostasis, embryonic development, cell proliferation, differentiation, and survival [96]. In the context of MM, the dysregulation of this pathway has profound effects on the pathogenesis of MBD, contributing to severe osteolytic lesions, impaired bone formation, and enhanced tumor progression within the BM microenvironment. The Wnt/β-catenin signaling pathway consists of two main branches: the canonical pathway and the non-canonical pathway, both of which play significant roles in cellular processes such as proliferation, differentiation, migration, and survival, as well as in bone homeostasis and disease, including MM-associated bone disease [97].

The canonical pathway is β-catenin-dependent and is primarily involved in regulating gene transcription. In this pathway, Wnt ligands (e.g., Wnt1, Wnt3a, and Wnt10b) bind to Frizzled (Fzd) receptors and the co-receptor Lrp5/6 on the cell surface. This binding inhibits the β-catenin destruction complex, which includes Axin, APC (adenomatous polyposis coli), GSK-3β (glycogen synthase kinase-3β), and CK1 (casein kinase 1). Under normal circumstances, this complex phosphorylates β-catenin, targeting it for ubiquitin-mediated degradation. When Wnt ligands are present, the destruction complex is disrupted, allowing β-catenin to accumulate in the cytoplasm. Stabilized β-catenin translocates into the nucleus, where it interacts with TCF/LEF (T-cell factor/lymphoid enhancer factor) transcription factors to activate target genes involved in OB differentiation, cell proliferation, and survival [98]. In bone biology, the canonical pathway promotes osteoblastogenesis and inhibits osteoclastogenesis by increasing the expression of OPG. In MM, this balance is disrupted as malignant PCs and the surrounding BMSCs secrete Wnt inhibitors such as DKK1, sFRPs (secreted Fzd-related proteins), and SOST, leading to reduced OB activity and increased OC-mediated bone resorption [97]. Dkk1 is a secreted glycoprotein that inhibits Wnt signaling by interacting with Lrp5/6 and Kringle-containing transmembrane protein 1 (Kremen1). This interaction forms a ternary complex, which facilitates the endocytosis and removal of Lrp5/6 from the plasma membrane [98]. sFRPs instead inhibit the canonical Wnt pathway by competing with Wnt proteins for binding to the Lrp-5/6 receptor, which subsequently leads to the degradation of β-catenin [22].

The non-canonical Wnt pathway operates independently of β-catenin and primarily regulates cytoskeletal dynamics, cell polarity, and migration. It can be subdivided into two main branches: the Wnt/PCP (planar cell polarity) pathway and the Wnt/Ca^{2+} pathway [97].

In the Wnt/PCP pathway, Wnt ligands (e.g., Wnt5a) bind to Fzd receptors and co-receptors such as Ror2 or Vangl. This triggers activation of Rho GTPases (e.g., RhoA, Rac, and Cdc42) and downstream kinases, such as JNK (c-Jun N-terminal kinase) and ROCK (Rho-associated protein kinase). The Wnt/PCP pathway regulates cytoskeletal organization, cell migration, and tissue polarity. In the context of cancer, including MM, this pathway is associated with tumor cell invasiveness and metastasis [96,99]. Moreover, MM cells suppress Ror2 expression in pre-OB by inhibiting non-canonical Wnt5a signaling, thereby impairing osteogenic differentiation [100].

In the Wnt/Ca^{2+} pathway, Wnt ligands (e.g., Wnt5a and Wnt11) activate Fzd receptors, leading to the release of intracellular calcium ions (Ca^{2+}). This activates calcium-dependent signaling molecules such as calmodulin-dependent protein kinase II (CaMKII), protein kinase C (PKC), and the transcription factor NFAT. The Wnt/Ca^{2+} pathway regulates processes like cell adhesion, migration, and inflammation [101,102]. The involvement of non-canonical Wnt signaling in MM was first highlighted in a study by Qiang et al. [103], which showed that Wnt3a significantly alters the morphology of MM cells by modulating cytoskeletal dynamics.

Inhibition of Wnt antagonists, such as neutralizing antibodies against DKK1 has demonstrated preclinical efficacy in restoring bone formation, reducing osteolysis, and controlling MM progression [51,104]. Activation of the Wnt pathway through GSK-3β inhibitors, including lithium chloride and small molecule inhibitors, can stabilize β-catenin, thereby promoting osteoblastogenesis and reducing OC activity [52,105]. While Wnt pathway activation is beneficial for bone repair, its effects on MM cells must be carefully considered. Some studies indicate that β-catenin stabilization in MM cells can promote cell proliferation, survival, and drug resistance, highlighting a potential pro-tumorigenic effect [106,107]. Conversely, other research suggests that Wnt pathway activation may suppress the development of MBD and reduce tumor burden in bone *in vivo* [52]. The specific molecular context of MM cells, including the presence of mutations and the state of the tumor microenvironment, may influence whether Wnt signaling exerts pro-tumorigenic or tumor-suppressive effects [97]. This dual role underscores the complexity of targeting the Wnt/β-catenin pathway in MM. Future research aims to further unravel the cross-talk between Wnt signaling and other pathways, including the RANKL/RANK/OPG axis, to optimize treatment strategies. In conclusion, the Wnt/β-catenin pathway represents a critical target in MBD, offering potential for restoring bone integrity and improving clinical outcomes.

3.3. *Cytokines signaling*

The immune system is deeply interconnected with bone health, relying on inflammatory cytokines to regulate skeletal homeostasis. Disruptions in the balance of these cytokines can have severe consequences for overall health. BM cells play a significant role in initiating and amplifying various signaling pathways that drive MM progression [108]. These pathways are often triggered by the release of soluble factors from both bone and stromal cells. Key soluble factors include IL-6, IGF-1, stromal cell-derived factor-1 (SDF-1), TNF-α, interleukin-8 (IL-8), interleukin-17 (IL-17), and VEGF. These molecules are involved in processes such as inflammation, immunosuppression, tumor development, and osteolysis, contributing to a range of pathological conditions [108].

IL-6, produced by BMSCs and MM cells, plays a dual role in the BM microenvironment: it promotes MM cell survival and proliferation while stimulating osteoclastogenesis through downstream activation of the

JAK/STAT3 and MAPK pathways [109]. *In vivo* research has demonstrated that IL-6 drives bone loss in both trabecular and endochondral bone. This effect is further exacerbated in the presence of MM cells and is linked to an increase in OC differentiation [110]. IL-6 is produced by BM cells in response to various stimuli, including TNF-α, IL-1, and IL-17.

Another pro-osteoclastogenic cytokine include CCL3, also known as MIP-1α. CCL3 is secreted by MM cells and BMSCs in the tumor microenvironment. It binds to its receptors CCR1 and CCR5 on pre-OCs, promoting their differentiation into mature OCs. Beyond stimulating OCs, CCL3 indirectly suppresses OB activity through upregulation of factors like DKK1 and SOST, which inhibit Wnt signalling [111]. A novel CCL3-HMGB1 signaling axis has been identified, where MM-derived CCL3 triggers the secretion of HMGB1 by osteocytes. This process is essential for the upregulation of RANKL in osteocytes, further promoting osteoclastogenesis and bone resorption [70]. CCL3 directly influences MM cells by binding to the CCR5 receptor, a process that supports their growth, survival, and migration. Additionally, CCL3 upregulates β1 integrin expression on MM cells, enhancing their adhesion to BMSCs. This strengthened interaction triggers BMSCs to secrete elevated levels of RANKL, IL-6, VEGF, and TNF-α, fostering myeloma cell proliferation, promoting angiogenesis, and accelerating bone destruction [112].

IL-3, a cytokine secreted by activated lymphocytes, exhibits elevated mRNA expression in myeloma cells and increased protein levels in the BM plasma of MM patients. It exerts a dual effect by promoting OC formation while inhibiting OB differentiation, thereby contributing to bone remodeling imbalance in MM [113]. When combined with CCL3 or RANKL, IL-3 markedly amplifies OC formation and bone resorption compared to the effects of CCL3 or RANKL alone. Additionally, IL-3 independently promotes the growth of myeloma cells, even in the absence of IL-6 [113,114].

IL-7 is primarily involved in the development and maintenance of immune cells. In MM, IL-7 contributes to bone disease by inhibiting OB activity, leading to reduced bone formation. Additionally, IL-7 indirectly promotes osteoclastogenesis by increasing the production of RANKL and decreasing OPG levels, favoring bone resorption [115,116].

IL-17 is a pro-inflammatory cytokine produced by T-helper 17 (Th17) cells. It has been shown to promote myeloma cell growth and colony formation via the IL-17 receptor, enhance adhesion to BMSCs, and increase growth *in vivo*. Furthermore, IL-17 promotes OC activation and induces osteolytic lesions by stimulating RANKL expression in BMSCs [117]. An anti-IL-17A monoclonal antibody (AIN 457) has been evaluated for its potential therapeutic effects in this context [118,119].

TGF-β promotes OC differentiation and activity, particularly when released from the bone matrix during resorption, creating a feedback loop that exacerbates bone degradation. Simultaneously, it suppresses OB differentiation and function by inhibiting Wnt signaling, leading to reduced bone formation and skeletal fragility [120]. It also modulates stromal and mesenchymal cells in the BM, creating a supportive niche for MM cell survival and proliferation. Additionally, TGF-β enhances the production of cytokines such as IL-6, which further drive MM cell growth and OC activation [121,122].

TNF-α is a pro-inflammatory cytokine with elevated levels observed in patients with MM. TNF-α promotes OC differentiation and activation by increasing the expression of RANKL in BMSCs and OBs. This interaction enhances osteoclastogenesis, resulting in increased bone resorption [42]. In the tumor microenvironment, TNF-α contributes to MM progression by inducing the transcription factor XBP1 in BMSCs, leading to increased production of VCAM1, RANKL, and IL-6. These factors enhance stromal cell support of MM cell growth and OC formation [42]. Inhibiting TNF-α activity may reduce OC-mediated bone resorption and disrupt the supportive tumor microenvironment, potentially impairing myeloma cell survival. Elevated TNF-α levels have been associated with disease severity and progression in MM, suggesting its potential as a biomarker for prognosis and treatment response [123].

3.4. Metabolic alterations in the bone marrow microenvironment: a spotlight on glutamine metabolism

Metabolism refers to the intricate network of biochemical processes that living cells rely on to produce energy and sustain growth and survival. It includes the synthesis and degradation of glucose, fatty acids, and amino acids, alongside energy production (ATP generation) and oxidative phosphorylation. In cancer cells, metabolic pathways are often reprogrammed to support tumor growth and cellular proliferation [124].

MM cells exhibit profound metabolic alterations, including changes in glucose, amino acid, and lipid metabolism, which drive tumor growth and influence the surrounding bone microenvironment [124]. The interplay between altered metabolism in MM cells and the bone microenvironment contributes to bone destruction and impaired remodeling. Glutamine (Gln) metabolism plays a critical role in MM progression and is intricately linked to the development of MBD (Fig. 3). MM cells exhibit “Gln addiction” due to the lack of Gln synthetase (GS), relying on extracellular Gln for energy production, biosynthesis, and maintaining redox balance [125,126]. It has been shown that blocking Gln uptake in MM cells through the sodium-dependent neutral amino acid transporter (ASCT2) inhibition significantly reduces cell growth both *in vitro* and *in vivo* models. Due to these alterations, the BM of MM patients is characterized by lower Gln and higher levels of its metabolite, glutamate (Glu) [126]. MM cells metabolize Gln through glutaminolysis, converting it into Glu via glutaminase (GLS) and subsequently into α-ketoglutarate (α-KG), which fuels the tricarboxylic acid (TCA) cycle. Increased Gln uptake and utilization in MM cells deprive other cells of the microenvironment of this critical nutrient. Gln depletion driven by MM promotes the induction of GS in MSCs, as observed in BM biopsies from MM patients and confirmed *in vitro* by co-culturing human MSCs with MM cells. It has been observed that during OB differentiation, the expression of *Slc38a2*, which encodes the SNAT2 transporter, was significantly upregulated in the presence of Gln, while its induction was markedly reduced in the absence of the amino acid. Similarly, GLS1 exhibited a comparable expression pattern, and its inhibition impaired the activation of osteogenic markers during MSC differentiation [4]. Indeed, skeletal stem cells depend significantly on Gln consumption and utilization during OB formation, making it a crucial factor in their proliferation, lineage specification, and differentiation [127]. Glutaminolysis is also important for OC differentiation. Research has demonstrated that CB-839, a potent and selective inhibitor of GLS, effectively impairs osteoclastogenesis and bone resorption. By inhibiting glutaminolysis, CB-839 reduces the availability of amino acids and nucleotides essential for OC differentiation and activity, leading to a decrease in OC-specific markers and a reduction in bone resorption activity. *In vivo*, administration of CB-839 prevented ovariectomy-induced bone loss in mice, suggesting its efficacy in conditions characterized by increased OC activity [128]. IL-17 has been shown to exacerbate bone loss in Gln-dependent pathways. A recent study revealed that blocking OC Gln transport effectively prevents IL-17-induced OC activation and bone loss, highlighting a previously unrecognized link between IL-17, Gln metabolism, and energy regulation [129]. Another study highlights the critical role of the amino acid transporter *Slc1a5* (ASCT2) in OC differentiation. Specifically, it shows that in *Slc1a5*-deficient mice, the formation of OCs from BM cells is impaired. This impairment is linked to the suppression of key signaling pathways involved in osteoclastogenesis, such as RANKL-induced activation of ERK (Extracellular Signal-Regulated Kinase), NFκB and NFATc1 [130]. In osteocytes, HIF-1α has been shown to transcriptionally upregulate significant expression of GLS, leading to the depletion of endogenous Gln reserves and an increase in Gln oxidation flux. Additionally, blocking glycolysis or supplementing Gln can restore mechanosensitivity during reloading in bones that were previously subjected to unloading. This restoration occurs by supporting the TCA cycle and rescuing the subsequent Ca^{2+} oscillatory response in osteocytes [131]. In summary, targeting Gln

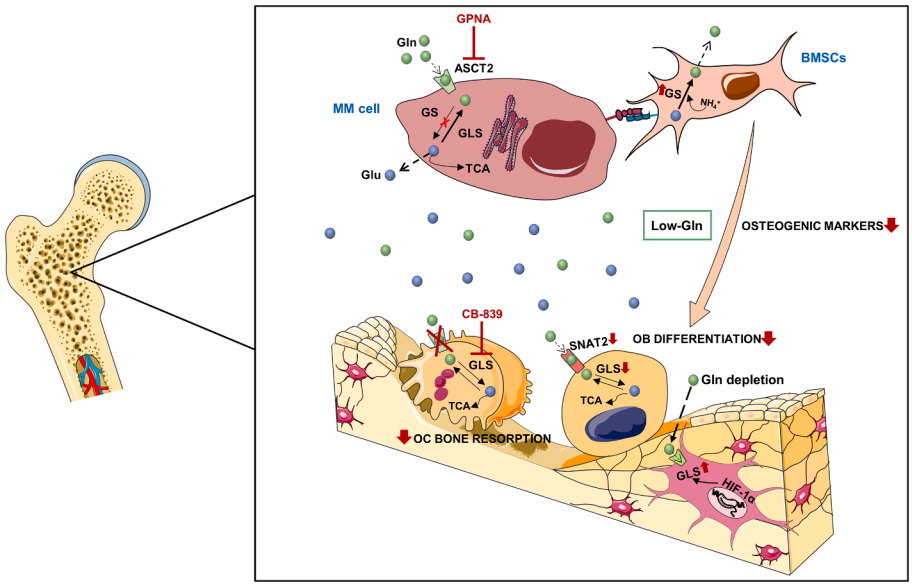

Fig. 3. Metabolic changes in the microenvironment of MM patients. MM cells exhibit elevated GLS levels and a deficiency in GS expression, making them highly reliant on extracellular Gln. This characteristic of malignant plasma cells leads to a BM microenvironment with reduced Gln levels, which in turn impairs OB formation and activity in patients with MM-related bone disease. Targeting Gln metabolism by inhibiting GLS or the ASCT2 transporter aims to disrupt key metabolic pathways that sustain myeloma cell growth and contribute to bone destruction. Abbreviations: ASCT2: Anti-Neutral amino acid transporter 2; BMSCs: Bone Marrow Stromal Cells; CB-839: Telaglenastat, GLS1 inhibitor; Gln: Glutamine; Glu: Glutamate; GLS: Glutaminase; GPNA: L-γ-Glutamyl-p-nitroanilide, selective inhibitor of ASCT2; GS: Glutamine Synthetase; MM cell: Multiple Myeloma cell; NH4+: Ammonium; OB: Osteoblast; OC: Osteoclast; SNAT2: Sodium-coupled neutral amino acid transporter 2; TCA: Tricarboxylic Acid Cycle.

metabolism offers a promising strategy to mitigate MBD. By modulating Gln availability or its downstream metabolic pathways, it may be possible to restore bone homeostasis and reduce skeletal damage in MM patients.

4. Clinical implications of targeting bone disease in multiple myeloma

For the treatment and prevention of MBD and the SREs in MM patients, OC inhibitors are the only drugs approved. These drugs include bisphosphonates as clodronate, pamidronate and zoledronic acid and the monoclonal antibody anti-RANKL (denosumab). The clinical implication of the use of OC inhibitors is to block the bone destruction and OC activity that represent the hallmark of the osteolytic bone disease in MM. Clinical studies in MM patients treated with bisphosphonates have demonstrated a significant reduction in bone pain, the incidence of SREs, and the need for surgical procedures and radiotherapy [132,133]. The reduction of SREs in MM patients may have an impact on the quality of life and the survival due to the reduction of the hospitalization. In MM patients with the evidence of osteolytic bone lesions, denosumab also demonstrated a reduction of SREs similar that of zoledronic acid. Furthermore, the progression-free survival (PFS) advantage of denosumab over zoledronic acid, as reported by Terpos et al. [134], is among the benefits observed in newly diagnosed MM, particularly in patients planning to undergo autologous stem cell transplantation (ASCT) and those receiving PI-based triplet regimens. A possible synergistic mechanism can be hypothesized between bortezomib and denosumab, suggested by the fact that both act on RANKL; bortezomib decreases circulating levels of RANKL and DKK-1 in MM patients [135]. PIs, including bortezomib, not only inhibit OC differentiation but also promote osteoblastogenesis and bone formation, demonstrating bone-anabolic activity. In turn OB stimulation have an anti-MM effect both *in vitro* and *in vivo* [136]. The improved PFS observed could be therefore due to the effective denosumab-induced inhibition of OC activity and subsequent decrease in OC-mediated stimulation of MM cell growth. Differences in the mechanism of action of denosumab and zoledronic acid, may explain the observed benefit of denosumab over zoledronic acid. Denosumab binds to RANKL, thus inhibiting OC formation and decreasing bone resorption, on the other hand zoledronic acid blocks protein prenylation, via inhibition of farnesyl pyrophosphate synthase inducing an increase of OC apoptosis and a reduction of OC activity without affecting OC formation. Relative inhibition of OCs is probably more complete with denosumab than with zoledronic acid. Moreover, it is well known that RANKL is a survival factor for dendritic cells and that RANKL inhibitors such as denosumab may affect dendritic cell survival and function [137,138]. Interestingly, it has recently been reported that dendritic cell-mediated activation induces activation-induced cytidine deaminase (AID)-dependent genomic instability in human MM cells. This effect is mediated by RANKL, as pretreatment with anti-RANKL antibodies resulted in AID inhibition [139]. These data show an important role for cell-associated RANKL mediated signaling in the induction of AID in MM cells that can be targeted by approaches such as RANKL inhibition. Therefore, it can also be hypothesized that these mechanisms may explain, at least in part, the observed effect of denosumab on PFS in MM patients undergoing ASCT.

Regarding the possible bone anabolic therapy in MM patients, previous studies have demonstrated that inhibiting DKK-1 with neutralizing antibodies (such as BHQ880) enhances bone formation and inhibits MM progression in preclinical models [51]. *In vitro*, BHQ880 promoted OB differentiation and reduced IL-6 secretion. In a severe combined immunodeficiency (SCID)-hu murine model of human MM, BHQ880 treatment led to a significant increase in the number of OBs, serum human osteocalcin levels, and trabecular bone. Although BHQ880 did not directly affect MM cell growth, it significantly inhibited MM cell proliferation in the presence of BMSCs *in vitro*. This inhibition was linked to reduced BMSC/MM cell adhesion and decreased IL-6 production. Additionally, BHQ880 increased β-catenin levels and decreased NF-κB activity in BMSCs. Notably, *in vivo*, BHQ880 also inhibited MM cell growth in the SCID-hu murine model [104]. Phase I clinical trial with the combination of BHQ880 with zoledronic acid and anti-myeloma therapy has been performed in patients with relapsed or refractory MM without significant clinical effects [140]. On the other hand, clinical

trial with antibody anti-SOST (romosozumab) are ongoing in MM patients with osteoporosis (ClinicalTrials.gov ID NCT05775094).

5. Conclusions

In conclusion, the pathophysiology of bone destruction in MM is a highly intricate process driven by the interplay of malignant PCs with the surrounding bone microenvironment. This interaction creates a vicious cycle in which PCs promote the recruitment and activation of OCs, while simultaneously inhibiting the differentiation and activity of OBs. This results in an imbalance that favors bone resorption over bone formation leading to the characteristic osteolytic lesions observed in MM patients. This disruption also creates a supportive niche that facilitates tumor growth and progression. Key molecular pathways are central to this process, with the RANK-RANKL-OPG axis being a primary driver of osteoclastogenesis and bone resorption, while the suppression of the Wnt/β-catenin signaling pathway hampers OB-mediated bone formation. Additional factors, such as cytokines and chemokines released by malignant cells and stromal cells and the metabolic changes established within the BM, further exacerbate this imbalance, creating a microenvironment that perpetuates bone destruction and enhances tumor cell survival. Such insights emphasize the need for a comprehensive understanding of the molecular and cellular mechanisms involved to develop effective therapeutic interventions aimed at breaking this destructive cycle.

Therapeutic strategies targeting these pathways, such as bisphosphonates, RANKL inhibitors, and agents modulating the Wnt pathway, have shown promise in mitigating bone damage and improving patient outcomes. Moreover, targeting Gln metabolism in MBD holds promise as a novel therapeutic strategy. By inhibiting enzymes like GLS, researchers aim to disrupt the metabolic pathways that support myeloma cell growth and bone destruction.

However, challenges remain, including variability in patient response, potential adverse effects, and the need for combination therapies to address the multifactorial nature of MM-associated bone disease. Future research should aim to further elucidate the underlying mechanisms driving this pathological process and identify novel therapeutic targets.

CRediT authorship contribution statement

Oxana Lungu: Writing – review & editing, Writing – original draft, Conceptualization. **Denise Toscani:** Writing – review & editing, Supervision. **Nicola Giuliani:** Writing – review & editing, Validation, Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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