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Effects of high-dose methotrexate on bone metabolism: A narrative literature review

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ABSTRACT

Methotrexate (MTX) play an important role in oncology, where it is used in high-dose therapy regimens. However, its associated dose-dependent toxicity, including MTX-osteopathy, poses a serious clinical problem, particularly in pediatric oncology. Even in patients with inflammatory rheumatic diseases receiving low doses, rare cases of MTX-osteopathy have been reported. This review provides an updated discussion of the side effects of high-dose MTX (HD-MTX), with the aim of improving understanding of the pathophysiology underlying its effects on bone, identifying potential supportive treatments, and highlighting new areas of research that could enhance the understanding and management of chemotherapy-related adverse effects.

Methodology: A review of the available literature was conducted to analyse the impact of HD-MTX on bone tissue.

Results: MTX disrupts bone homeostasis and microarchitecture through a variety of cell-specific mechanisms. These effects trigger a cascade of negative outcomes, including abnormalities in macrometric and structural bone histomorphometric parameters, as well as impaired bone healing. Few studies have evaluated the protective effects of adjunctive therapies, and those that do exist demonstrate only partial protection. This highlights the need for further research to develop more effective strategies to prevent MTX-induced bone toxicity.

Conclusions: Available data suggest that the effects of HD-MTX involve multiple mechanisms that disrupt bone homeostasis, including impaired osteoblast function, enhanced osteoclastogenesis, altered osteocyte viability, increased bone marrow adipogenesis, and endothelial damage. These changes lead to reduced bone density, compromised microarchitecture, and impaired healing. This highlights the importance of multi-targeted strategies to prevent MTX-induced bone damage and improve skeletal outcomes.

1. Introduction

Methotrexate (MTX), originally developed in the 1940s as a folate antagonist, has been one of the key drugs in oncology practice for decades and has been used to treat a range of malignant neoplasms, including osteosarcoma, acute leukemia, non-Hodgkin's lymphoma, and primary CNS lymphoma (Bleyer, 1978; Ferreri et al., 2004; Huennekens, 1994). Despite rapid advances in chemotherapeutics and oncological therapy, MTX is still commonly administered at high-doses for the treatment of malignant tumors in children and adults (Paul et al., 2016; Brown et al., 2020; Joerger et al., 2012; Christensen et al., 2012; Howard et al., 2016). Although a universally accepted definition of high-dose methotrexate (HD-MTX) is lacking, clinical guidelines generally define it as a dosage of $\geq$ 500 mg/m^2 (Howard et al., 2016). In contrast, low-dose methotrexate (5–25 mg/week) is considered a disease-modifying agent in various autoimmune disorders, including rheumatoid arthritis and other inflammatory arthropathies, systemic lupus erythematosus, Crohn's disease, vasculitis, atopic dermatitis, and psoriasis (Minaur et al., 2002; Singh et al., 2016). HD-MTX exerts its therapeutic effects by inhibiting the dihydrofolate reductase (DHFR), a key enzyme involved in cell replication, purine nucleotide and thymidylate synthesis, and subsequently in DNA and RNA syntheses (Olsen, 1991; Raimondi et al.,

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2019). Despite its many therapeutic benefits, HD-MTX is associated with a wide range of adverse effects and clinical risks. The most common toxicities include hepatotoxicity, acute lung injury, nephrotoxicity, neurotoxicity, pancytopenia and dysregulation of the gut microbiota (Marin et al., 2022; Albrecht and Muller-Ladner, 2010). The negative effects of HD-MTX chemotherapy on bones may not initially seem concerning in oncological practice, but the development of fractures in 9-13,5% of children (Ecklund et al., 1997; Ahn et al., 2015; Hogler et al., 2007), delayed fracture healing, and impaired bone growth (van der Sluis et al., 2002) might have significant long-term consequences which are often underestimated. This is particularly relevant for children, as HD-MTX is administered during the years of active skeletal growth, well before long bones have fully matured and peak bone mass have been achieved. Unfortunately, to our knowledge, there are currently no clear data on the effect of HD-MTX on the risk of osteopathy or fractures in cohorts of adult cancer survivors.

Despite the relatively large number of studies devoted to HD-MTX toxicity, the mechanisms underlying its effects on bone tissue are still not fully elucidated. The most pronounced side effect of HD-MTX on bone in clinical practice is classified as MTX-osteopathy, which was first described over 40 years ago in patients treated for acute leukemia (Ragab et al., 1970), defined by the clinical triad of bone pain, osteoporosis, and stress fractures (Ragab et al., 1970; Cegiela et al., 2005; Maenaut et al., 1996; Preston et al., 1993; Robin et al., 2021; Stevens et al., 2001). The successful treatment and improvements in oncology over the last decades lead to an increasing number of cancer survivors. Therefore, the awareness of potential short and long-term effects on the skeleton is crucial.

In this review, we summarize the main findings of studies investigating the effects and mechanisms of methotrexate on bone metabolism and current strategies for its prevention.

1.1. Methodology

We conducted a literature search of the PubMed, Scopus, and Web of Science databases (until December 31, 2024) using search terms including "methotrexate," "bone," "osteopathy," "fracture," "osteoblast," "osteoclast," "osteocyte," "chondrocyte," and "bone marrow". This was supplemented by manual screening of reference lists from key publications. The inclusion criteria were: (1) studies reporting the pathophysiological effects of high-dose methotrexate used as chemotherapy on bone, and (2) studies evaluating the potential bone-protective effects of adjunctive treatments administered in combination with methotrexate. A total of 442 relevant articles were identified. These were independently reviewed by two authors to ensure they met the inclusion criteria. After excluding studies that did not meet these criteria, 92 studies were included in our review.

1.2. Clinical effects of HD-MTX on bone

Clinical data on the effects of HD-MTX on bone are limited, and most available clinical data comes from children with acute lymphoblastic leukemia (ALL), the most common childhood malignancy. In children treated for ALL, and therefore with HD-MTX among other therapies, fracture and osteonecrosis rate increases substantially, reaching up to 13,5% and 12,1% respectively, not only during treatment but also afterward and during the maintenance phase (Hogler et al., 2007). Fractures typically occur at a median of 14.92 months after diagnosis, predominantly in weight-bearing bones (Hogler et al., 2007; Elmantaser et al., 2010). However, this cohort of patients is exposed to multiple risk factors affecting the developing skeleton, such as reduced physical activity, impaired nutrition, low body mass index, hormonal deficiencies, and concomitant treatments, including other chemotherapeutics and glucocorticoids (Hogler et al., 2007; Schini et al., 2024). In particular, glucocorticoid co-treatment in ALL is strongly associated with skeletal comorbidities (Elmantaser et al., 2010). Another common detrimental complication which is nearly inseparably linked to glucocorticoid treatment in ALL children is an increased risk for osteonecrosis (Hogler et al., 2007; Mattano Jr. et al., 2012).

Comparable radiological findings, as well as insufficiency fractures, have been described in 9% of children with osteosarcoma. The most commonly affected sites of fractures were the distal radius and tibia, proximal humerus, calcaneus, and pubic bone. This effect was not directly dose-dependent and was substantially more likely to affect younger patients (mean age 9.2 years compared to 14.9 years) (Ecklund et al., 1997).

Data on bone mineral density (BMD) are conflicting. Not glucocorticoids or vitamin D, but HD-MTX was found to have a negative, dose-related effect on BMD among pediatric ALL survivors at the beginning of their long-term follow-up care (Yamanishi et al., 2024). A recent study on childhood cancer survivors (CCS) after HD-MTX treatment in Switzerland indicated musculoskeletal complications in 56% of patients. 61% of those had reduced BMD and nearly all of them had concomitantly received glucocorticoid treatment. However, a recent meta-analysis evaluating three studies on long-term effects on BMD in CCS identified no significant reduction of BMD (Daetwyler et al., 2022). Therefore, since patient populations in these studies are small and data in the literature limited, screening guidelines for CCS regarding BMD are not uniform (C.O. Group, 2018; Brunold et al., 2024; Hudson et al., 2021; Skinner et al., 2007).

In infants with CNS tumors under three years of age, very high dosages of MTX (cumulative up to 135 g/m^2) were administered to postpone radiation treatment. These patients experienced toxic side effects including osteopenia, thick dense provisional zones of calcification, growth arrest lines in the lower extremities, and corner fractures (Meister et al., 1994).

Unfortunately, there is very little clinical data on the impact of HD-MTX on bone in adults. It has only been shown that in adult patients with osteosarcoma, MTX reduced radial BMD and bone mineral content (BMC) dose-dependently, primarily at the distal point of trabecular bone but not at the midpoint (Gnudi et al., 1988).

2. MTX effects on bone marrow

2.1. Vascular endothelial growth factor (VEGF)

HD-MTX causes significant bone marrow (BM) sinusoidal endothelium damage by inhibiting BM-derived endothelial cells (BMECs) proliferation and migration, inducing BMECs apoptosis, and reducing the tube-forming abilities of sinusoidal endothelial cells (Peymanfar et al., 2022a; S.Y.-W. Hassanshahi et al., 2019), downregulating of expression of angiogenesis marker CD-31 (Hassanshahi et al., 2019) as well as dilation and regression of microvasculature in the BM with significant increase in cortical vasculature, pore volume and the number of vasculature canals (Peymanfar et al., 2022a; S.Y.-W. Hassanshahi et al., 2019; Hassanshahi et al., 2019) with significant increases in sinusoid diameter and sinus volume/marrow volume ratio (S.Y.-W. Hassanshahi et al., 2019). However, HD-MTX does not significantly induce BMECs production of nitric oxide (NO) (Peymanfar et al., 2022a), known as a pro-angiogenic factor (MacLauchlan et al., 2011) and a main regulatory molecule for normal and pathological vasculature remodelling (Miller et al., 2009). But it significantly reduces the expression of the vascular endothelial growth factor (VEGFa) mRNA, the modulator of endothelial cell proliferation and migration (Georgiou et al., 2012a) in BMECs (Peymanfar et al., 2022a; Hassanshahi et al., 2019). Increased microvasculature dilation and dysfunction following MTX chemotherapy accompanied by an elevation of the production and release of TNF-α from osteoblasts and BM cells, which is known to cause endothelial cell dysfunction (Peymanfar et al., 2022a).

2.2. Notch2 pathway

Peymanfar et al. found that HD-MTX caused no significant changes in Notch1 levels, but a significant upregulation of Notch2 mRNA (Peymanfar et al., 2022b) and an obvious elevation of the spatiotemporal expression pattern of active Notch2 intracellular domain (NICD2) protein level, which was most predominantly expressed in bone lining osteoblasts and osteocytes in rat metaphyseal bone (Peymanfar et al., 2022b). Upregulation of NICD2 was also found in rat BMECs (Peymanfar et al., 2022a). Furthermore, it has been demonstrated that Notch2 overactivation leads to the dilation and dysfunction of microvasculature and the induction of osteoclastogenesis by promoting NFATc1 activity (Fukushima et al., 2008) with a remarkable upregulation of the Notch target genes HeyL, Hes1 and Hey1 as well as Jag 1 and Delta-like ligand 1 in rat bone marrow mesenchymal stromal cells (BMSCs) (Peymanfar et al., 2022a; Peymanfar et al., 2022b).

2.3. Wnt pathway

In metaphyseal bone, HD-MTX increases mRNA expression of Wnt antagonists secreted frizzled-related protein-1 (sFRP-1), dickkopf-1 (Dkk-1), survivin and sclerostin (SOST) (Peymanfar et al., 2022b; Georgiou et al., 2012b). HD-MTX also causes reduction in nuclear β-catenin protein in BMSCs, with decreased mRNA levels of β-catenin target genes Lef-1 and cyclin D1. The mRNA expression of low-density lipoprotein-related receptors 5 and 6 (LRP5/6) antagonist Dkk-1 was found to be not significantly increased in metaphyseal bone (Georgiou et al., 2012b). These findings demonstrate an important role of Notch2 and Wnt/β-catenin signalling in HD-MTX chemotherapy-induced cellular changes to the BM microenvironment (Peymanfar et al., 2022b; Georgiou et al., 2012b).

In the secondary spongiosa, HD-MTX induces BM sinusoidal endothelial cells apoptosis with upregulation of the expression of not only the pro-apoptotic gene Bax but also the anti-apoptotic gene Bcl-2 (S.Y.-W. Hassanshahi et al., 2019). Moreover, increased expression of the Bcl-2 gene has been found in the growth plate cartilage of the tibia (Xian et al., 2007). It was suggested that the overexpression of the Bcl-2 gene may be involved in minimizing the induction of apoptosis (Xian et al., 2007).

2.4. Bone marrow cellularity

HD-MTX increases the expression of matrix metalloproteinase (MMP-9) in whole metaphyseal bone, which can directly degrade C-X-C motif chemokine ligand-12 (CXCL12) (Georgiou et al., 2012a; Georgiou et al., 2010), reducing its level in the BM with dysregulation of CXCL12/CXCR4 chemotactic signalling, contributing to decreased BM cellularity (Georgiou et al., 2012a). In addition, HD-MTX reduces BM CXCL12 protein levels (despite its increased mRNA expression in BMSCs) and reduces C-X-C motif chemokine ligand-4 (CXCR4) mRNA expression correlating with a reduction of BM cellularity (Georgiou et al., 2012a; Georgiou et al., 2012b). This suggests that potential deregulation of CXCL12/CXCR4 signalling may occur in this HD-MTX chemotherapy-induced stress, partially associated with the reduced BM hematopoietic cells density observed in the BM cavity (Georgiou et al., 2012b). In addition, it reduces BM differentiation potential (Georgiou et al., 2012b) by decreasing the proliferation of BMSCs, preosteoblasts, osteoblasts (Xian et al., 2007; Fan et al., 2011; Davies et al., 2002; Uehara et al., 2001; Georgiou et al., 2012c) and marrow pericytes, but dramatically increases BMSCs differentiation towards adipocytes (Peymanfar et al., 2022b; Georgiou et al., 2012b; Georgiou et al., 2012c; Fan et al., 2012; Raghu Nadhanan et al., 2013; Raghu Nadhanan et al., 2014; Zhang et al., 2021a; Zhang et al., 2022; Marques et al., 2013; Georgiou et al., 2015). As a result, HD-MTX administration causes a switch from osteogenesis to adipogenesis in the BM resulting in an increase in adiposity in the long bones (Zhang et al., 2022; Fan et al., 2009) with a dramatic increase in adipocyte number and density (Peymanfar et al., 2022b; Raghu Nadhanan et al., 2014; Lee et al., 2017; King et al., 2015) and a reduction of total BM cellularity (Georgiou et al., 2012a; Fan et al., 2011; Georgiou et al., 2012c).

Indeed, HD-MTX chemotherapy increases the expression of master regulatory transcription factors associated with adipogenic differentiation – proliferator activator activated receptor-γ (PPAR - γ) mRNA (Peymanfar et al., 2022b; Georgiou et al., 2012c; Fan et al., 2012), and the expression of the early adipogenic regulator C/EBPα (Zhang et al., 2021a; Zhang et al., 2022; Lee et al., 2017; King et al., 2015), fatty acid binding protein 4 (FABP4) (Georgiou et al., 2012b; Raghu Nadhanan et al., 2013) and Peroxisome proliferator-activated receptor γ (PPARγ) expression mainly involved in fatty acid storage, glucose metabolism and adipogenesis regulation (Zhang et al., 2021a; Zhang et al., 2022).

2.5. microRNAs (miRNAs)

Recently, the first studies have been published on the effects of HD-MTX on miRNAs involved in the regulation of bone homeostasis, such as osteogenesis and adipogenesis. They showed that HD-MTX upregulates miR-155-5p, miR-154-5p, miR-344 g, miR-6215 and miR-6315, which are promising candidates associated with MTX-induced bone defects (Zhang et al., 2022). The expression of Smad2, a key mRNA target of miR-6315, was significantly reduced and showed a clear inverse correlation with miR-6315 levels (Zhang et al., 2022). Furthermore, the action of miR-6315 involved in osteogenesis/adipogenesis regulation might be partially due to its modulation of the TGF-β/Smad2 signalling pathway (Zhang et al., 2021b). HD-MTX decreases distal-less homeobox 5 (DLX5) mRNA expression (Zhang et al., 2022) and reduces the expression of miR-542-3p, which promotes increased adipogenesis (Zhang et al., 2021a). The influence of microRNAs in the development of MTX osteopathy requires further research.

2.6. Mechanical stimulation

Although the role of mechanical stimulation in bone development and homeostasis is well established, a review of the current literature reveals that only a single study to date has investigated its effects on bone cells exposed to MTX. Elliot et al. demonstrated that MTX dose-dependently suppresses the electrophysiological response of human bone cells to mechanical stimulation. This was accompanied by reduced production and release of interleukin-1β (IL-1β), decreased mRNA expression of bone morphogenetic protein-4 (BMP-4), and a consistent, though statistically non-significant, increase in type I collagen mRNA levels. However, MTX does not inhibit the downstream effects of IL-1β on target cells (Elliot et al., 2004). These changes suggest that MTX suppresses the response of human bone cells to mechanical stimuli by reducing the differentiation/anabolic response (Elliot et al., 2004).

3. Effects on the osteoblast cell lineage

Published data indicate that HD-MTX chemotherapy has a multifactorial negative effect on osteoblast metabolism (Wheeler et al., 1995), decreases osteoblast function (Wheeler et al., 1995; May et al., 1994), inhibits osteoblast proliferation (Scheven et al., 1995), and decreases bone formation (Fan et al., 2011; Fan et al., 2012; Lee et al., 2017; May et al., 1994) with dose-dependent effects that are especially pronounced in the early stages of development (Cegiela et al., 2005; Gnudi et al., 1988; Cavalcanti et al., 2014; Poutoglidou et al., 2021). Human and animal studies found that HD-MTX suppresses supply of osteoblastic progenitor cells (Xian et al., 2007; Fan et al., 2011), affects early osteoblastic cells and inhibits their differentiation into osteoblasts but has little influence on either the function or proliferation of late-stage osteoblasts (Xian et al., 2007; Davies et al., 2002; Uehara et al., 2001). Partially in line with the in vitro data, animal studies have shown that HD-MTX dramatically reduce the expression of osteogenesis marker

genes, including alkaline phosphatase (ALP) (Uehara et al., 2001; Peymanfar et al., 2022c), runt-related transcription factor 2 (Runx2), Osterix (Osx), Cyclin D1 (CcnD1), osteocalcin (OCN) (Georgiou et al., 2012c; Zhang et al., 2021a; Peymanfar et al., 2022c) and suppresses Wnt 10b (Peymanfar et al., 2022c), which is known as a positive regulator of mesenchymal stem cell differentiation, osteoblast differentiation, and bone formation (Bennett et al., 2007), and is generally considered an indispensable factor in the maintenance of bone homeostasis (Liu et al., 2022). The effects of HD-MTX on osteoblasts may be mediated via Notch and Wnt signalling as it induces the Notch2 target gene Hes1 in pre-osteoblastic cells which closely correlates with an inhibition in osteoblast differentiation and function (see above) (Peymanfar et al., 2022c). In addition, it induces the expression of the Wnt antagonists secreted frizzled-related protein 1 (sFRP1), Dickkopf 1 (Dkk1), SOST (encodes sclerostin) and survivin (Peymanfar et al., 2022b; Georgiou et al., 2012b). In contrast, the expression of Collagen type 1 alpha 1 (Col1a1), cell surface ligand Jagged1 (Jag1), Notch1 mRNA and Notch target gene Hey1 remained unchanged (Peymanfar et al., 2022c). MTX also affects preosteoblastic cell viability and activates preosteoblastic cell apoptosis (Xian et al., 2007; Zhang et al., 2021b; Shandala et al., 2012; Corrado et al., 2015), but the signalling pathways are unknown.

At the late stage of differentiation, HD-MTX causes a downregulation of Runx2, Wnt 10b, Col1a, *CCND1*, OCN and β-catenin 1 (CTNNB1), Notch ligand Jag2, and Notch1 mRNA levels, together with an upregulation of sFRP1, Dkk1, Notch ligand Jag1, Notch2, Hes1 and Hey1 mRNA levels, without significantly changing ALP mRNA levels. These results confirm that HD-MTX suppresses signalling of genes regulating osteogenesis at all stages of cell differentiation but, to a greater extent, at the early stages of differentiation (Peymanfar et al., 2022c).

The effects of HD-MTX on osteoblast density are controversial. Some studies report a reduction in osteoblast and preosteoblast surface density in the primary (Fan et al., 2012; Fan et al., 2009; Shandala et al., 2012) and secondary spongiosa (Xian et al., 2007; Friedlaender et al., 1984). However, other studies did not find significant reductions in osteoblast numbers in the secondary spongiosa (Georgiou et al., 2012b; Raghu Nadhanan et al., 2013; King et al., 2015).

4. Effects on the osteocyte cell lineage

Osteocytes are descended from mature, matrix-producing osteoblasts (Robling and Bonewald, 2020). The effects of methotrexate on osteocytes are among the least studied, despite the fact that the osteocytes are the longest-lived and most numerous cells in bone tissue, and play a central role in regulating bone metabolism and the balance of bone formation and bone resorption in bone (re)modelling (Robling and Bonewald, 2020; Tsourdi et al., 2018; Plotkin and Bellido, 2016).

It was found that HD-MTX chemotherapy increases apoptosis among osteocytes within trabeculae in the primary spongiosa and to a lesser extent in the secondary spongiosa (Xian et al., 2007; Shandala et al., 2012; Xian et al., 2008). Furthermore, HD-MTX induces a significant increase in apoptotic osteocytes in bone trabeculae of juvenile rats, accompanied by a 35% loss of trabecular bone (Shandala et al., 2012).

Shandala et al. 2012 unexpectedly found that increased levels of osteocyte apoptosis in HD-MTX-treated bones coincided with an increase in the total number of osteocytes ("healthy" plus apoptotic) and correlated with the up-regulation of expression of the osteocyte-specific genes DMP1 and SOST (Shandala et al., 2012). But most "healthy" osteocytes possessed shorter and fewer dendrites, and none of these osteocytes were positive for sclerostin, whose expression was immunodetected only in the cortical zone of the trabecular bone (Shandala et al., 2012). Apoptotic osteocytes have a great capacity to trigger the formation of osteoclasts, potentially through secreted cytokines IL-6 and IL-11, suggesting the possibility of cross-talk between apoptotic osteocytes and osteoclasts (Shandala et al., 2012).

Peymanfar et al. 2022 found no significant changes in osteocyte lacunar porosity and osteocyte lacunar number, but a significant increase in vascular canal porosity, average vascular canal number, and a decrease in vascular canal separation using a rat model of HD-MTX chemotherapy (Peymanfar et al., 2022a).

5. Effects on the osteoclast cell lineage

Studies of the effects of HD-MTX on the expression of key genes regulating osteoclastogenesis in rodent bone samples have shown an upregulation of the main osteoclastogenic factor – receptor activator for nuclear factor kappa B ligand (RANKL) with an increase in the RANKL/OPG osteoprotegerin (OPG) ratio (Fan et al., 2012; Raghu Nadhanan et al., 2014; Fan et al., 2009; Lee et al., 2017; King et al., 2015). It is well known that the RANK–RANKL–OPG system is a complex signalling pathway that plays a critical role in bone metabolism and remodelling (Lacey et al., 1998). Overall, the obtained results indicate the activation of the pro-osteoclastogenic signalling system RANK–RANKL–OPG by HD-MTX, with subsequent stimulation of osteoclastogenesis.

Furthermore, HD-MTX increases the level of pro-osteoclastogenic pro-inflammatory cytokines TNF-α (Peymanfar et al., 2022a), IL-1β, IL-6 (Raghu Nadhanan et al., 2013; King et al., 2015; King et al., 2012) and IL-11 in metaphysis bones (Shandala et al., 2012). However, it did not cause significant differences in the levels of anti-inflammatory cytokines IL-4 and IL-10 and mRNA expression in bone (Raghu Nadhanan et al., 2013).

HD-MTX-induced osteoclastogenesis results in both a significant increase in the numbers of differentiated osteoclasts and in the pool of osteoclast precursor cells in BM (Peymanfar et al., 2022b; Georgiou et al., 2012b; Raghu Nadhanan et al., 2013; King et al., 2015; Shandala et al., 2012), and an increase in the number of osteoclasts in metaphyseal primary and in secondary spongiosa (Peymanfar et al., 2022b; Georgiou et al., 2012b; Raghu Nadhanan et al., 2013; Lee et al., 2017; King et al., 2012).

6. Effects on the chondrocyte cell lineage

Direct effects of HD-MTX on cellular processes in the growth plate and in metaphyseal bone are likely a key element of the untoward skeletal effects of MTX chemotherapy in children, impacting bone lengthening and suppressing longitudinal growth and bone mass accumulation (Xian et al., 2007; Fan et al., 2011; Su et al., 2023).

Early animal studies observed that a short course of HD-MTX did not affect lactate dehydrogenase (LDH) release from chondrocytes, did not change the proportion of viable chondrocytes in cartilage explants, and did not appreciably influence the rate of chondrocyte protein and proteoglycan synthesis, proteoglycan breakdown, or the hydrodynamic size of newly synthesized proteoglycans or total proteoglycans. However, MTX dose-dependently increased the uptake of [3H]-thymidine, and decreased the incorporation of [3H]-d-uridine into chondrocytes, suggesting inhibition of thymidylate-synthetase activity, which could negatively affect chondrocyte DNA metabolism (Neidel et al., 1998).

It was later found that HD-MTX reduces collagen II expression, suppresses the proliferation of growth plate chondrocytes in the proliferative zone, and induces apoptosis in regions of the growth plate where it does not normally occur (Xian et al., 2007; Fan et al., 2012; Xian et al., 2008; Fan et al., 2014; Su et al., 2021). Counting of cells per chondrocyte column revealed a significant reduction of chondrocyte numbers after HD-MTX treatment (Fan et al., 2012).

In young rats treated with HD-MTX, an increased expression of CXCL12 was found in the tibial growth plate, and more resorbing chondroclasts were present at the border between the hypertrophic growth plate and metaphyseal bone, leading to bone growth impairments. This shows an association between chondrocyte apoptosis and stimulated osteoclastic migration following HD-MTX treatment, which may be linked to CXCL12 induction (Su et al., 2021).

Induction of chondrocyte apoptosis is accompanied by significant induction of expression of anti-apoptotic molecule Bcl-2 in growth plate

but not significant downregulation of expression of pro-apoptotic gene Bax, and does not significantly upregulate death receptor Fas and Fas ligand (Xian et al., 2007; Fan et al., 2012). Xian et al. 2008 suggested that the Fas death receptor pathway may be involved in HD-MTX-induced apoptosis in growth plate, and that Bcl-2 upregulation of the Bax–Bcl-2 system may be involved in minimizing apoptotic induction (Xian et al., 2008). Obviously, the few existing studies with unclear results do not allow us to reliably identify the effects of HD-MTX on the mechanisms that regulate chondrocyte apoptosis, and further research is required.

HD-MTX effects on bone at the molecular and cellular level are summarised in Fig. 1.

7. Bone material properties

7.1. Bone growth & trabecular bone volume

Published studies show that MTX induces dose-dependent changes in various bone macroscopic and histomorphometric parameters (summarised in Fig. 2). Studies on animal models confirm that HD-MTX chemotherapy leads to abnormalities in bone growth. This affects transverse growth (cross-sectional area) as well as length growth (growth plate affected) and leads to a decrease in long bone mass, diminished bone mineral and calcium content (Cegiela et al., 2005; Xian et al., 2007; Nowinska et al., 2007), and corresponding deterioration of mechanical properties (Cegiela et al., 2005; Nowinska et al., 2007; van Leeuwen et al., 2003; Van Leeuwen et al., 2003).

In rodents, structural changes in or near the growth plate in the femur and tibia are well documented: each of the different zones of the growth plate is affected by HD-MTX. These changes in resting, proliferative and hypertrophic zone are responsible for a significant reduction in growth plate thickness (Fan et al., 2012; Raghu Nadhanan et al., 2013; Fan et al., 2014; Van Leeuwen et al., 2003; Iqbal et al., 2003). The height of the primary spongiosa decreases, while BV/TV remains constant within primary spongiosa (Fan et al., 2012; Raghu Nadhanan et al., 2013; Fan et al., 2009; Lee et al., 2017; Xian et al., 2008). This is accompanied by a reduction in trabecular bone volume in the secondary spongiosa, as well as a decrease in metaphyseal height (Raghu Nadhanan et al., 2013; Lee et al., 2017; King et al., 2015; Shandala et al., 2012; Su et al., 2023; Fan et al., 2014; van Leeuwen et al., 2003). The reduction in BV/TV is caused by a decreased trabecular thickness and trabecular number as well as an increased trabecular separation (Peymanfar et al., 2022b; Fan et al., 2012; Lee et al., 2017; King et al., 2015; Fan et al., 2014).

7.2. Bone mineral density

In ex vivo mineralization assays it has been shown that HD-MTX chemotherapy significantly inhibits matrix mineralization (Georgiou et al., 2012b; Uehara et al., 2001; Raghu Nadhanan et al., 2013; Zhang et al., 2021a; May et al., 1994). In histomorphometry and in ex vivo osteoclastogenesis assays, MTX was associated with an increase in osteoclast density (Georgiou et al., 2012b; Uehara et al., 2001; Fan et al., 2009; King et al., 2012). In contrast, histomorphometric parameters of bone formation were reduced, including osteoblast number (Uehara et al., 2001), as well as mineralizing surface, mineral apposition rate and

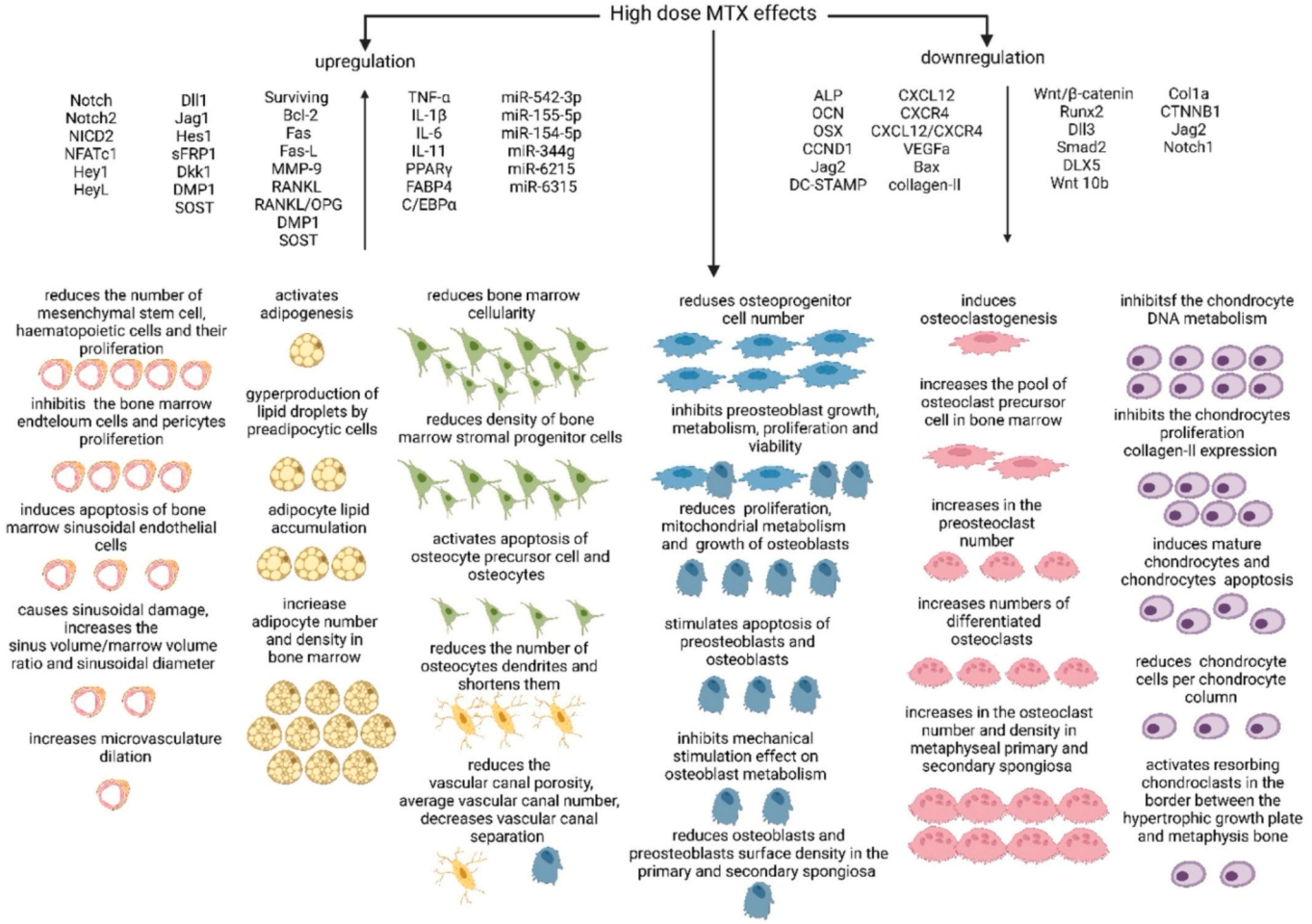

Fig. 1. Effects of high dose methotrexate at the molecular and cellular levels based on reviewed articles.

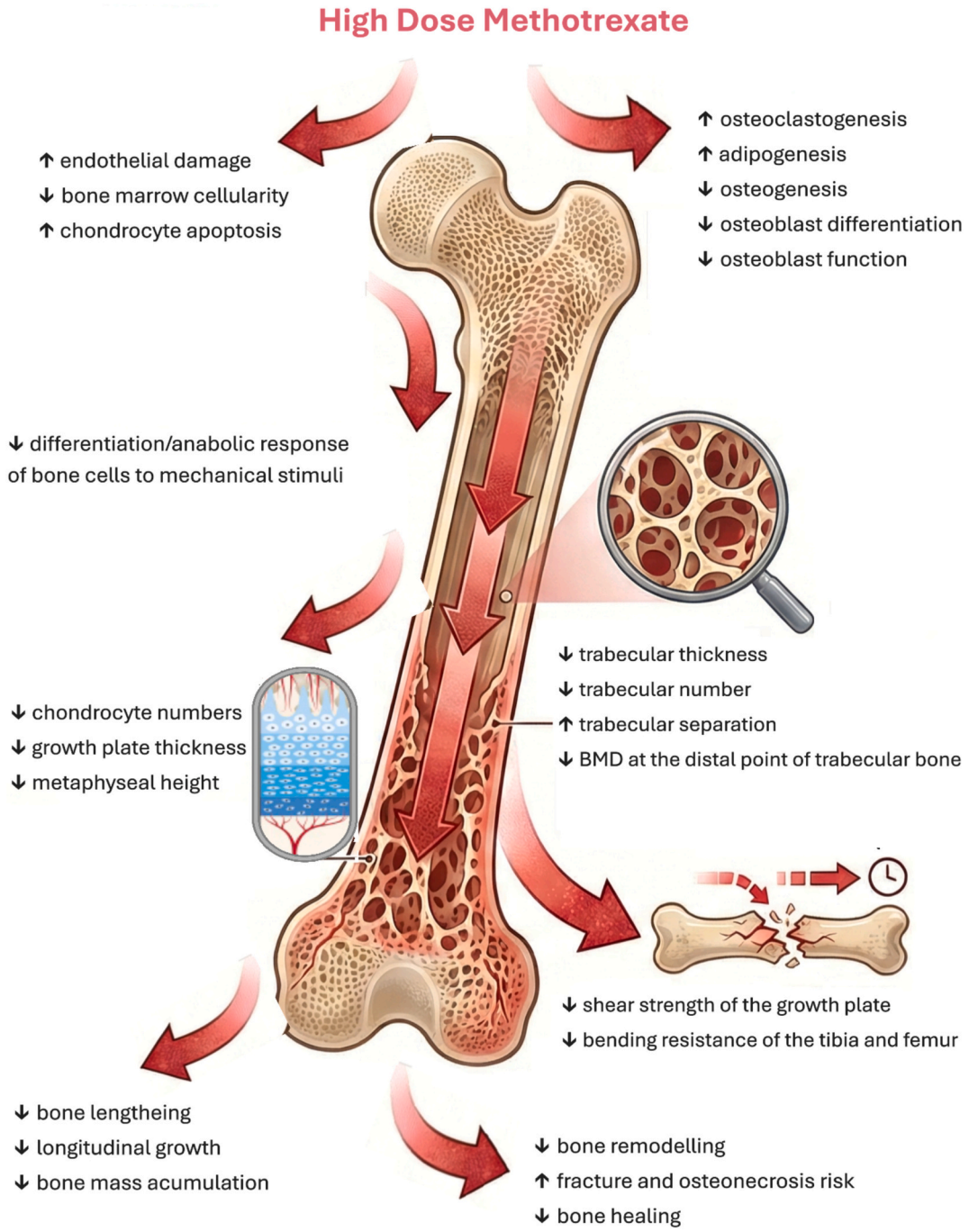

Fig. 2. Effects of high dose MTX on bone macrometric and structural histomorphometric parameters based on reviewed articles.

bone formation rates (Liu et al., 2015). In rats, this leads to a reduction of BMD in the femur and in the vertebrae, but no significant BMD difference was found at other sites (Liu et al., 2015; Spadaro et al., 2006).

There is insufficient data on the impact of HD-MTX on BMD in humans. It has only been found that, in adult patients with osteosarcoma, HD-MTX has a negative, dose-dependent effect on radial BMD and BMC, primarily at the distal point of trabecular bone, but not at the midpoint (Gnudi et al., 1988). In a pediatric ALL cohort, HD-MTX was found to have a negative, dose-dependent effect on BMD at the beginning of long-term follow-up (Yamanishi et al., 2024). However, no significant reduction in BMD was found years after the last HD-MTX treatment in childhood cancer survivor cohort (Daetwyler et al., 2022).

7.3. Bone mechanical properties & fracture repair

In rodents, HD-MTX chemotherapy leads to negative effects on the mechanical competence of long bones. In rats, a reduction in the shear strength of the growth plate and in the bending resistance of the tibia and femur have been reported after MTX treatment (Cegiela et al., 2005; Nowinska et al., 2007; Van Leeuwen et al., 2003). This confirms the role of HD-MTX in the increased fracture risk in humans. Noteworthy, one study from Spadaro et al. in 2006 found no significant differences in peak moment, bending rigidity, or energy to fracture in rat femoral midshaft after HD-MTX treatment using three-point bending tests (Spadaro et al., 2006).

HD-MTX was also reported to delay fracture repair. In a rat model of mandibular condyle fracture, histological analysis revealed diminished

bone neoformation, impaired mandibular condyle repair, and degenerative articular changes (atrophy of the fibrocartilage and the presence of fibrous tissue in the joint space) present 90 days after fracture (Cavalcanti et al., 2014). The mechanism by which MTX delays bone healing and remodelling is poorly understood and requires further research. An analysis of the available literature indicates a lack of human data on the effect of HD-MTX on fracture repair. However, it can be assumed that its key importance lies in its ability to inhibit the metabolism and proliferation of osteoblasts and chondrocytes, which play a crucial role in callus formation.

8. Management of MTX bone effects

8.1. Folinic acid

There is currently a lack of research examining the potential bone protective effect of adjunctive treatment in combination with HD-MTX. Most of the research has focused on the potential protective effect of folinic acid (FA) – the preferred antidote for MTX poisoning. It reduces gut absorption and mitigates the cellular toxicities of HD-MTX, while also replenishing intracellular folate stores (Hamed et al., 2022). Also, high FA doses during HD-MTX treatment were associated with increased risk of relapse and reduced cure rates in childhood ALL (Skarby et al., 2006). However, in the pediatric transplantation setting, late FA treatment seemed safe with regard to relapse in malignant disease, graft-versus-host disease after hematopoietic stem cell transplantation and rejection. But FA supplementation in doses >185 mg/m^2 were recognized as risk factors for graft rejection in nonmalignant disease (Lindqvist et al., 2013).

FA has been shown to have at least a partial bone protective effect during HD-MTX treatment (Fan et al., 2011; Fan et al., 2009; Xian et al., 2008). It was found that FA reduces MTX up-regulation of adipogenesis regulatory genes PPAR-g (Fan et al., 2012; Raghu Nadhanan et al., 2014) and FABP4 mRNA in BMSCs, which contributes to the prevention of adipocyte increase (Raghu Nadhanan et al., 2014), decreases bone marrow adiposity (Fan et al., 2012; Raghu Nadhanan et al., 2014) and enhances proliferation of BMSCs and progenitor cells (Xian et al., 2008).

On the other hand, FA preserved the downregulation of OCN mRNA reduction (Raghu Nadhanan et al., 2014) and increased osteoblast differentiation (Fan et al., 2009; Xian et al., 2008) with prevention of osteoblast apoptosis, which contributes to normalization of osteoblast density within the primary (Fan et al., 2012; Fan et al., 2009) and secondary spongiosa (Raghu Nadhanan et al., 2014). In addition, FA has moderate protective effects against HD-MTX damage in preserving proliferation of osteoblasts and pre-osteoblasts and in limiting the degree of osteocyte apoptosis, particularly in the primary spongiosa (Xian et al., 2008). FA suppress the osteoclastogenic potential of HD-MTX by downregulation of IL-1 (Fan et al., 2012; Raghu Nadhanan et al., 2014), IL-6, and TNF-α expression and RANKL/OPG ratio (Raghu Nadhanan et al., 2014; Fan et al., 2009). As a result, FA can, at least partially, reduce the negative effect of MTX on the reduction of trabecular bone volume in primary and secondary spongiosa and preserve the primary spongiosa height (Fan et al., 2012; Raghu Nadhanan et al., 2014; Fan et al., 2009). In addition, FA can preserve collagen-II mRNA expression (Xian et al., 2008), prevent HD-MTX-induced chondrocyte apoptosis, and preserve chondrocyte columnar arrangement and number in the growth plate. This suggests that folinic acid supplementation during HD-MTX treatment can alleviate growth plate (Fan et al., 2012; Xian et al., 2008) and metaphyseal damage and therefore may be potentially useful in pediatric patients who are at risk of skeletal growth suppression due to MTX chemotherapy (Fan et al., 2012).

8.2. Omega-3 polyunsaturated fatty acids

Fish oil rich in omega-3 polyunsaturated fatty acids (FO) and soy products rich in isoflavone genistein supplementation were found to be as effective as FA in inhibiting osteoclastogenic cytokine expression, osteoclast formation, BM adiposity, and in preventing bone loss during HD-MTX treatment (Raghu Nadhanan et al., 2013; Raghu Nadhanan et al., 2014). FO was also found to be more effective than FA in down-regulating FABP4 and IL-6, and in stimulating Osx and OCN expression. Additionally, FO is more effective at maintaining trabecular thickness and preventing loss of trabecular bone volume within the primary and secondary spongiosa (Raghu Nadhanan et al., 2014).

Unfortunately, there is currently a gap in research into the potential protective role of other drugs used to treat osteoporosis such as bisphosphonates, RANKL inhibitors, calcitonin and hormone therapy on bone when using HD-MTX chemotherapy. We found only one study that assessed the protective effect of the selective estrogen-receptor modulator Raloxifene on bone mechanical properties in male rats. It found that Raloxifene administration resulted in a stimulation of bone mineralization, measured as an increase in the ratio of femur mass to body mass, and by an increase in the mineral content and the ratio of the bone mineral content to bone mass. As a result, Raloxifene increases both the ultimate and breaking load values of the whole femur, as well as the load resulting in the femoral neck fracture (Nowinska et al., 2007).

It is obvious that further research is needed to develop maintenance treatment regimens for more effective prevention of the adverse effects of MTX chemotherapy on bones.

9. Conclusion

Although high doses of methotrexate have long been used in oncology practice, the clinical significance and pathophysiology of its effect on bone tissue remains poorly understood. Most studies have been conducted in vitro or on animal models, with a critical lack of human studies and clinical data on the effects of HD-MTX chemotherapy on human bone tissue. Available data suggest that HD-MTX involves multiple mechanisms that disrupt bone homeostasis, including impaired osteoblast function, enhanced osteoclastogenesis, altered osteocyte viability, bone marrow adipogenesis, and endothelial damage. These lead to reduced bone density, poor microarchitecture, and impaired healing. While few studies have explored adjunctive therapies, the results suggest only partial protection. This underscores the need for multi-targeted strategies to prevent MTX-induced bone damage and improve skeletal outcomes.

CRediT authorship contribution statement

Anton Sokhan: Writing – original draft, Visualization, Conceptualization. **Markus A. Hartmann:** Writing – review & editing. **Stéphane Blouin:** Writing – review & editing. **Reinhold G. Erben:** Writing – review & editing. **Jochen Zwerina:** Writing – review & editing. **Judith Haschka:** Writing – review & editing, Writing – original draft. **Martina Behanova:** Writing – review & editing. **Roland Kocijan:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

In preparing this manuscript, ChatGPT (OpenAI, GPT-4) was employed to assist with grammar corrections and language polishing. After that, the author reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare no competing interests.

Data availability

No data was used for the research described in the article.

References

Ahn, J.H., Cho, W.H., Lee, J.A., Kim, D.H., Seo, J.H., Lim, J.S., 2015. Bone mineral density change during adjuvant chemotherapy in pediatric osteosarcoma. Ann. Pediatr. Endocrinol. Metab. 20 (3), 150–154.

Albrecht, K., Muller-Ladner, U., 2010. Side effects and management of side effects of methotrexate in rheumatoid arthritis. Clin. Exp. Rheumatol. 28 (5 Suppl 61), S95–101.

Bennett, C.N., Ouyang, H., Ma, Y.L., Zeng, Q., Gerin, I., Sousa, K.M., Lane, T.F., Krishnan, V., Hankenson, K.D., MacDougald, O.A., 2007. Wnt10b increases postnatal bone formation by enhancing osteoblast differentiation. J. Bone Miner. Res. 22 (12), 1924–1932.

Bleyer, W.A., 1978. The clinical pharmacology of methotrexate: new applications of an old drug. Cancer 41 (1), 36–51.

Brown, P., Inaba, H., Annesley, C., Beck, J., Colace, S., Dallas, M., DeSantes, K., Kelly, K., Kitko, C., Lacayo, N., Larrier, N., Maese, L., Mahadeo, K., Nanda, R., Nardi, V., Rodriguez, V., Rossoff, J., Schuettpelz, L., Silverman, L., Sun, J., Sun, W., Teachey, D., Wong, V., Yanik, G., Johnson-Chilla, A., Ogba, N., 2020. Pediatric acute lymphoblastic leukemia, version 2.2020, NCCN clinical practice guidelines in oncology. J. Natl. Compr. Cancer Netw. 18 (1), 81–112.

Brunold, K., Otth, M., Scheinemann, K., 2024. Late effects of high-dose methotrexate in childhood cancer survivors: a Swiss single centre observational study. Discov. Oncol. 15 (1), 17.

C.O. Group, 2018. Long-Term Follow-Up Guidlines for Survivors of Childhood, Adolescent and Young Adult Cancers, Version 5.0.

Cavalcanti, S.C., Correa, L., Mello, S.B., Luz, J.G., 2014. The effect of methotrexate on the bone healing of mandibular condylar process fracture: an experimental study in rats. J. Craniomaxillofac. Surg. 42 (7), 1133–1139.

Cegiela, U., Sliwinski, L., Kaczmarczyk-Sedlak, I., Folwarczna, J., 2005. In vivo effects of high-dose methotrexate on bone remodeling in rats. Pharmacol. Rep. 57 (4), 504–514.

Christensen, A.M., Pauley, J.L., Molinelli, A.R., Panetta, J.C., Ward, D.A., Stewart, C.F., Hoffman, J.M., Howard, S.C., Pui, C.H., Pappo, A.S., Relling, M.V., Crews, K.R., 2012. Resumption of high-dose methotrexate after acute kidney injury and glucarpidase use in pediatric oncology patients. Cancer 118 (17), 4321–4330.

Corrado, A., Neve, A., Marucci, A., Gaudio, A., Cantatore, F.P., 2015. Combined effects of infliximab and methotrexate on rheumatoid arthritis osteoblastic cell metabolism. Clin. Exp. Med. 15 (3), 277–283.

Daetwyler, E., Bargetzi, M., Otth, M., Scheinemann, K., 2022. Late effects of high-dose methotrexate treatment in childhood cancer survivors-a systematic review. BMC Cancer 22 (1), 267.

Davies, J.H., Evans, B.A., Jenney, M.E., Gregory, J.W., 2002. In vitro effects of chemotherapeutic agents on human osteoblast-like cells. Calcif. Tissue Int. 70 (5), 408–415.

Ecklund, K., Laor, T., Goorin, A.M., Connolly, L.P., Jaramillo, D., 1997. Methotrexate osteopathy in patients with osteosarcoma. Radiology 202 (2), 543–547.

Elliot, K.J., Millward-Sadler, S.J., Wright, M.O., Robb, J.E., Wallace, W.H., Salter, D.M., 2004. Effects of methotrexate on human bone cell responses to mechanical stimulation. Rheumatology (Oxford) 43 (10), 1226–1231.

Elmantaser, M., Stewart, G., Young, D., Duncan, R., Gibson, B., Ahmed, S.F., 2010. Skeletal morbidity in children receiving chemotherapy for acute lymphoblastic leukaemia. Arch. Dis. Child. 95 (10), 805–809.

Fan, C., Cool, J.C., Scherer, M.A., Foster, B.K., Shandala, T., Tapp, H., Xian, C.J., 2009. Damaging effects of chronic low-dose methotrexate usage on primary bone formation in young rats and potential protective effects of folinic acid supplementary treatment. Bone 44 (1), 61–70.

Fan, C., Georgiou, K.R., King, T.J., Xian, C.J., 2011. Methotrexate toxicity in growing long bones of young rats: a model for studying cancer chemotherapy-induced bone growth defects in children. J. Biomed. Biotechnol. 2011, 903097.

Fan, C.M., Foster, B.K., Hui, S.K., Xian, C.J., 2012. Prevention of bone growth defects, increased bone resorption and marrow adiposity with folinic acid in rats receiving long-term methotrexate. PLoS One 7 (10), e46915.

Fan, C., Garcia, M., Scherer, M., Tran, C., Xian, C.J., 2014. Potential roles of metallothioneins I and II in protecting bone growth following acute methotrexate chemotherapy. J. Chemother. 26 (1), 37–48.

Ferreri, A.J., Guerra, E., Regazzi, M., Pasini, F., Ambrosetti, A., Pivnik, A., Gubkin, A., Calderoni, A., Spina, M., Brandes, A., Ferrarese, F., Rognone, A., Govi, S., Dell'Oro, S., Locatelli, M., Villa, E., Reni, M., 2004. Area under the curve of methotrexate and creatinine clearance are outcome-determining factors in primary CNS lymphomas. Br. J. Cancer 90 (2), 353–358.

Friedlaender, G.E., Tross, R.B., Doganis, A.C., Kirkwood, J.M., Baron, R., 1984. Effects of chemotherapeutic agents on bone. I. Short-term methotrexate and doxorubicin (adriamycin) treatment in a rat model. J. Bone Joint Surg. Am. 66 (4), 602–607.

Fukushima, H., Nakao, A., Okamoto, F., Shin, M., Kajiya, H., Sakano, S., Bigas, A., Jimi, E., Okabe, K., 2008. The association of Notch2 and NF-kappaB accelerates RANKL-induced osteoclastogenesis. Mol. Cell. Biol. 28 (20), 6402–6412.

Georgiou, K.R., Foster, B.K., Xian, C.J., 2010. Damage and recovery of the bone marrow microenvironment induced by cancer chemotherapy - potential regulatory role of chemokine CXCL12/receptor CXCR4 signalling. Curr. Mol. Med. 10 (5), 440–453.

Georgiou, K.R., Scherer, M.A., King, T.J., Foster, B.K., Xian, C.J., 2012a. Deregulation of the CXCL12/CXCR4 axis in methotrexate chemotherapy-induced damage and recovery of the bone marrow microenvironment. Int. J. Exp. Pathol. 93 (2), 104–114.

Georgiou, K.R., King, T.J., Scherer, M.A., Zhou, H., Foster, B.K., Xian, C.J., 2012b. Attenuated Wnt/beta-catenin signalling mediates methotrexate chemotherapy-induced bone loss and marrow adiposity in rats. Bone 50 (6), 1223–1233.

Georgiou, K.R., Scherer, M.A., Fan, C.M., Cool, J.C., King, T.J., Foster, B.K., Xian, C.J., 2012c. Methotrexate chemotherapy reduces osteogenesis but increases adipogenic potential in the bone marrow. J. Cell. Physiol. 227 (3), 909–918.

Georgiou, K.R., Nadhanan, R.R., Fan, C.M., Xian, C.J., 2015. Methotrexate-induced bone marrow adiposity is mitigated by folinic acid supplementation through the regulation of Wnt/beta-catenin signalling. J. Cell. Physiol. 230 (3), 648–656.

Gnudi, S., Butturini, L., Ripamonti, C., Avella, M., Bacci, G., 1988. The effects of methotrexate (MTX) on bone. A densitometric study conducted on 59 patients with MTX administered at different doses. Ital. J. Orthop. Traumatol. 14 (2), 227–231.

Hamed, K.M., Dighriri, I.M., Baomar, A.F., Alharthy, B.T., Alenazi, F.E., Alali, G.H., Alenazy, R.H., Alhumaidi, N.T., Alhulayfi, D.H., Alotaibi, Y.B., Alhumaidan, S.S., Alhaddad, Z.A., Humadi, A.A., Alzahrani, S.A., Alobaid, R.H., 2022. Overview of methotrexate toxicity: a comprehensive literature review. Cureus 14 (9), e29518.

Hassanshahi, M., Su, Y.W., Khabbazi, S., Fan, C.M., Chen, K.M., Wang, J.F., Qian, A., Howe, P.R., Yan, D.W., Zhou, H.D., Xian, C.J., 2019. Flavonoid genistein protects bone marrow sinusoidal blood vessels from damage by methotrexate therapy in rats. J. Cell. Physiol. 234 (7), 11276–11286.

Hogler, W., Wehl, G., van Staa, T., Meister, B., Klein-Franke, A., Kropshofer, G., 2007. Incidence of skeletal complications during treatment of childhood acute lymphoblastic leukemia: comparison of fracture risk with the General Practice Research Database. Pediatr. Blood Cancer 48 (1), 21–27.

Howard, S.C., McCormick, J., Pui, C.H., Buddington, R.K., Harvey, R.D., 2016. Preventing and managing toxicities of high-dose methotrexate. Oncologist 21 (12), 1471–1482.

Hudson, M.M., Bhatia, S., Casillas, J., Landier, W., C.S.O.G.A.S.O.F.P.H.O. Section On Hematology/Oncology, 2021. Long-term follow-up care for childhood, adolescent, and young adult cancer survivors. Pediatrics 148 (3).

Huennekens, F.M., 1994. The methotrexate story: a paradigm for development of cancer chemotherapeutic agents. Adv. Enzym. Regul. 34, 397–419.

Iqbal, M.P., Ahmed, M., Umer, M., Mehboobali, N., Qureshi, A.A., 2003. Effect of methotrexate and folinic acid on skeletal growth in mice. Acta Paediatr. 92 (12), 1438–1444.

Joerger, M., Huitema, A.D., Illerhaus, G., Ferreri, A.J., 2012. Rational administration schedule for high-dose methotrexate in patients with primary central nervous system lymphoma. Leuk. Lymphoma 53 (10), 1867–1875.

King, T.J., Georgiou, K.R., Cool, J.C., Scherer, M.A., Ang, E.S., Foster, B.K., Xu, J., Xian, C.J., 2012. Methotrexate chemotherapy promotes osteoclast formation in the long bone of rats via increased pro-inflammatory cytokines and enhanced NF-kappaB activation. Am. J. Pathol. 181 (1), 121–129.

King, T.J., Shandala, T., Lee, A.M., Foster, B.K., Chen, K.M., Howe, P.R., Xian, C.J., 2015. Potential effects of phytoestrogen genistein in modulating acute methotrexate chemotherapy-induced osteoclastogenesis and bone damage in rats. Int. J. Mol. Sci. 16 (8), 18293–18311.

Lacey, D.L., Timms, E., Tan, H.L., Kelley, M.J., Dunstan, C.R., Burgess, T., Elliott, R., Colombero, A., Elliott, G., Scully, S., Hsu, H., Sullivan, J., Hawkins, N., Davy, E., Capparelli, C., Eli, A., Qian, Y.X., Kaufman, S., Sarosi, I., Shalhoub, V., Senaldi, G., Guo, J., Delaney, J., Boyle, W.J., 1998. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93 (2), 165–176.

Lee, A.M., Shandala, T., Soo, P.P., Su, Y.W., King, T.J., Chen, K.M., Howe, P.R., Xian, C. J., 2017. Effects of resveratrol supplementation on methotrexate chemotherapy-induced bone loss. Nutrients 9 (3).

van Leeuwen, B.L., Hartel, R.M., Jansen, H.W., Kamps, W.A., Hoekstra, H.J., 2003. The effect of chemotherapy on the morphology of the growth plate and metaphysis of the growing skeleton. Eur. J. Surg. Oncol. 29 (1), 49–58.

Lindqvist, H., Remberger, M., Harila-Saari, A., Winiarski, J., Sundin, M., 2013. Folinic acid supplementation in higher doses is associated with graft rejection in pediatric hematopoietic stem cell transplantation. Biol. Blood Marrow Transplant. 19 (2), 325–328.

Liu, Y., Cui, Y., Chen, Y., Gao, X., Su, Y., Cui, L., 2015. Effects of dexamethasone, celecoxib, and methotrexate on the histology and metabolism of bone tissue in healthy Sprague Dawley rats. Clin. Interv. Aging 10, 1245–1253.

Liu, J., Xiao, Q., Xiao, J., Niu, C., Li, Y., Zhang, X., Zhou, Z., Shu, G., Yin, G., 2022. Wnt/beta-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct. Target. Ther. 7 (1), 3.

MacLauchlan, S., Yu, J., Parrish, M., Asoulin, T.A., Schleicher, M., Krady, M.M., Zeng, J., Huang, P.L., Sessa, W.C., Kyriakides, T.R., 2011. Endothelial nitric oxide synthase controls the expression of the angiogenesis inhibitor thrombospondin 2. Proc. Natl. Acad. Sci. USA 108 (46), E1137–E1145.

Maenaut, K., Westhovens, R., Dequeker, J., 1996. Methotrexate osteopathy, does it exist? J. Rheumatol. 23 (12), 2156–2159.

Marin, G.E., Neag, M.A., Burlacu, C.C., Buzoianu, A.D., 2022. The protective effects of nutraceutical components in methotrexate-induced toxicity models-an overview. Microorganisms 10 (10).

Marques, C., Teixeira, D., Cunha, A., Meireles, M., Pestana, D., Keating, E., Calhau, C., Monteiro, R., Faria, A., 2013. Methotrexate enhances 3T3-L1 adipocytes hypertrophy. Cell Biol. Toxicol. 29 (4), 293–302.

Mattano Jr., L.A., Devidas, M., Nachman, J.B., Sather, H.N., Hunger, S.P., Steinherz, P.G., Gaynon, P.S., Seibel, N.L., G. Children's Oncology, 2012. Effect of alternate-week versus continuous dexamethasone scheduling on the risk of osteonecrosis in paediatric patients with acute lymphoblastic leukaemia: results from the CCG-1961 randomised cohort trial. Lancet Oncol. 13 (9), 906–915.

May, K.P., West, S.G., McDermott, M.T., Huffer, W.E., 1994. The effect of low-dose methotrexate on bone metabolism and histomorphometry in rats. Arthritis Rheum. 37 (2), 201–206.

Meister, B., Gassner, I., Streif, W., Dengg, K., Fink, F.M., 1994. Methotrexate osteopathy in infants with tumors of the central nervous system. Med. Pediatr. Oncol. 23 (6), 493–496.
Miller, T.W., Isenberg, J.S., Roberts, D.D., 2009. Molecular regulation of tumor angiogenesis and perfusion via redox signaling. Chem. Rev. 109 (7), 3099–3124.
Minaur, N.J., Jefferiss, C., Bhalla, A.K., Beresford, J.N., 2002. Methotrexate in the treatment of rheumatoid arthritis. I. In vitro effects on cells of the osteoblast lineage. Rheumatology (Oxford) 41 (7), 735–740.
Neidel, J., Sova, L., Schroers, B., Sintermann, F., Manzke, O., Bohlen, H., 1998. Effects of methotrexate on normal articular cartilage in vitro and in vivo. Ann. Rheum. Dis. 57 (7), 414–421.
Nowinska, B., Cegiela, U., Folwarczna, J., Sliwinski, L., Kaczmarczyk-Sedlak, I., Pytlik, M., Janiec, W., 2007. Effects of raloxifene on development of the methotrexate-induced changes in bone mechanical properties of male rats. Pharmacol. Rep. 59 (3), 359–364.
Olsen, E.A., 1991. The pharmacology of methotrexate. J. Am. Acad. Dermatol. 25 (2 Pt 1), 306–318.
Paul, S., Kantarjian, H., Jabbour, E.J., 2016. Adult acute lymphoblastic leukemia. Mayo Clin. Proc. 91 (11), 1645–1666.
Peymanfar, Y., Su, Y.W., Hassanshahi, M., Xian, C.J., 2022a. Therapeutic targeting Notch2 protects bone Micro-vasculatures from methotrexate chemotherapy-induced adverse effects in rats. Cells 11 (15).
Peymanfar, Y., Su, Y.W., Xian, C.J., 2022b. Notch2 blockade mitigates methotrexate chemotherapy-induced bone loss and marrow adiposity. Cells 11 (9).
Peymanfar, Y., Su, Y.W., Hassanshahi, M., Xian, C.J., 2022c. Methotrexate treatment suppresses osteoblastic differentiation by inducing Notch2 signaling and blockade of Notch2 rescues osteogenesis by preserving Wnt/beta-catenin signaling. J. Orthop. Res. 40 (10), 2258–2270.
Plotkin, L.I., Bellido, T., 2016. Osteocytic signalling pathways as therapeutic targets for bone fragility. Nat. Rev. Endocrinol. 12 (10), 593–605.
Poutoglidou, F., Pourzitaki, C., Manthou, M.E., Samoladas, E., Malliou, F., Saitis, A., Kouvelas, D., 2021. Effects of long-term methotrexate, infliximab, and tocilizumab administration on bone microarchitecture and tendon morphology in healthy Wistar rats. Cureus 13 (4), e14696.
Preston, S.J., Diamond, T., Scott, A., Laurent, M.R., 1993. Methotrexate osteopathy in rheumatic disease. Ann. Rheum. Dis. 52 (8), 582–585.
Ragab, A.H., Frech, R.S., Vietti, T.J., 1970. Osteoporotic fractures secondary to methotrexate therapy of acute leukemia in remission. Cancer 25 (3), 580–585.
Raghu Nadhanan, R., Skinner, J., Chung, R., Su, Y.W., Howe, P.R., Xian, C.J., 2013. Supplementation with fish oil and genistein, individually or in combination, protects bone against the adverse effects of methotrexate chemotherapy in rats. PLoS One 8 (8), e71592.
Raghu Nadhanan, R., Fan, C.M., Su, Y.W., Howe, P.R., Xian, C.J., 2014. Fish oil in comparison to folinic acid for protection against adverse effects of methotrexate chemotherapy on bone. J. Orthop. Res. 32 (4), 587–596.
Raimondi, M.V., Randazzo, O., La Franca, M., Barone, G., Vignoni, E., Rossi, D., Collina, S., Inhibitors, D.H.F.R., 2019. Reading the past for discovering novel anticancer agents. Molecules 24 (6).
Robin, F., Cadiou, S., Albert, J.D., Bart, G., Coiffier, G., Guggenbuhl, P., 2021. Methotrexate osteopathy: five cases and systematic literature review. Osteoporos. Int. 32 (2), 225–232.
Robling, A.G., Bonewald, L.F., 2020. The osteocyte: new insights. Annu. Rev. Physiol. 82, 485–506.
S.Y.-W. Hassanshahi, M., Fan, C.-M., Khabbazi, S., Hassanshahi, A., Xian, C.J., 2019. Methotrexate chemotherapy–induced damages in bone marrow sinusoids: an in vivo and in vitro study. J. Cell. Biochem. 120, 3220–3231.
Scheven, B.A., van der Veen, M.J., Damen, C.A., Lafeber, F.P., Van Rijn, H.J., Bijlsma, J. W., Duursma, S.A., 1995. Effects of methotrexate on human osteoblasts in vitro: modulation by 1,25-dihydroxyvitamin D3. J. Bone Miner. Res. 10 (6), 874–880.
Schini, M., Johansson, H., Harvey, N.C., Lorentzon, M., Kanis, J.A., McCloskey, E.V., 2024. An overview of the use of the fracture risk assessment tool (FRAX) in osteoporosis. J. Endocrinol. Investig. 47 (3), 501–511.
Shandala, T., Shen Ng, Y., Hopwood, B., Yip, Y.C., Foster, B.K., Xian, C.J., 2012. The role of osteocyte apoptosis in cancer chemotherapy-induced bone loss. J. Cell. Physiol. 227 (7), 2889–2897.
Singh, J.A., Saag, K.G., Bridges Jr., S.L., Akl, E.A., Bannuru, R.R., Sullivan, M.C., Vaysbrot, E., McNaughton, C., Osani, M., Shmerling, R.H., Curtis, J.R., Furst, D.E., Parks, D., Kavanaugh, A., O'Dell, J., King, C., Leong, A., Matteson, E.L., Schousboe, J.T., Drevlow, B., Ginsberg, S., Grober, J., Clair, E.W. St, Tindall, E., Miller, A.S., McAlindon, T., 2016. 2015 American College of Rheumatology Guideline for the treatment of rheumatoid arthritis. Arthritis Rheum. 68 (1), 1–26.
Skarby, T.V., Anderson, H., Heldrup, J., Kanerva, J.A., Seidel, H., Schmiegelow, K., H. Nordic Society of Paediatric, Oncology, 2006. High leucovorin doses during high-dose methotrexate treatment may reduce the cure rate in childhood acute lymphoblastic leukemia. Leukemia 20 (11), 1955–1962.
Skinner, R., Wallace, W.H., Levitt, G., 2007. Long-term follow-up of children treated for cancer: why is it necessary, by whom, where and how? Arch. Dis. Child. 92 (3), 257–260.
van der Sluis, I.M., van den Heuvel-Eibrink, M.M., Hahlen, K., Krenning, E.P., de Muinck Keizer-Schrama, S.M., 2002. Altered bone mineral density and body composition, and increased fracture risk in childhood acute lymphoblastic leukemia. J. Pediatr. 141 (2), 204–210.
Spadaro, J.A., Damron, T.A., Horton, J.A., Margulies, B.S., Murray, G.M., Clemente, D.A., Strauss, J.A., 2006. Density and structural changes in the bone of growing rats after weekly alendronate administration with and without a methotrexate challenge. J. Orthop. Res. 24 (5), 936–944.
Stevens, H., Jacobs, J.W., Van Rijk, P.P., De Klerk, J.M., 2001. Methotrexate osteopathy demonstrated by Technetium-99m HDP bone scintigraphy. Clin. Nucl. Med. 26 (5), 389–391.
Su, Y.W., Fan, J., Fan, C.M., Peymanfar, Y., Zhang, Y.L., Xian, C.J., 2021. Roles of apoptotic chondrocyte-derived CXCL12 in the enhanced chondroclast recruitment following methotrexate and/or dexamethasone treatment. J. Cell. Physiol. 236 (8), 5966–5979.
Su, Y.W., Lee, A.M.C., Xu, X., Hua, B., Tapp, H., Wen, X.S., Xian, C.J., 2023. Methotrexate chemotherapy causes growth impairments, vitamin D deficiency, bone loss, and altered intestinal metabolism-effects of calcitriol supplementation. Cancers (Basel) 15 (17).
Tsourdi, E., Jahn, K., Rauner, M., Busse, B., Bonewald, L.F., 2018. Physiological and pathological osteocytic osteolysis. J. Musculoskelet. Neuronal Interact. 18 (3), 292–303.
Uehara, R., Suzuki, Y., Ichikawa, Y., 2001. Methotrexate (MTX) inhibits osteoblastic differentiation in vitro: possible mechanism of MTX osteopathy. J. Rheumatol. 28 (2), 251–256.
Van Leeuwen, B.L., Verkerke, G.J., Hartel, R.M., Sluiter, W.J., Kamps, W.A., Jansen, H. W., Hoekstra, H.J., 2003. Chemotherapy decreases epiphyseal strength and increases bone fracture risk. Clin. Orthop. Relat. Res. 413, 243–254.
Wheeler, D.L., Vander Griend, R.A., Wronski, T.J., Miller, G.J., Keith, E.E., Graves, J.E., 1995. The short- and long-term effects of methotrexate on the rat skeleton. Bone 16 (2), 215–221.
Xian, C.J., Cool, J.C., Scherer, M.A., Macsai, C.E., Fan, C., Covino, M., Foster, B.K., 2007. Cellular mechanisms for methotrexate chemotherapy-induced bone growth defects. Bone 41 (5), 842–850.
Xian, C.J., Cool, J.C., Scherer, M.A., Fan, C., Foster, B.K., 2008. Folinic acid attenuates methotrexate chemotherapy-induced damages on bone growth mechanisms and pools of bone marrow stromal cells. J. Cell. Physiol. 214 (3), 777–785.
Yamanishi, A.D., Determan, D., Kuo, D.J., 2024. Dose-related effect of chemotherapy on bone mineral density among pediatric acute lymphoblastic leukemia survivors. J. Pediatr. Pharmacol. Ther. 29 (1), 53–60.
Zhang, Y.L., Liu, L., Su, Y.W., Xian, C.J., 2021a. miR-542-3p attenuates bone loss and marrow adiposity following methotrexate treatment by targeting sFRP-1 and Smurf2. Int. J. Mol. Sci. 22 (20).
Zhang, Y.L., Liu, L., Su, Y.W., Xian, C.J., 2021b. miR-6315 attenuates methotrexate treatment-induced decreased osteogenesis and increased adipogenesis potentially through modulating TGF-beta/Smad2 signalling. Biomedicines 9 (12).
Zhang, Y., Liu, L., Pillman, K.A., Hayball, J., Su, Y.W., Xian, C.J., 2022. Differentially expressed miRNAs in bone after methotrexate treatment. J. Cell. Physiol. 237 (1), 965–982.