HHS Public Access

Author manuscript

Arch Oral Biol. Author manuscript; available in PMC 2025 January 14.

Published in final edited form as:

Arch Oral Biol. 2024 March ; 159: 105875. doi:10.1016/j.archoralbio.2023.105875.

Rodents as an animal model for studying tooth extraction-related medication-related osteonecrosis of the jaw: assessment of outcomes

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Abstract

Objective: To assess the outcomes of several rodent animal models for studying tooth extraction-related medication-related osteonecrosis of the jaw (MRONJ).

Design: After a search of the databases, 2004 articles were located, and 118 corroborated the inclusion factors (in vivo studies in rodents evaluating tooth extraction as a risk factor for the development of MRONJ).

Results: Numerous studies attempting to establish an optimal protocol to induce MRONJ were found. Zoledronic acid (ZA) was the most used drug, followed by alendronate (ALN). Even when ZA did not lead to the development of MRONJ, its effect compromised the homeostasis of the bone and soft tissue. The association of other risk factors (dexamethasone, diabetes, and

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Declaration

All authors made a significant contribution to this article. Conceptualization: F.P.S.G. and H.H.; Methodology: H.H. and F.A.S.; Formal analysis: H.H.; Investigation: H.H. and H.R.M.; Data curation: H.H.; Writing: H.H. and H.R.M.; Writing, review, and editing: H.H., and F.P.S.G.; Supervision: F.P.S.G., S.I.P., and F.A.S.; Project administration: F.P.S.G. All authors reviewed the manuscript.

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.

CRediT authorship contribution statement

Guastaldi Fernando P.S.: Project administration, Supervision, Writing – review & editing, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft. **Souza Francisley A.:** Methodology, Writing – review & editing, Conceptualization, Formal analysis, Writing – original draft. **Matheus Henrique R.:** Investigation, Writing – review & editing, Data curation, Formal analysis, Methodology. **Hadad Henrique:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Validation, Visualization. **Pai Sara I.:** Writing – review & editing, Data curation, Formal analysis, Methodology.

tooth-related inflammatory dental disease) besides tooth extraction also played a role in the development of MRONJ. In addition, studies demonstrated a relationship between cumulative dose and MRONJ.

Conclusions: Both ZA and ALN can lead to MRONJ in rodents when equivalent human doses (in osteoporosis or cancer treatment) are used. Local oral risk factors and tooth-related inflammatory dental disease increase the incidence of MRONJ in a tooth extraction-related rodent model.

Keywords

MRONJ; ONJ; BRONJ; Bisphosphonates; Antiresorptive

1. Introduction

Controlling the dosages and frequency allows nitrogen-containing bisphosphonates (such as zoledronate acid [ZA], alendronate [ALN], pamidronate [PAM], etc.) and anti-RANKL antibodies (e.g., denosumab) to be used either for managing hypercalcemia and bone metastases in patients with cancer (Duran et al., 2017; Van Poznak et al., 2017) or for preventing fragility fractures in osteoporotic patients (Yu et al., 2020). The antiresorptive drugs (ARs) include a class of drugs called bisphosphonates, and they are related to the occurrence of a potentially severe adverse event, medication-related osteonecrosis of the jaw (MRONJ). MRONJ has been defined by the American Association of Oral and Maxillofacial Surgery (AAOMS) as the presence of exposed bone in the maxillofacial region for a period longer than 8 weeks in patients with no history of radiation therapy or metastatic disease in the jaws which have been treated with ARs (Ruggiero et al., 2022).

The incidence is higher in patients with cancer who receive anti-resorptive drugs (2–5%), but the incidence of MRONJ in patients using antiresorptives in osteoporotic dosages is not negligible (0.01–0.03%) (Khan et al., 2015; Coropciuc et al., 2023). Besides the systemic impact of antiresorptive drugs on bone metabolism, local oral risk factors such as tooth extraction and major dental surgery, inflammatory/infectious dental disease (e.g., periodontal and periapical abscess), ill-fitting removable dental prostheses, or possibly dental implants are required concurrently to trigger MRONJ (Khan et al., 2015; Hasegawa et al., 2017; Otto et al., 2018, 2021; Castillo et al., 2021; Wan et al., 2021). There is broad consensus that the incidence of MRONJ is higher in patients with cancer who take antiresorptives than in patients with osteoporosis who take antiresorptives and that the ten-fold higher cumulative absorbed dose of antiresorptives in patients with cancer than in patients with osteoporosis seems likely to play a role. However, a complete understanding of how local oral risk factors interact with antiresorptives to cause MRONJ is not yet in place. Importantly, although it is more common at high dosages, a similar progression from health to disease can be expected when comparing MRONJ in cancer and osteoporosis patients (Khan et al., 2015; Wan et al., 2021), thus indicating that these local factors are central players which are fundamental to the occurrence of MRONJ.

The mechanistic interactions between the potent suppression of osteoclast activity and patient-related local risk factors are critical to fully comprehending the pathophysiology

of MRONJ while seeking further precise therapeutic approaches for its prevention and treatment. Therefore, an appropriate animal model is a reasonable step for in vivo validation. The superiority of large animal models over rodents has been proven in some scenarios (Lunney et al., 2021). However, the interspecies comparison performed by Pilawski et al. (2021) provided accurate information assuring equivalency in the morphology and physiology of pristine alveolar bone when comparing mice, rats, mini-pigs, and humans. Furthermore, Pan et al. (2020) supported the hypothesis that the underlying mechanisms of alveolar bone healing following tooth extraction are histologically equivalent and conserved between mice and mini pigs. Therefore, the inherent financial benefit and easy manipulation, combined with the non-superiority of pigs over mice, substantiate the use of rodents as a human biomedical model for studying the processes related to tooth socket healing.

The wide variety of drugs, dosages, administration routes, frequency of administration, and presence of local/systemic predisposing factors results in significant heterogeneity among the pre-clinical models used for studying tooth extraction-related MRONJ (Aguirre et al., 2021). Only by knowing what to expect in a specific animal scenario can a pre-clinical experiment accurately mimic equivalent clinical situations, thus providing a basis for translational research. Therefore, this review aims to organize and summarize data from multiple rodent models for studying tooth extraction-related MRONJ.

2. Methods

A systematic screening process was used to select the articles to be included, from which the data were collected and shown narratively. The search included articles reporting rodent studies evaluating the development of MRONJ in a tooth extraction model published up to April 1, 2023. Ethical approval was not necessary.

2.1. Search criteria

The search was performed in PubMed/MEDLINE, Embase, and Cochrane Library by applying the following strategy: (rodents OR rat OR mice OR mouse) AND (zoledronic acid OR bisphosphonate OR anti-resorptive drugs OR rank ligand antibody OR denosumab) AND (control OR saline solution) AND (osteonecrosis OR osteonecrosis of the jaw OR necrosis OR microtomographic OR microct OR bone volume OR bone parameters OR histologic OR histomorphometry OR immunobiological OR in vivo).

2.2. Inclusion and exclusion criteria

The following criteria were applied to assess the eligibility of the studies: in vivo studies in rodents evaluating tooth extraction as a risk factor for the development of MRONJ through clinical and complementary imaging (x-ray and micro-tomography), and histopathologic analyses were included. Articles which did not mention doing a histopathologic-based examination for necrotic bone in the region of the tooth extraction socket in each animal were excluded. Reviews, clinical studies, technical notes, abstracts, in vitro studies, and studies not published in English were not eligible. Articles lacking proper control groups were not included.

2.3. Data extraction

After removing duplicates, two reviewers independently assessed titles and abstracts before evaluating full texts and applying the inclusion and exclusion criteria. A third reviewer was consulted in case of disagreement. The following data were extracted: types of drugs; dose, frequency, and duration of both pre- and post-extraction treatment; route of administration; animal species, gender, and age; the presence of associated risk factors; surgical site-related characteristics (which tooth, number of extractions, and location [maxilla or mandible]; and the reported outcomes.

3. Results

3.1. Search results and study characteristics

A total of 2004 articles were identified (1810 from PubMed/MED-LINE, 194 Embase). After removing duplicates, 1568 publications were screened by title and abstract. A total of 67 full texts were selected. Eighteen papers were excluded due to the absence of a control group, 7 due to the lack of a tooth extraction, and 8 due to reference to a previously described model. Hence, 49 articles meeting the inclusion and exclusion criteria were included in this review, and 69 papers were added after a manual search, totaling 118 articles.

Data demonstrated 73 rat studies (Table 1–4) and 45 mouse studies (Table 5). The rat studies are divided into three tables for ZA: a) Physiologic Doses (N = 14); b) Supraphysiologic Doses (N = 23); and c) Extremely Supraphysiologic Doses (N = 27); and one Table (#4) (N = 12) for ALN rat studies. The ZA rat studies span 25–4500% of the ZA oncology dose. The mouse studies span ZA doses of 25–5200% of the mouse ZA oncology dose. The ALN rat studies span absorbed doses of 1.7–23333% of the ALN rat osteoporosis dose. Pre-extraction treatment covers 0–147 days but focuses on 14–21 days. Post-extraction treatment covers 3–105 days but focuses on 28–42 days.

In the first category (rats), the studies were stratified by the drug (ZA, ALN, or other drugs) and by the dosages of these drugs used by which authors, considering not only the mg/kg of the individual dose as it was administered but also the total number of doses given and the period over which they were administered. Thus, the ZA doses were categorized considering the percentage (%) of rat ZA oncology dose (80 μg/kg ZA/monthly IV), according to Aguirre et al. (2021) and Gasser et al. (2008) into physiologic (≤200% of the rat ZA oncologic dose) (Group P), supraphysiologic (>200% to <1000% of the rat ZA oncologic dose) (Group S), and extremely supraphysiologic (≥1000% of the rat ZA oncologic dose) (Group ES) cumulative absorbed dose groups. For ALN, the percentage of drug used was calculated based on rat osteoporotic dose (120 μg/kg/monthly SC) with consideration of route of administration (SC or oral) (Seedor et al., 1991). In the second category (mice), each author's ZA percentage was calculated based on the mouse ZA oncology dose, which is ~540 μg/kg/month IV (Pozzi et al., 2009; Park et al., 2015; Aguirre et al., 2021).

Thirty-six studies (30%) combined tooth extraction with other risk factors, such as dexamethasone (DX) administration (Sonis et al., 2009; Kikuri et al., 2010; Bi et al., 2010; Ali-Erdem et al., 2011; Abtahi et al., 2012; Zhao et al., 2012; Berti-Couto et al., 2013;

Kuroshima, Yamashita, 2013; Jabbour et al., 2014; Kaibuchi et al., 2016; Yanık et al., 2016; Jung et al., 2019; Mergoni et al., 2019; Tamari et al., 2019; Movahedian Attar et al., 2020; Adachi et al., 2020; Sanda et al., 2022; Liu et al., 2021; Gao et al., 2021; Yoshioka et al., 2022), senescence in animals (Statkievicz et al., 2018; Biguetti et al., 2019; Paulo et al., 2020; Liu et al., 2021), vitamin D deficiency (Hokugo et al., 2010), repeated trauma (Howie et al., 2015), ovariectomy with estrogen deficiency (Kim et al., 2015), diabetes (Takaoka et al., 2015; Zhang et al., 2015), and local oral risk factors such as inflammatory dental disease [periodontitis (Kim et al., 2018; Soundia et al., 2018; Statkievicz et al., 2018; Ervolino et al., 2022; Williams et al., 2022), periapical lesions (Song et al., 2016; Hadaya et al., 2018; Bolette et al., 2019; Du et al., 2022), and perirradicular disease (Soundia et al., 2016)].

Intraperitoneal, intravenous (tail vein), and subcutaneous were the main routes for administering the drugs, with oral dosing of alendronate even attempted several times. The following drug administration frequencies were used most: once/week, twice/week, and daily. Molars, sometimes more than one, were by far the most frequent tooth extracted. Two-thirds came from the maxilla, while one-third came from the mandible. The most frequent was the first molar.

The following outcome parameters were used to characterize MRONJ and microscopic osteonecrosis events in and around the extraction sites: clinical evaluation, histological and immunohistochemical analyses, x-ray, micro-tomography, and biochemical analysis of the blood. The comprehensive findings are reported below and in Tables 1–5.

3.2. Experimental models in rats

3.2.1. Zoledronic Acid (ZA)

3.2.1.1. Physiologic (Group P).: Fourteen studies using doses ≤ 200% of rat ZA oncology doses were included in this section (Table 1). The rat ZA oncology dose is 80 μg/kg/monthly IV (Aguirre et al., 2021; Gasser et al., 2008). For convenience, all study data in tables are arranged in order of increasing cumulative absorbed dose rate. Only Guevarra et al. (2015), Marino et al. (2012), Ali-Erdem et al. (2011), Tamari et al. (2019), and Sonis et al. (2009) used doses significantly inferior to the oncologic dose (25%, 33%, 38%, 38%, and 38% of the rat ZA oncology dose, respectively), which is 2–4X the rat ZA osteoporosis dose.

Guevarra et al. (2015) used two IV administrations of 0.02 mg/kg of ZA at 4-week intervals, and after 4 weeks post-operative, it was verified open wound with MRONJ signs in 38% of the sample. Interestingly, authors performed vascular perfusion followed by microCT, which demonstrated that ZA decreased connectivity and branching and a decrease in the ordered pattern of blood vessels when compared to control. Marino et al. (2012) reported that administration of ZA alone (0.02 mg/kg, intravenous) three weeks before and three weeks after tooth extraction led to the exposed bone in 75% and necrotic bone in 100% of rats at 8 weeks after tooth extraction. Their findings supporting MRONJ were both clinical (bone exposure and sequestration) and histological (presence of empty lacunae, inflammatory infiltration, and bacterial colonization).

Ali-Erdem et al. (2011), using ZA and dexamethasone (ZA 0.0075 mg/kg once/week for 3 weeks and dexamethasone 1.0 mg/kg once/-week for 3 weeks), noted that 60% of the sample

had bone necrosis in ZA group. Histological analysis demonstrated that ZA led to bone necrosis, decreased percentage of new bone area, and exacerbated inflammatory process ($p < 0.05$) compared to the control group. The incidence of MRONJ was 60% in the ZA+DX group vs. 32% in the control group ($P < 0.015$). Moreover, the combination of ZOL+DX (0.0075 mg/kg + 1 mg/kg, once/week over 11 weeks) therapy increased the incidence of the chronic wound and MRONJ in a rat model (Tamari et al., 2019), characterized by a higher area of necrosis and number of empty lacuna ($p < 0.05$), decreased number of blood vessels and reduction in the VEGF and CD31 expression when compared to control. Similarly, Sonis et al. (2009), using the same protocol, reported that the rats that received ZA+DX remained with non-healed extraction sites, and in general, the lesion was characterized by exposed bone, erythema and rolled edematous borders.

The same dose of ZA (140 μg ZA/Kg in 2 months, 87% of the rat ZA oncologic dose) was used in 4 studies under different designs, which were as follows: *Wistar–Albino* rats (Curra et al., 2016; Cardoso et al., 2018), *Sprague–Dawley* rats with vitamin D deficiency (Hokugo et al., 2010), and *Sprague–Dawley* rats with diabetes (Takaoka et al., 2015). Curra et al. (2016) reported bone exposure in 40%, osteolysis, loss of integrity of the alveolar walls (micro-tomography), and osteonecrosis events with microbial and inflammatory infiltration (histology) in 100% of the samples. Similarly, Cardoso et al. (2018) showed that 40% of the sample had bone exposure, and 16% had bone exposure and suppuration. MicroCT demonstrated that ZA treatment led to osteolysis, fractures, or loss of socket integrity in all samples. A decrease in VEGF expression was also noted.

Although the study by Hokugo et al. (2010) reported delayed socket healing and the presence of necrotic bone and bone sequestrum (micro-tomography) in the ZA group (14.3% of animals), the incidence of these events in animals affected by both ZA and vitamin D deficiency was significantly higher (67%). Similarly, Takaoka et al. (2015) proposed a tooth extraction model related to MRONJ in combination with type 2 diabetes mellitus. The protocol used by Takaoka et al. (2015) (612.5 μg ZA/Kg in 8.75 months, 87% of the rat ZA oncologic dose) induced substantial damage to the socket (100% of bone exposure [clinically], and 37% of animals with necrotic bone [histologically]) compared to ZA alone, in which necrotic bone tissue was found in only 12.5% of the samples.

Also, osteoid matrix deposition and mild neutrophil infiltration with the same dose (ZA, 0.160 mg/kg over 8 weeks, IP, 100% of rat ZA oncology dose) were reported by Silva et al. (2015), who observed MRONJ restricted to one sample. In addition, Zhu et al. (2023) conducted an experiment using 133–200% of the rat ZA oncologic dose (0.08 mg/kg, once/week for 8 weeks, with 16 or 24 total weeks observation). They demonstrated that MRONJ signs could be clinically found (exposed bone) in 25% of the sample and histologically (necrotic bone) in 75%. Also, the period of 8 weeks of drug holiday was evaluated in this study and had no effect. In the same way, Brierly et al. (2019), using two doses of ZA (0.1 mg/kg at a three week interval), found typical signs of MRONJ, such as nonvital bone, empty lacunae.

On the other hand, Biasotto et al. (2010) (using 200% of the rat ZA oncologic dose) found that the weekly intravenous administration of 0.04 mg/kg ZA (for 5 weeks) led to clinical

exposure of the bone and expansion of the original defect (post-extraction socket). Micro-tomography identified irregular cortical contour and bone destruction, while non-vital bone and peripheral resorption without inflammatory infiltration were indicated by histologic examination.

3.2.2. Supraphysiologic (Group S)—This section includes studies using doses > 200% of the rat ZA oncology dose to < 1000% of the rat ZA oncology dose (Table 2).

Elsayed et al. (2018) demonstrated that ZA (0.08 mg/kg/week for 13 weeks, IV, 289% of rat ZA oncologic dose) affected gingival repair leading to open wounds, microCT evidenced bone sequestration, and a higher percentage of empty lacuna was noted ($p < 0.0001$) when compared to control. The administration of ZA (0.06 mg/kg/week over 7 weeks, 300% of the rat ZA oncologic dose) led to MRONJ and exposed bone in all samples (Zandi et al., 2016) and occasional fistulas. In this same study, tooth extraction was performed at different time points (0, 7, 14, 21) after the beginning of the ZA administration, and results demonstrated that after 4 weeks, all groups present MRONJ. Likewise, Borke et al. (2015), also using 300% of the rat ZA oncologic dose (0.06 mg/kg/week for 2 weeks), highlighted that after 4 and 8 weeks of tooth extraction, 100% and 75% of the wounds presented exposed bone with incomplete closure in ZA group, also histological findings were characterized by necrotic bone with empty lacunae, general tissue disorganization, and lymphocytic infiltration. Imada et al. (2019), using the same protocol as Borke et al. (2015), also found exposed bone in 86% and 57% of the animals treated with ZA after 3 and 8 weeks (respectively). It is also noted that the ZA impaired the volume of new bone formation, and after 8 weeks, 86% of the sample were diagnosed with MRONJ.

Although Vidal-Gutiérrez et al. (2017) applied only two doses of 0.06 mg/kg on the 7th and 14th day postoperatively, intramuscular (300% of the rat ZA oncologic dose), data demonstrated that ZA led to MRONJ, confirmed by the bone exposure, and a histologically evident ulcerative lesion with bone necrosis, sequestrum, and bacterial colonization, as well as radiographic evidence of osteolytic lesions, extensive destruction, and evident sequestrum. In the same way, Zandi et al. (2015) found that intraperitoneal delivery of 0.06 mg/kg/week of ZA for 4 weeks (300% of the rat ZA oncologic dose) resulted in clinical bone exposure or fistula in 85% of the samples and histological discontinuity of the epithelium, the presence of inflammatory infiltrate, sequestra, and compromised new bone formation as compared with the control.

Zandi et al. (2017) used their previous ZA protocol (Zandi et al., 2016), but now, over 12 weeks, it was demonstrated bone exposure or fistula in 80% of the extraction sites. Also, histological findings suggest that ZA-impaired bone remodeling, epithelial covering, necrotic bone, and inflammatory infiltrate were noted in 83% of the sample.

Howie et al. (2015) and Yang et al. (2015) used the same ZA dose (400% of the rat oncologic dose). However, while the first used a protocol of 1040 μg ZA/Kg in 2 months, the second used 3 different protocols: 320 μg ZA/Kg in 1 month, 2) 560 μg ZA/Kg in 1.75 months, and 3) 1200 μg ZA/Kg in 3.75 months. Howie et al. (2015) (0.080 mg/kg weekly, 13 weeks, intravenous) used a model of repeated trauma (2 independent extractions

at distinct time points) in which the ZA group exhibited progressive mucosal dehiscence with bone exposure sites. Histologically, empty osteocyte lacunae were found in 27% of the specimens but with no detectable bacterial colonization in the extraction site. Micro-tomography showed no bone formation within the socket and substantial fragmentation of the alveolar bone extending from the extraction site. Yang et al. (2015) demonstrated that ZA (0.08 mg/kg weekly for 3, 7, and 15 weeks, intravenous) resulted in the presence of necrotic bone in all samples, inflammatory infiltration in most samples (6/8), and soft tissue impairment in some samples (3/8).

Also, the data obtained by Gong et al. (2017) demonstrated that ZA (0.08 mg/kg/week over 12 weeks, 400% of the rat oncologic dose) led to 65% and 45% of bone exposure after 4 and 12 weeks (respectively) after tooth extraction. Histological findings demonstrating necrotic bone and mucosal disruption. Janovszky et al. (2015), using 0.08 mg/kg/week of ZA over 8 weeks, evidenced histological signs of osteonecrosis, such as the absence of nuclear staining in the osteocytes, necrotic bone, increased inflammatory infiltration, and granulation tissue formation. Sequestrum formation was present in 60% of the ZA-treated animals. Similarly, Elsayed et al. (2020), using 0.08 mg/kg/week of ZA over 13 weeks, showed exposed necrotic bone 8 weeks after tooth extraction. Although Isaias et al. (2021) have not observed bone exposure using ZA (0.2 mg/kg/week over 10 weeks), they demonstrated deleterious effects in bone repair, such as necrotic bone, empty lacunae, and increased TNF-α expression.

When combining ovariectomy with ZA administration (1500 μg ZA/Kg in 3.75 months, 500% of the rat oncologic dose), Kim et al. (2015) reported that the incidence of MRONJ was as high as 78%. Histological evidence demonstrated extensive ulcerative lesions accompanied by exposed and necrotic bone with sequestrum, and bacterial colonies were observed. Barba-Recreo et al. (2014) used 900 μg ZA/Kg in 2.25 months, which corresponded to 500% of the rat oncologic dose (applied 0.1 mg/kg, once/week), only 25% of their samples presented osteonecrosis and incomplete epithelial healing, and 50% of the samples showed disrupted alveolar bone (micro-tomography). Also, Silva et al. (2015) demonstrated histological evidence of bone necrosis in all samples ($p = 0.0004$, when compared to the control group), associated with large bone sequestra and intense inflammatory infiltrate.

Kuroshima et al. (2014), using 570% of the ZA oncologic dose (150 μg ZA/Kg in 0.33 months), demonstrated that ZA suppressed bone resorption and retained the necrotic bone within the socket. They also highlighted the impact of ZA in impairing soft tissue healing and decreasing the number of blood vessels. Additionally, micro-tomography showed the presence of osteolytic lesions, extensive destruction, and disorganization of trabecular patterns with cortical disruption and sequestrum formation. Similarly, dexamethasone plus ZA (670% of the rat ZA oncology dose) led to a greater incidence (90%) of open wounds with clinical signs of inflammation and sequestrum (micro-tomography) than ZA alone (62.5%; no bone sequestrum [micro-tomography]) (Jabbour et al., 2014).

Using 750% of the rat ZA oncologic dose, Ferreira et al. (2020) showed that ZA (0.6 mg/kg every 4 weeks over 20 weeks) delayed bone remodeling, leading to 100% of the

sample presenting bone sequestration, but only 8% with exposed bone. Also, Maahs et al. (2011) (0.6 mg/kg every 4 weeks over 12 weeks, 750% of the rat ZA oncologic dose) found a 100% loss of mucosal integrity combined with osteonecrosis lesions in 80% of their sample. The study by de Almeida et al. (2018) using 1800 μg ZA/Kg in 0.9 months, which also corresponded to 750% of the rat ZA oncologic dose, reported a complete absence of epithelialization of the extraction socket and histological findings, indicating a high incidence of osteonecrosis (70%), intense inflammatory infiltrate, and the presence of microbial colonies.

Continuing with 750% of the rat ZA oncology dose, Vasconcelos et al. (2012) used 0.6 mg/kg of ZA every 4 weeks over 17 weeks and found the absence of epithelial healing and signs of osteonecrosis in 100% of the sample. Again, with 750% of the rat ZA oncology dose, Silveira et al. (2016), using ZA (0.6 mg/kg every 4 weeks over 16 weeks), induced osteonecrosis (92% of the samples). Finally, histological data from Kosach et al. (2020) demonstrated that ZA (0.18 mg/kg/week over 6 weeks, 900% of rat ZA oncologic dose) led to a necrotic and inflammatory process in bone repair, characterized by the spread of loose fibrous tissue, and an uneven alternation of compact and spongy tissue.

3.2.3. Extremely Supraphysiologic (Group ES)—Studies using doses ≥ 1000% of rat ZA oncologic doses are included in this section (Table 3). Initially, Kolpakova et al. (2017) used ZA (1080% of the rat oncologic dose) and reported bone sequestration and disrupted cortical walls under microCT analysis. The histological sections highlighted necrotic lesions and inflammatory infiltration when ZA (0.36 mg/kg once + 0.18 mg/kg weekly, 4 weeks, intravenous) was administered.

The protocol of ZA+DX (0.066 mg/kg + 5 mg/kg three times a week over 4 weeks) was performed by Adachi et al. (2020), Kaibuchi et al. (2016), and Sanda et al. (2022). These 3 studies showed ZA+DX led to exposed necrotic bone with incomplete restoration of epithelial continuity in 100% of the sample and increased area of necrotic bone in these histological findings. Also, Kaibuchi et al. (2016) demonstrated microCT signs of delayed and irregular bone healing and reduced expression of VEGF, HGF, RANKL, and OPG.

Still approaching papers using 1000% of the ZA oncologic dose, Yanık et al. (2016) modified the concentration of ZA+DX (0.1 mg/kg + 1 mg/kg, twice a week over 7 weeks); however, MRONJ occurred in all ZA-treated rats by 7weeks post extraction, and bone exposure occurred in some ZA-treated rats. The incidence of both differed only from the non-ZA controls. Also, Liu et al. (2021) demonstrated that ZA+DX (0.2 mg/kg + 2 mg/kg once a week over 8 weeks) delayed soft tissue healing characterized by open wounds and exposed bone. MicroCT also showed signs of bone resorption and absence of bone regeneration for animals treated with ZA, and MRONJ was diagnosed in 83% of the sample. Gao et al. (2021) used ZA+DX (0.2 mg/kg + 5 mg/kg once a week over 16 weeks) and induced classic osteonecrosis signs, such as incomplete mucosal healing and bone exposure in all ZA-treated rats. Also, microCT revealed irregularities in alveolar bone and disruption of cortical bone, and histology pointed to almost 90% of rats with necrotic bone with empty lacunae.

Although Poubel et al. (2018a) and Cui et al. (2020) used very similar protocols of ZA (0.125 mg/kg twice/week over 4 weeks and 0.125 mg/kg twice/week over 5 weeks, respectively – both 1250% of the rat ZA oncologic dose), Poubel et al. (2018a) did not report clinical signs of MRONJ. Still, it was demonstrated that a moderate to severe inflammatory infiltrate was identified in 63% of the ZA-treated animals, and 75% of the sample showed bone sequestration. However, Cui et al. (2020) found that 80% of rats had bone exposure at five weeks after tooth extraction, a decrease in the percentage of bone volume, and histological findings demonstrated hollow bone and irregular alignment of trabecular bone.

Then, increasing the dose up to 1500% of the rat ZA oncologic dose, Barba-Recreo et al. (2013); (2014) highlighted the impact of the cumulative dose on the expected outcomes. Using 2700 μg ZA/Kg in 2.25 months, they found that 80% of the samples presented osteonecrosis, decreased vascularization, and incomplete epithelial healing (histology), and 100% of the samples displayed disrupted alveolar bone under micro-tomography, showing higher incidence and more severe lesions than when they used 500% of the rat ZA oncology dose (Table 2). Interestingly, the data presented by de Souza et al. (2022) found not only a high percentage of bone exposure (91%) and moderate inflammatory infiltrate but an increase in the expression of IL-6, TNF-α, and caspase3; histological sections showed 52% fewer viable osteocytes and more empty lacunae.

Both studies by Dayisoylu et al., (2013, 2014) used the same protocol of ZA (0.1 mg/kg, MWF, over 8 weeks), corresponding to 1500% of the rat oncologic dose. ZA impaired bone repair in these studies, leading to unhealed exposed bone and pus formation. Also, minimal inflammatory process around the periodontal area was noted. Similarly, Silva et al. (2015), animals using 2500% of the rat ZA oncology dose, reported that ZA exhibited radiolucent areas, while the histological findings of all animals were compatible with osteonecrosis lesions (bone sequestrum and intense inflammation infiltrate). Enhancing the ZA dose up to 3000% of the rat oncologic dose, Ersan et al. (2014) could not find bone exposure. Still, their ZA protocol of 0.2 mg/kg, MWF, over 6 weeks reduced alveolar bone width and increased osteonecrosis area compared to control.

Fourteen studies in this category associated ZA treatment with another risk factor besides tooth extraction. The factors considered were concomitant use of DX, which were associated with 1000% (Adachi et al., 2020; Kaibuchi et al., 2016; Sanda et al., 2022; Yanık et al., 2016; Liu et al., 2021; Gao et al., 2021) or with 1500% of the ZA rat oncologic dose (Mergoni et al., 2019; Jung et al., 2021); senescence and periodontitis, which were associated with 1500% of the ZA rat oncologic dose (Statkievcz et al., 2018); senescence and diabetes, which were associated with 1500% of the ZA rat oncologic dose (Ervolino et al., 2022); age, which was associated with 1500% of the ZA rat oncologic dose (Paulo et al., 2020); periapical disease, which was associated with 2000% of the ZA rat oncologic dose (Hadaya et al., 2018); experimental periodontal disease, which was associated with 2000% of the ZA rat oncologic dose (Soundia et al., 2018); and periapical lesions, which were associated with 4500% of the ZA rat oncologic dose (Bolette et al., 2019).

The association of ZA+DX (0.1 mg/kg+1 mg/kg, 3x/week over 9 weeks, 1500% of the rat ZA oncologic dose) was used by Mergoni et al. (2019). At 8d post-extraction, one of the shortest post-extraction follow-up periods studied, they reported impaired wound healing and significantly more rats with necrotic bone in the ZA group than in the control group, with half the samples in the ZA group showing exposed bone. The same ZA+DX protocol was used by Jung et al. (2021), which demonstrated 50% of rats with bone exposure at 21 days after tooth extraction in ZA+DX-treated animals and histological signs of necrotic bone, empty lacunae, and decreased number of vessels.

Similar results were reported by Statkievicz et al. (2018) (0.1 mg/kg every Monday, Wednesday, and Friday (MWF), 7 weeks, IP, 1500% of rat oncologic dose) when proposing a model of MRONJ associating ZA administration, periodontitis, and tooth extraction in senile female rats. Their model led to oral bone exposure in some samples (clinically), with histological findings which suggest that ZA impairs post-extraction socket healing and causes MRONJ.

The data from Ervolino et al. (2022) showed that 100% of their sample presented MRONJ with an area of exposed bone. In addition, ZA negatively affected the tissue repair process, presenting histological characteristics consistent with MRONJ-like lesions, a moderate/severe inflammatory response in the entire extension of connective tissue and bone tissue, and an association with more TRAP-positive cells. Likewise, Paulo et al. (2020) found that in the follow-up 21 days after tooth extraction, the ZA group contained 57% of the cases as presenting gingival discontinuity and 28% of those with abscesses and purulent content. In the same period, animals from the ZA group had a lower bone density coefficient when compared to the control group ($p = 0.001$). Histological findings from the ZA group showed alterations in the epithelium, such as a decrease in the thickness and areas of discontinuity of the mucosa associated with the absence of bone tissue in the extraction site. However, immature bone was noted in the most coronal region of the surgical site, surrounded by inflammatory cells and hemorrhagic areas, suggestive of osteomyelitis.

Hadaya et al. (2018) reported bone exposure and a lack of socket healing in ZA-treated animals. Their results strongly supported a synergistic contribution of severe inflammatory dental disease and tooth extraction to MRONJ pathogenesis since experimental periapical disease (EPD) enhanced osteonecrosis-like lesions, and these were associated with the bacterial presence in areas of osteonecrotic alveolar bone, highlighting the importance of infection in tooth extraction-related MRONJ. Very similar data were presented by Soundia et al. (2018), who demonstrated through microCT that experimental periodontitis (EP) and ZA administration could compromise socket healing and decrease the percentage of bone volume fraction (BV/TV). Histologically, ZA and EP increased osteonecrosis areas, with more empty osteocyte lacunae in alveolar bone associated with marked inflammatory response and increased matrix metallopeptidase 9 and 13 (MMP9 and MMP13) expression. Similarly, Bolette et al. (2019) performed pulpal exposure before the tooth extraction model to study the influence of infection in MRONJ. Data from this tooth extraction model demonstrated that when the combination of mandible and infection was present, MRONJ significantly increased ($p = 0.0074$) compared to maxillae and infection. The prevalence

of MRONJ was 88% in the ZA+pulpal exposure group vs. 50% in the ZA without pulpal exposure group.

3.2.4. Alendronate (ALN)—Twelve studies used ALN as a potential drug to induce MRONJ (Table 4). The rat "osteoporosis dose" of ALN, which is 120 μg/kg/month/SC (Seedor et al., 1991), was used in this review to classify these studies according to their cumulative absorbed doses. This dose completely stops ovariectomy-induced bone loss. It is usually given at 15 μg/kg twice weekly, SC. It is equivalent to the ZA dose (8 μg/kg/month IV) quoted in Gasser et al. (2008).

Of the 12 studies in this category, only half reported MRONJ (Conte Neto et al., 2013; Conte-Neto et al., 2016; Gonçalves et al., 2023; Berti-Couto et al., 2013; Abtahi et al., 2012; Movahedian Attar et al., 2020). First, Conte Neto et al. (2016) demonstrated that using 8000 μg ALN/Kg SC over 2 months, corresponding to 3333% of the ALN rat osteoporosis dose, led to MRONJ following tooth extraction. Their findings were clinical bone exposure, necrosis (histology), and radiographic sclerosis of the bone with 1 mg/kg SC, once/week for 8 weeks. Conte Neto et al., 2013 also demonstrated the association of ALN (63000 μg ALN/kg SC in 2.25 months, 23333% of the rat osteoporosis dose) with necrotic bone. In that model, ALN induced ONJ-like lesions in rodents. After 28 days, the authors observed bone exposure, partial epithelial coverage, and infection. When examined histologically, animals treated with ALN presented significantly increased necrotic bone ($p < 0.01$). Similar findings were also reported by Gonçalves et al. (2023), which demonstrated that ALN (1 mg/kg/day SC over 90 days, 16700% of the rat ALN osteoporosis dose) impaired bone and soft tissues in the post-extraction sockets. Also, the histological analysis pointed to necrotic bone, absence of osteocytes, empty gaps, and lack of epithelial covering.

It is noteworthy that Berti-Couto et al. (2013) showed that using a dose of ALN 100x lower (0.05 mg/kg SC once a week, over 16 weeks, 167% of the osteoporotic rat dose) than Gonçalves et al. (2023), was not able to induce osteonecrosis. However, when a DX (5 mg/kg/day) or 10% D-glucose anhydrous treatment to induce diabetes was associated with ALN administration, the rates of osteonecrosis were 28% and 78%, respectively. Notably, while 2800 μg ALN/Kg SC in 0.5 months, which corresponded to 4666% of osteoporotic rat dose, did not induce MRONJ, the combination of this same cumulative dose of ALN (0.2 mg/kg daily for 14 days) with DX (1 mg/kg daily for 14 days) led to bone exposure (clinical) and discontinuity of the overlying epithelium, inflammatory infiltration, and distinguishable sequestrum (histology) (Abtahi et al., 2010). Movahedian Attar et al. (2020) also showed that ALN+DX (0.2 mg/kg/day SC + 1 mg/kg/day for 14 d), 4666% of the rat ALN osteoporosis dose, induced open wounds, necrotic exposed bone, and infection.

MRONJ did not occur following tooth extraction in Maahs et al. (2011), most likely because the absorbed dose of ALN was only 1.67% of the rat ALN osteoporosis dose. Histological findings (percentage of vital bone, the amounts of connective and epithelial tissues, microbial colonies, and inflammatory infiltrate) were similar between groups (test [5 cycles of 0.6 mg/kg ALN every 28 days, oral gavage)] and control). Similarly, Isaias et al. (2021) used several protocols of rat ALN varying between 2.1–16.7% of the rat ALN osteoporosis dose. Mustakim et al. (2022) (167% of the ALN osteoporosis dose) reported

no signs of MRONJ, and histological findings demonstrated normal healing of the tooth extraction site.

However, Aguirre et al. (2010) (using doses ranging between 100–1000% of the rat ALN osteoporotic dose) and Altundal & Güvener (2004) (using an SC dose of 5833% of the rat ALN osteoporotic dose) found a decrease in bone formation and vascularity with no signs of exposed or necrotic bone.

3.3. Experimental models in mice

This section includes studies designed using mice, ranging from 25–2200% of the mouse ZA oncologic dose. The mouse ZA oncology dose is 540 μg/kg/month IV (Pozzi et al., 2009; Park et al., 2015; Aguirre et al., 2021). The routes of injection used in the 46 studies are as follows: IV (26), SC (10), and IP (10).

4. Summary

- At the lowest dose, 25% of the mouse ZA oncology dose, a single study reported no necrotic or exposed bone.
- Nine studies applied ZA at approximately 75% of the mouse ZA oncology dose. Though two found necrotic bone at 28d after tooth extraction, after 7–21d pre-treatment plus continued treatment with ZA, two others found no necrotic bone. Five others added either cyclophosphamide or DX to ZA. While all five reported necrotic bone, only three noted exposed bone.
- Six studies used doses ranging from 93–173% of the mouse ZA oncology dose. One added DX. All six reported some degree of necrotic bone at 14–112d after tooth extraction, after 7–21d pre-treatment, and generally continued ZA treatment, after tooth extraction, but only three reported exposed bone.
- Twenty-one studies gave a ZA dose equal to 185–225% of the mouse oncology dose. Six reported necrotic bone after 7–21d pre-treatment and 7–56d after tooth extraction with continued treatment. Fifteen others applied cofactors, including periodontitis, periapical lesions, DX, diabetes, or senescence after 7–21d pre-treatment and saw an increased amount of necrotic bone compared to ZA alone at 7–84 days post-extraction. In 75% of the studies, exposed bone was reported, but often only in 30–50% of the mice.
- Four studies applied a dose equal 370% of the mouse ZA oncology dose. All reported necrotic bone after 7–21d pre-treatment and 7–21d after tooth extraction and continued treatment. None reported exposed bone.
- Five studies used 740% or more of the mouse ZA oncology dose. Four reported necrotic bone after 7–21d pre-treatment and 7–21d after tooth extraction and continued treatment. Three reported exposed bone. The one which reported no necrotic bone pre-treated for 7d and only looked at 5d post-extraction.

Initially, no signs of bone necrosis or exposed bone were found in the studies by Hokugo et al. (2019), which used the lowest dose in this section (25% of the mouse ZA oncologic

dose). The data demonstrate that there was no ZA-related loss of mucosal integrity, swelling of mucosal tissue, or necrotic bone area inside the socket, even increasing the ZA dose up to 74% of the ZA mouse oncologic dose (0.3 mg/kg once over 3 weeks). Hayano et al. (2020) also used 74% of ZA mouse oncologic dose (0.05 mg/kg twice a week, over 5 or 7 weeks), and found no MRONJ or bone exposure. However, when cyclophosphamide was added to ZA at this dose, Hayano saw 100% bone exposure with necrotic bone and empty lacunae. The percentage of collagen fibers and the amount of inflammatory infiltrate were also affected. Similarly, the 700 μg ZA/Kg dose in 1.75 months, used by Kozutsumi et al. (2022) (74% of the ZA mouse oncologic dose), did not lead to bone exposure. However, decreased epithelial thickness and rete ridge length, increased area of non-vital bone, and increased percentage of bone volume, number, thickness, and separation of trabeculae were observed in the ZA group compared to the control group.

Data obtained by Sun et al. (2016) demonstrated that ZA (0.5 mg/kg once, 120% and 70% of the ZA mouse oncologic dose) enhanced the percentage of necrotic bone at 2 and 4 weeks after tooth extraction. However, when Sun et al. (2016) gave 0.5 mg/kg ZA once and looked at three days post-tooth extraction (220% of mouse ZA oncology dose), they found no MRONJ or bone exposure, most likely because insufficient time had passed after tooth extraction to allow features depicting MRONJ and bone exposure to develop. Igarashi et al. (2023) showed that the use of ZA (0.05 mg/kg twice a week, over 3 weeks, 74% of the ZA mouse oncologic dose), associated or not to CYP, was associated with the appearance of necrotic bone, but not exposed bone. Kuroshima et al. (2021) demonstrated that ZA+CYP (0.05 mg/kg + 150 mg/kg twice a week, over 7 weeks, 74% of the ZA mouse oncologic dose) reduced the percentage of living bone and enhanced necrotic bone, empty lacunae, and PMN cells. Also, Kuroshima et al. (2018a), (2018b), using a similar methodology, demonstrated that ZA could not induce MRONJ unless associated with CYP. Likewise, Kuroshima, Yamashita (2013) showed that a combination of ZA + melphalan (MEL) (0.05 mg/kg + 7 mg/kg once a week, over 7 weeks) led to MRONJ and exposed necrotic bone.

Similarly, Park et al. (2015) used ZA (0.54 mg/kg once IV; 80% of mouse ZA oncology dose) one week before tooth extraction, then did necropsies at 4 days, 1 week, 2 weeks, and 4weeks post-extraction. Both control and ZA groups showed necrotic bone at 4d, 1week, and 2weeks post-extraction. However, by 4weeks post-extraction only the ZA group exhibited necrotic and exposed bone. Importantly, Park et al.'s data, like that of Mergoni et al. (2019) in the rat, indicate that necrotic bone not related to ZA can be found up to 2weeks after tooth extraction in mice. The combination of ZA+DX (0.125 mg/kg + 5 mg/kg once a week, over 10 weeks, 93% of the ZA mouse oncologic dose) used by Yu et al. (2020) showed histological and morphological impacts, such as 78% with necrotic bone as 56% of the sample presented bone exposure. Hokugo et al. (2019) found a significant effect of ZA on MRONJ incidence, but not bone exposure, when the dose was increased to 0.5 mg/kg in 0.75 months (124% of ZA mouse oncologic dose) and 0.7 mg/kg in 0.75 months (173% of ZA mouse oncologic dose) since the data demonstrated inflammation in the oral mucosa, and connective tissue, signs of bone necrosis (around 30% of the alveolus compromised by non-vital bone). Kaneko et al. (2023) used 148% of the mouse ZA oncology dose, finding an increase in the percentage of necrotic bone and empty lacunae which was even more evident when an anti-VEGFA antibody was added. These authors did not find exposed bone. Using

the same percentage of the mouse ZA oncologic dose, however, in a different protocol (0.1 mg/kg twice a week, over 12 weeks), Córdova et al. (2016) showed changes in bone healing, such as exposed and necrotic bone in 21% of the ZA-treated animals.

Using ZA in a dose of 185% of mouse oncologic dose, Zhu et al. (2019), Shen et al. (2022), Zhang et al. (2013), Williams et al. (2014), Kim et al. (2017), and Taniguchi et al. (2019) demonstrated similar results, in which, MRONJ and bone exposure was found in the samples. The number of empty lacunae and percentage of necrotic bone was consistently higher in ZA-treated mice when compared to the control group. Similarly, Yoshioka et al. (2022), using ZA at the above dose + DX (5 mg/kg twice/week over three weeks), showed significant increases in BV/TV, Tb. Th, and Tb.N compared to control, and a substantial decrease in Tb.Sp. Additionally, a higher percentage of bone necrosis and empty lacunae were found. Also, in a previous study, Kim et al. (2017) using ZA at the above dose for 4–5 weeks, including only 2–3 weeks after tooth extraction did not find MRONJ, but retrieved the unhealed osteomucosal tissues in the tooth extraction socket for gene expression profiling and found overexpression of IL-36, which is related to inhibition of collagen expression. Similarly, Zhang et al. (2015) (0.125 mg/kg twice/week, over 4 weeks) demonstrated that ZA resulted in open sockets with impaired mucosal coverage restricted to 37.5% of the samples, residual necrotic alveolar bone, and delayed bone healing. When this dose was associated with diabetes, residual necrotic alveolar bone, and delayed bone healing were more prominent in the ZA group, and bone exposure was also reported.

Kikiuri et al. (2010), using ZA + DX (0.125 mg/kg + 5 mg/kg twice/week over 3 or 8 weeks) or ZA isolated (0.125 mg/kg twice/week over 8 weeks), 185% of mouse ZA oncologic dose, demonstrated an incomplete mucosal healing with open sockets and exposed bone (50% of the sample for ZA+DX and 30% for ZA) in the extraction sockets after 8 weeks. Histological findings evidenced increased inflammation, necrotic bone, fibrosis, and absence of epithelium. Likewise, Zhao et al. (2012) demonstrated, using C3H/HeJ mice at 185% of the mouse ZA oncologic dose, incomplete mucosal healing, and necrotic bone in open sockets with 20% of exposed bone in samples treated with ZA (0.125 mg/kg twice/week over 3 weeks) and 45% in samples treated with ZA+DX (0.125 mg/kg + 5 mg/kg twice/week over 3 weeks).

Also using the above dose, Bi et al. (2010) demonstrated that angiogenesis and bone remodeling were suppressed by ZA (0.125 mg/kg twice/week over 6 or 15 weeks) associated with DX (5 mg/kg once a week) which induced sclerotic and necrotic bone. Then, Kim et al. (2018) used ligature-induced experimental periodontitis in mice receiving ZA (0.125 mg/kg twice/week over 7 weeks) and demonstrated that the periodontal inflammatory conditions exacerbate the amount of bone loss, number of empty lacunae, and percentage of necrotic bone. Biguetti et al. (2019), using the above dose in 129 Sv mice (ZA 0.25 mg/kg weekly for 4 weeks), reported a model of MRONJ in which ZA disturbed the socket healing; delayed blood clot and debris removal; and increased inflammatory infiltration, ensuing bone sequestrum, and the presence of empty lacunae. Corroborating these data, Mahamoud et al. (2021), using 1000 μg ZA/Kg in 1 month, showed the reduced quantity and quality of the inorganic matrix within the alveolar sockets and the presence of osteonecrosis lesions in the ZA group.

Similarly, Song et al. (2016) associated pulp exposure with inducing periapical disease in mice receiving ZA (0.125 mg/kg + 5 mg/kg twice/week over 6 weeks) and found that periapical periodontitis exacerbates MRONJ. Also, a more recent study by Williams et al. (2020) applied ligature-induced periodontitis in mice receiving ZA (0.125 mg/kg twice/week over 6 or 13 weeks, 185% of mouse oncologic dose). The data suggested that the induced inflammation led to more MRONJ since microCT evidenced bony sequestrum, and histological analysis revealed increased bone necrosis and the number of empty lacunae. Interestingly, this article showed that the duration of inflammation can be associated with the amount of necrotic bone in mice receiving ZA.

Du et al. (2022) created periapical disease in mice receiving ZA (0.125 mg/kg + 5 mg/kg twice/week over 9 weeks) and found that this protocol led to a higher incidence of MRONJ than in ZA-treated mice without periapical abscess. Wu et al. (2022) used ZA (0.125 mg/kg twice a week, over 7 or 11 weeks, 185% of ZA mouse oncologic dose) associated with periodontitis caused by *Porphyromonas gingivalis* and showed MRONJ signs without bone exposure. Using 185% of ZA mouse oncologic dose, associated with DX (5 mg/kg), Yoshioka et al. (2022) demonstrated that the percentage of necrotic bone and empty lacunae was greater than ZA alone with no signs of bone exposure. Hokugo et al. (2019), using a dose of ZA (0.9 mg/kg, once, over 3 weeks), which corresponds to 225% of the ZA mouse oncologic dose, demonstrated abnormal swelling in the mucosal tissue, and inflammatory infiltrate in connective tissue, and signs of bone necrosis (around 30% of the alveolus occupied by non-vital bone).

Using ZA (0.25 mg/kg twice a week over 4 weeks), Chen et al. (2021) (370% of mouse ZA oncologic dose), showed a significant ZA-related increase in necrotic bone and apoptosis markers. Then, Soma et al., (2021, 2022) demonstrated that ZA (0.5 mg/kg once/week over 8 weeks, which corresponds to 370% of ZA mouse oncologic dose) led to 60% of the alveolar socket with non-vital bone. Also, Kozutsumi et al. (2022), using 3500 μg ZA/Kg in 1.75 months, which corresponded to 370% of the ZA mouse oncologic dose, highlighted the greater incidence of bone necrosis and a more significant number of empty lacunae ($p < 0.01$ and $p < 0.001$, respectively), as well as a lower amount of vital bone ($p < 0.05$) in the ZA group as compared with the control. Also, in this same paper, when ZA was increased to 740% of the ZA mouse oncologic dose (7000 μg ZA/Kg in 1.75 months), dose dependency was shown, since this dose significantly impaired bone healing after tooth extraction and further increased necrotic bone and number of empty lacunae.

Zheng et al. (2023) used ZA (0.5 mg/kg twice a week over 4 weeks, 740% of ZA mouse oncologic dose) and showed necrotic bone exposure and decrease in bone volume (microCT) in the ZA group. Also, the expression of IL-1 and IL-6 was highly elevated in ZA-treated animals. Kobayashi et al. (2010) (0.25 mg/kg daily, 11 days, subcutaneous) demonstrated that ZA (1455% of ZA mouse oncologic dose) delayed wound healing and inhibited osteogenesis and angiogenesis, as shown by the decreased expression of platelet endothelial cell adhesion molecules. This dose also affected migration and cell viability, resulting in open sockets prone to bacterial infiltration. These authors failed to find MRONJ and exposed bone, quite likely because they only examined tissue at five days post-extraction. Also, Zhao et al. (2023) used ZA (1 mg/kg, MWF, over 4 weeks, 2222% of

ZA mouse oncologic dose) with periodontitis, finding a higher number of empty lacunae and significantly more necrotic bone and 30% of samples with exposed bone, when compared to ZA alone.

4.1. Other drugs

This section included only the paper by Soundia et al. (2016). Their study used OPG/Fc antibody (10 mg/kg, twice/week for 8 weeks, IP) in 9-week-old male mice, followed by tooth extraction in the maxillae. It was reported that this dose of OPG/Fc antibody could lead to MRONJ with teeth extraction, especially with a periradicular disease. This protocol showed significant alveolar bone loss and a 70% rate of mucosal defects with exposed bone; histologically, the bone presented more empty osteocyte lacunae and osteonecrotic areas than in the control group.

5. Discussion

MRONJ, a potentially serious condition that requires the coexistence of systemic and local oral risk factors to develop, is common in patients with cancer (2–5%) and rare in patients with osteoporosis (0.01–0.03%) who take antiresorptive medications. Accordingly, the most frequent systemic risk factor is antiresorptive medication. Patients with cancer receive a ten-fold greater cumulative antiresorptive dose than patients with osteoporosis. It has previously been established that such profound inhibition of bone resorption exists at oncology doses of antiresorptives, that further increases in dose cause no additional inhibition of bone resorption. Tooth extraction is the major procedural event and tooth-related inflammatory dental disease is the major local tissue condition consistently identified as local oral risk factors for MRONJ in humans.

Over 100 studies of tooth extraction-related MRONJ in mice and rats are summarized here. Approximately 20 of these studies were previously reviewed (Poubel et al., 2018b; Kuroshima et al., 2019). We review here studies that used zoledronate (ZA) and alendronate (ALN), two widely available bisphosphonate-class antiresorptives used in humans. The specific drugs used in humans can be easily applied pre-clinically in rodents. Their pharmacology in both humans and rodents is well-documented, particularly surrounding the rat and mouse equivalents of the oncology dose in humans. ZA and ALN are excellent representative antiresorptives. Pre-clinical studies with a second major antiresorptive class used often in humans, RANKL antibodies, are difficult because the agents used in humans are fully humanized antibodies, which are not efficacious in rats. Thus, it is not practical to apply the specific RANKL inhibitor drugs used in humans in rodent experiments, because those drugs are biologically-inactive in rodents. The results have been similar when custom-developed rodent-specific RANKL inhibitors (e.g., OPG/Fc antibody) have been applied. Still, such pre-clinical experiments will continue to be rare because rodent-specific RANKL agents are not widely available.

The current studies have tested a range of antiresorptive doses covering more than four orders of magnitude of cumulative absorbed dose, including the absorbed doses equivalent to those given to patients with osteoporosis and cancer. The study repetition at key dose levels in mice is excellent, with ten studies using approximately 75% of the mouse ZA

oncology dose and 21 studies using about twice the mouse ZA oncology dose. Study repetition at key dose levels for the rat is very good, with six studies using approximately the rat ZA oncology dose and 14 studies using 2–4X the rat ZA oncology dose.

A large range of pre-extraction treatment periods that focus on 21–28d but include up to 147 days has been tested. A large range of post-extraction observation periods that focus on 28–56d but extend to 105 days has also been used. About two-thirds of studies continued anti-resorptive treatment during the post-extraction observation period.

Though many general histopathologic observations are recorded in these publications (e.g., signs of inflammation, fibrosis, lymphocytic infiltration, microbial colonies. swelling, mucosal dehiscence, suppuration, fistula, non-vital bone, osteolysis, number of empty lacunae, sequestrum, etc.), the most specific and crucial endpoints related to MRONJ are: a) necrotic alveolar bone in and around the tooth extraction socket; and b) exposed bone in the oral cavity. Of these two endpoints, the more important is necrotic bone, because the presence of necrotic bone after a reasonable post-extraction time period in this type of model, probably a minimum of 4weeks, can be accepted as diagnostic of MRONJ. It is important to note that investigators who examined tissue from the tooth extraction site at less than 21 days post-extraction often reported necrotic bone in the control group, making the attributing necrotic bone after tooth extraction in ZA-treated rats or mice more difficult.

Authors using this type of model generally monitored exposed bone in the oral cavity, most likely because, as a marker for underlying necrotic bone, it is the hallmark of human MRONJ. However, exposed bone, whenever it is seen, is still only a marker. The earliest histopathologic studies of tissues from human MRONJ cases showed that underlying necrotic bone was generally present in patients with exposed alveolar bone. Biopsy to formally identify necrotic bone in humans thought to have MRONJ is rarely done today, to avoid negatively altering the course of the putative MRONJ lesion.

Using exposed bone as an MRONJ marker should be considered insufficient for diagnosing MRONJ in rodents, because rodents can always be necropsied and the bone tissue which could contain necrotic bone can be made available for histopathologic studies. This review actually found that exposed bone is a less consistent finding than necrotic bone in this type of model. Such inconsistency may tempt readers to view this type of model as somehow not relevant to human MRONJ, because exposed bone is not its main feature. This review maintains that necrotic bone is the principal lesion in MRONJ and that the ability to directly examine each animal for necrotic bone itself makes this type of model very strong and extremely relevant to human MRONJ. It is straightfordefineso save the appropriate tissue, prepare multiple anatomically well-positioned serial-like 4 μm thick H&E-stained sections at an interval which faithfully represents the status of the who tooth extraction site, and do a systematic, microscope-based search for necrotic bone in and around the tooth extraction site, looking for fields of contiguous empty osteocyte lacunae in bone tissue that define necrotic bone tissue (Fondi, Franchi, 2007). Unlike human MRONJ studies, these pre-clinical studies directly document the existence of necrotic bone. Quantitative measurements, including “empty lacunae/mm2”and “% of empty lacunae,” are often used

as supportive data for an MRONJ diagnosis, ultimately comprising a very relevant model. MicroCT imaging which can reveal sequestra and sclerotic bone may also be helpful.

It is important to highlight here that even when osteonecrosis was not reported by the authors, many studies included in this review have shown that antiresorptive drugs, especially ZA, affect bone repair since they can impair bone and soft tissues, modify the expression of growth factors necessary for vascularization, such as VEGF, as well as delay the mucosal coverage, by decreasing cell migration and increasing toxicity and impairing bone repair.

It is very encouraging for the models that MRONJ was not reported by three studies of up to five months duration that applied ≤ 25% of the ALN rat osteoporosis dose. Considering the rarity of MRONJ in patients with osteoporosis who take antiresorptives, it is unsurprising that MRONJ was not reported by two other studies of up to thirteen weeks duration that applied ≤ 167–1000% of the ALN rat osteoporosis dose.

When ZA doses in the range of the oncology dose in mice and rats were used, MRONJ was reported more frequently than with doses in the range of the osteoporosis dose. Adding simultaneous systemic risk factors (e.g., dexamethasone, diabetes) or simultaneous local oral risk factors (localized experimental periodontitis, periapical abscess, or P. gingivalis) generally increased the % of cases in which MRONJ was seen in individual study reports. When ZA doses two-fold and more above the ZA oncology dose were applied, MRONJ incidence generally approached 100%, particularly when local oral risk factors were added.

Finding MRONJ in ZA-only groups may imply that surgical trauma to the bone that leaves behind traumatically detached pieces that die and are resorbed abnormally slowly is one cause of MRONJ. The presence of necrotic bone in both control and ZA groups at 4–14d post-extraction (Park et al., 2015) could be interpreted as a sign of procedure-related dead bone in this type of model. Their finding of no necrotic bone in control animals at 4weeks post-extraction may also signal that procedure-related dead bone is removed in a timely fashion only in the absence of ZA. However, since other local risk factors, such as inflammatory dental disease, increase MRONJ incidence, other local risk factors are also important in this type of model.

Data in Group P demonstrated that 14 studies used doses of 33–200% of the rat ZA oncology dose. Only five continued ZA treatment after tooth extraction. All studies found some degree of ZA-related MRONJ, though usually with an incidence of far less than 100%. Eleven reported exposed bone. In Group SP (Table 2), all studies using > 200% of the rat ZA oncologic dose to < 1000% of the rat ZA oncologic dose reported MRONJ. However, ZA's impact on bone resorption, soft tissue healing, and angiogenic potential was highlighted. While the use of supraphysiologic and extremely supraphysiologic ZA doses has proven indispensable during model development, when it was necessary to convincingly demonstrate that antiresorptive-treated mice and rats could express necrotic bone after tooth extraction, it would be helpful in the future to execute experiments concerning tooth extraction-related MRONJ that focus on models that have both local oral risk factors (trauma and infection) present in the extraction of most human teeth, possibly facilitating the more

reliable development of MRONJ while simultaneously using more physiologic doses. Using physiologic ZA doses would increase the likelihood that the animal model did not rely on unknown metabolic pathways affected by ZA to create MRONJ.

Regarding experiments performed in mice (Table 5), it can be highlighted that data obtained from studies using doses above 125% of the mouse ZA oncology dose are very consistent, with all studies reporting MRONJ. Eight of 11 studies with doses ranging from 33–100% of the mouse ZA oncology dose reported MRONJ, though generally with an incidence less than 100%. All but one found exposed bone. In seven of the eight studies reporting MRONJ, it was necessary to add another risk factor to ZA, such as DX (n = 2), melphalan (n = 1), or cyclophosphamide (n = 5), in order for ZA to be associated with MRONJ. It is worth noting that the study by Hokugo et al. (2019) investigated various doses of ZA in mice (0.1, 0.3, 0.5, 0.7, and 0.9 mg/kg once over 3 weeks) and suggested that a particular threshold concentration of bioavailable BP dose in the jawbone is necessary to trigger osteonecrosis pathogenesis, which in their model, was 0.5 mg/kg.

Although ALN is the most common drug used to treat osteoporosis worldwide (Paiva-Fonseca et al., 2014), epidemiological studies demonstrate that the incidence of MRONJ associated with ALN is low (0.01–0.03%) (Sedghizadeh et al., 2009; Abtahi et al., 2012; Ruggiero et al., 2022). In this review, only 6 studies using ALN reported the occurrence of MRONJ (Table 4). Conte Neto et al. (2013), using 1 mg/kg SC daily for 8 weeks (23333% of the rat ALN osteoporosis dose), and Conte-Neto et al. (2016), using a much lower dose, 1 mg/kg SC once/wk for 8 weeks (3333% of the rat ALN osteoporosis dose), found more necrotic, exposed bone associated with infection and inflammation at 4wks post-tooth extraction in the alveolar bone tissue of ALN rats than in control rats, in both experiments. All other ALN studies reported no clinical signs of MRONJ (Altundal & Güvener, 2004; Aguirre et al., 2010; Maahs et al., 2011; Isaias et al., 2021; Mustakim et al., 2022). However, these studies reported either delayed initial healing or decreased bone volume and vascularization (Altundal & Güvener, 2004; Aguirre et al., 2010), reduced number of osteoclasts per measured area (Altundal & Güvener, 2004). The cumulative absorbed ALN dose delivered in these studies was often less than 25% of the osteoporosis dose of ALN (Maahs et al., 2011; Isaias et al., 2021), a dose so low that MRONJ could not reasonably be expected to occur. In another case (Altundal & Güvener, 2004), there was no pre-treatment with ALN, which does not parallel customary experience in humans. Three studies using 167–4666% of the rat ALN osteoporosis dose saw no MRONJ or exposed bone in their ALN-only group, but reported MRONJ when ALN and DX were co-administered (Berti-Couto et al., 2013; Abtahi et al., 2012; Movahedian Attar et al., 2020).

Glucocorticoids have often been reported as a risk factor for MRONJ in humans taking antiresorptives (Ruggerio et al., 2022). Appropriately, investigators applied DX as a systemic risk factor in 12 ZA rat studies, three ALN rat studies, and seven ZA mouse studies of tooth extraction-related MRONJ. In all the experiments with BP-only contrast groups, DX increased the prevalence of necrotic bone and bone exposure compared to the BP-only contrast group.

Commentary on the mechanism by which this occurs is appropriate. Glucocorticoids inhibit the inflammatory process and are used to suppress the immune system in multiple clinical situations, including in patients with rheumatoid arthritis and in cancer patients who require supportive care (Yuan et al., 2023; Yennurajalingam & Bruera, 2014; Giles et al., 2018). It is well-known that glucocorticoids like dexamethasone have major negative effects on bone (Liu et al., 2015). Glucocorticoids reduce bone formation, cause bone loss, and increase fracture risk. Osteoporosis doses of BPs stop bone loss in patients taking glucocorticoids. However, despite the known negative direct effects of glucocorticoids on the skeleton, it seems more likely to us that their role in MRONJ is mediated by their well-documented negative influence on wound healing (Johnston, 1990; Beer et al., 2000; Poetker & Reh, 2010; Harris et al., 2015).

Both glucocorticoids and diabetes are known for delaying wound healing (Jiao et al., 2015; Chen et al., 2022). Diabetes also increased MRONJ prevalence in BP treated rodent models of tooth extraction related MRONJ reported here (Berti-Couto et al., 2014; Takaoka et al., 2015; Zhang et al., 2015). A portion of antiresorptive-related MRONJ cases may thus result from the failure of oral events that would ordinarily resolve, to heal in a timely fashion, leading to MRONJ. It is also possible that anti-angiogenic properties of BPs, which have been identified mostly through in vitro studies (Wood et al., 2002; Lang et al., 2016; Guirguis et al., 2023; Gulcu, Akkaya, 2022; Bullock et al., 2022), present as failed wound healing, causing MRONJ when doses at and above the oncology dose are employed.

Though some have proposed that adding DX to models of BP-induced tooth extraction-related MRONJ produces a more reliable model (Sonis et al., 2009; Jabbour et al., 2014), one must remember that the majority of MRONJ cases in humans occur in the absence of glucocorticoid administration. Using DX as a standard way to increase the incidence of MRONJ in pre-clinical studies introduces a systemic risk factor not present in most human MRONJ.

In consideration of the multifactorial nature of MRONJ, a role in the pathophysiology of the condition ought to be assigned to the trauma caused by surgical procedures in the oral cavity (e.g., tooth extractions), since previous studies (Hokugo et al., 2010; Barba-Recreo et al., 2014; Park et al., 2015) demonstrated that ZA alone does not create an environment sufficient to the spontaneous onset of MRONJ. Also, mimicking the clinical scenario, the presence of infected teeth is a frequently found factor included in this review and deserves recognition, as the data demonstrated here highlight how presence of tooth–related inflammatory dental disease increases the prevalence of MRONJ in rodents which undergo tooth extraction during treatment with ZA (Soundia et al., 2016; Hadaya et al., 2018; Soundia et al., 2018; Song et al., 2018; Statkievicz et al., 2018; Kim et al., 2018; Bolette et al., 2019; Ervolino et al., 2022; Du et al., 2022; Williams et al., 2022). Also noteworthy are the results published by Williams et al., 2022, which show that the duration of the inflammatory process is associated with increased MRONJ in ZA-treated mice.

The role of antiresorptives and their cumulative dose as a systemic risk factor in triggering MRONJ is clearly stated in the literature. However, the systemic drug by itself is unlikely to prompt spontaneous bone sequestration (Kahn et al., 2015; Ruggiero et al., 2022). The

cumulative dose of antiresorptive and the duration of therapy are more important than the specific drug itself and its dosing interval (Thumbigere-Math et al., 2012). Cumulative dose and extended duration combine as a potent enabler of MRONJ only when they are associated with concurrent local oral risk factors, such as tooth-related inflammatory dental disease (e.g., periodontal or periapical abscess), tooth extraction, or use of removable dental prostheses (Hasegawa et al., 2017; Otto et al., 2018). Other systemic risk factors such as glucocorticoid therapy and diabetes play occasional roles as noted above.

In rats, ZA led to MRONJ regardless of the doses used (physiologic [Sonis et al., 2009; Hokugo et al., 2010; Biasotto et al., 2010; Ali-Erdem et al., 2011; Marino et al., 2012; Takaoka et al., 2015; Silva et al., 2015; Curra et al., 2016], supraphysiologic [Maahs et al., 2011; Barba-Recreo et al., 2014; Jabbour et al., 2014; Ho is very low but still wie et al., 2015; Kim et al., 2015; Yang et al., 2015; Zandi et al., 2015; Silva et al., 2015; Vidal-Gutiérrez et al., 2017; de Almeida et al., 2018], and extremely supraphysiologic [Bolette et al., 2019; Barba-Recreo et al., 2014; Barba-Recreo et al., 2015; Silva et al., 2015; Kolpakova et al., 2017; Hadaya et al., 2018; Soundia et al., 2018; Statkievcz et al., 2018; Paulo et al., 2020; Liu et al., 2021; Ervolino et al., 2022;], and when associated with dexamethasone [Sonis et al., 2009; Ali-Erdem et al., 2011; Jabbour et al., 2014]). This is not entirely unexpected, as the lowest ZA dose tested was actually 250% of the rat ZA osteoporosis dose. It is well-known that MRONJ incidence in humans treated with osteoporosis doses of antiresorptives though very low, is still above zero, at 0.01–0.03%.

All pre-clinical models included in this review emphasized histopathologic examination that revealed the presence of necrotic bone. In animal models, the standard for MRONJ calls for histopathological detection of fields of adjacent empty lacunae in bone tissue (Fondi & Franchi, 2007; Kuroshima et al., 2019). Using pattern recognition to identify necrotic bone tissue has long been part of the pathologist's arsenal for judging the viability of bone tissue, supported by the quantification of the empty lacunae/mm^2 of bone tissue and % of empty osteocyte lacunae. Authors using this type of model can consider presenting clinical (e.g., bone exposure) and imaging data as secondary support for the MRONJ diagnosis established by finding necrotic bone during histopathologic examination.

6. Conclusion

This review of approximately 120 studies supports the conclusion that ZA and ALN lead to MRONJ at rodent-equivalent doses to those used in humans with osteoporosis or, in the case of ZA, cancer, when pre-treatment and post-treatment observation times relative to tooth extraction are adequate in rats and mice. The tested doses range from 1.7% of the ALN rat osteoporosis dose to 4500% of the rat ZA oncology dose. Adding a local oral risk factor, inflammatory dental disease, to tooth extraction at the same dose of ZA causes a higher incidence of MRONJ. These rodent models are noteworthy because, unlike in humans, it is reasonable to use a histopathologic approach to diagnosing MRONJ in each animal.

Investigators who choose a physiologic dose of ZA for a tooth extraction-related MRONJ rat or mouse model face the likelihood that the incidence of MRONJ in their ZA groups may be only 10–40%. The protocol might call for two IV ZA doses per month, with tooth extraction

occurring four weeks after the first dose and necropsy occurring four to six weeks after tooth extraction. Though this will necessitate relatively large group sizes for MRONJ incidence findings in comparator groups to achieve statistical significance compared to the ZA group, investigators can be reasonably certain that the ZA environment in the experiment occurs in humans with MRONJ.

Investigators who choose a supraphysiologic dose of ZA for a tooth extraction-related MRONJ rat or mouse model can achieve an incidence of MRONJ in their ZA groups of 70–100%. The specific protocol would resemble the above timing. Though this will allow smaller group sizes for any findings about MRONJ incidence in comparator groups to achieve statistical significance compared to the ZA group, investigators will be less certain that the ZA environment in the experiment is relevant to what occurs in humans with MRONJ. With interest in using pre-clinical models to test treatments for MRONJ on the rise, one should consider that MRONJ induced by supraphysiologic doses of ZA may occur because of unique pathophysiologic mechanisms not invoked by physiologic doses of ZA. Such MRONJ might thus be more treatment resistant than MRONJ induced by physiologic ZA doses, making potentially efficacious MRONJ treatments wrongly appear unsuccessful.

Investigators who choose a physiologic dose of ZA for a tooth extraction-related MRONJ rat or mouse model could consider adding a relevant local oral risk factor such as inflammatory dental disease, an intervention that would likely increase the incidence of MRONJ in the ZA groups into the 60–80% range. The specific protocol would resemble the above timing. This will allow smaller group sizes for findings about MRONJ incidence in comparator groups to achieve statistical significance compared to the ZA group while maintaining a ZA environment in the experiment that occurs in humans with MRONJ. Given the data presented in this manuscript, this type of experiment is reasonable to consider.

Acknowledgments

The São Paulo State Foundation for Research (FAPESP) awarded Dr. Henrique Hadad a Ph.D. scholarship (#2019/22657-8), and the scholarship granted by the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES), within the scope of the Program CAPES-PrInt; process number #88887.570145/2020–00.

Funding

This work was partly supported by the Educational and Research Fund, Department of Oral and Maxillofacial Surgery, Massachusetts General Hospital (Boston, MA).

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Table 1

Characteristics of studies evaluating Zoledronic Acid [Physiologic Dose (≤200% of rat ZA oncology dose)].

Author/Year	Dose	% of rat ZA Oncology dose	Route	Animal/ Gender/Age	Risk Factor	Surgical Site	MRONJ	Bone Exposure	Pre-XT (d)	Post-XT (d)	Post-XT treat
Guevarra et al. (2015)	0.02 mg/kg−monthly 8wks40 μg/kg 2 m	25	IV	SD/F/RB	No	Md/1 M	Yes(38%)	Yes(38%)	28	28, 56, 84	No
Marino et al. (2012)	0.02 mg/kg every 21d − 3wks40 μg/kg 1.5 m	33	IV	SD/F/8 m	No	Md/1 M	Yes	Yes (75%)	21	28, 56	No
Ali-Erdem et al. (2011)	0.0075 mg/kg once/wk − 3wks22.5 μg/kg 0.75 m	38	SC	WA/M/5w	DX	Md Mx/ 1–3 M	Yes1	No	14	28	No
Tamari et al. (2019)	0.0075 mg/kg−1X/wk −11wks83 μg/kg 2.75 m	38	SC	Lewis/ F&M /13w	DX	Mx/1 M	Yes (52%)	Yes	14	56	Yes
Sonis et al. (2009)	0.0075 mg/kg once/wk − 3wks 22.5 μg/kg 0.75 m	38	SC	SD/F/11w	DX	Md Mx/1–3 M	Yes	Yes5	21	14, 28	No
Cardoso et al. (2019)	0.035 mg/kg every 2 wks − 13 wks	54	IV	WA/M/8w?	No	Mx/In	Yes (7%)	Yes (40%)	42	21, 35, 49	No
Hokugo et al. (2010)	0.035 mg/kg every 2 wks − 11wks210 μg/kg 2.75 m	87	IV	SD/#/7w	Yes *	Mx/1–3 M	Yes2	Yes	21	7, 14, 28, 56	Yes
Curra et al. (2016)	0.035 mg/kg every 2 wks − 8 wks140 μg/kg 2 m	87	IV	WA/M/10w?	No	Mx /In	Yes	Yes	63	14, 28, 42	No
Takaoka et al. (2015)	0.035 mg/kg every 2 wks − 29 wks525 μg/kg 7.5 m	87	IV	SD/M/5w	Yes γ	Mx/1–3 M	Yes3	Yes	147	14, 28, 56	Yes
Silva et al. (2015)	3 cycles of 0.04 mg/kg once/wk + 1 cycle 5 wks later160 μg/kg 2 m	100	IV	WA/M/8w	No	Md/1 M	Yes4	No	42	28	Yes
Zhu et al. (2023)	0.08 mg/kg/wk − 8 wks − 16wks640 μg/kg 4m 0.08 mg/kg/wk − 8 wks − 24wks640 μg/kg 6 m	133,133	IP	SD/F/8mo	Yes°	Md/1–3 M	Yes (75%) Yes (89%)	NoNo	5656 + 56 H	5656	NoNo
Brierly et al. (2019)	0.1 mg/kg− 3wks − 6wks200 μg/kg 1.5 m	166	IV	SD/?/9w	No	Md/1 M	Yes	Yes	21	56	No
Biasotto et al. (2010)	0.04 mg/kg once/wk − 5 wks200 μg/kg 1.25 m	200	IV	WA/M/13w6	No	Mx/1 M	Yes	Yes	14	56	Yes

Rat ZA oncology dose is 0.08 mg/kg IV/month (Aguirre et al., 2022).

Column MRONJ: authors' interpretation of their results (*Yes* means treated rats developed osteonecrosis).

Column Risk Factor:

* means vitamin D deficiency;

γ means diabetes associated.

Column MRONJ and Bone Exposure, 1 means 60% of samples had osteonecrosis; 2 means 14% of samples in the VitD+ /ZA group had osteonecrosis, while 67% in VitD-/ZA had osteonecrosis; 3 means 17% of samples in ZA + diabetes group had osteonecrosis and 100% of samples in ZA + diabetes had osteonecrosis and bone exposure; 4means 14% of samples had osteonecrosis; 5 40% of samples had bone exposure; 6 means “skeletally mature”.

Abbreviation: ZA, zoledronate; DX, dexamethasone, SC, subcutaneous; IP, intraperitoneal; IV, intravenous; SD, *Sprague Dawley* rats; WA, *Wistar Albinus* rats; M, male; #, not reported; w, wks; m, mos; Mx, maxilla; Md, mandible; 1 M, fist molar; 2 M, second molar; 3 M, third molar; 1–3 M, first, second, and third molar; In, incisor; Pre-XT: pre-extraction treatment period; Post-XT: post-extraction period; Post-XT Treat: drug treatment applied during post-extraction period.

Table 2

Overall characteristics of studies evaluating Zoledronic Acid [Supraphysiologic Dose (>200%<1000% of rat ZA oncology dose)].

Author/Year	Dose	% of rat ZA Oncology dose	Route	Animal	Risk Factor	Surgical Site	MRONJ	Bone Exposure	Pre-XT (d)	Post-XT (d)	Post-XT treat
Elsayed et al. (2018)	0.08 mg/kg/− 1X/wk − 13wks (1040 μg/kg 4.5 m)	289	IV	SD/F/10–12mo	No	Md/1–2 M	Yes	Yes	91	35	No
Zandi et al. (2016)	0.06 mg/kg− 1X/wk − 7wks420 μg/kg 1.75 m	300	IP	WA/M/10w	No	Md/1 M	Yes(100%)	Yes(100%)	0, 7, 14, 21	28	Yes
Borke et al. (2015)	0.06 mg/kg−1X/wk 2wks120 μ/kg 0.5 m	300	IV	SD/F/12w	No	Md/1 M	Yes(100%)	Yes(100%)	7	28	No
Imada et al. (2019)	0.06 mg/kg/wk −2wks 120 μg/kg 0.5 m	300	IV	SD/F/10w	No	Md/1 M	Yes	Yes	14	21 or 56	No
Vidal-Gutierrez et al., 2017	0.06 mg/kg every 7d − 2 cycles120 μg/kg 0.5 m	300	IM	WA/M/10w	No	Mx/2 M	Yes	Yes	21	14, 28, 42	No
Zandi et al. (2015)	0.06 mg/kg once/wk − 5 wks300 μg/kg 1.25 m	300	IP	WA/M/10w	No	Md/1 M	Yes	Yes(85%)	28	56	No
Zandi et al. (2017)	0.06 mg/kg− 1X/wk − 12wks720 μg/kg 3 m	300	IP	WA/M/13w	No	Md/1 M	Yes(83%)	Yes(80%)	84	56	No
Gong et al. (2017)	0.08 mg/kg−1X/wk −12wks960μg/kg 3 m	400	IV	SD/F/9w	No	Md Mx/1 M	Yes	Yes(45%)	14	70	Yes
Yang et al. (2015)	0.08 mg/kg once/wk − 3 wks240 μg/kg 0.75 m 0.08 mg/kg once/wk − 7 wks560 μg/kg 1.75 m 0.08 mg/kg once/wk − 15 wks1200 μg/kg 3.75 m s	400400400	IV	SD/M/9w	No	Mx/1 M	Yes	Yes	212121	72884	YesYesYes
Janovszky et al. (2015)	0.08 mg/kg− 1X/wk − 8wks640 μgkg 2 m	400	IV	WA/M/8w	No	Md/1–2 M	Yes(60%)	Yes(100%)	21	42	Yes
Isais et al., 2021	0.2 mg/kg/wk − 3 wks + 1 wk800 μg/kg 2.5 m	400	IV	WA/M/9w	No	Md/1 M	Yes	No	42	28	No
Elsayed et al. (2020)	0.08 mg/kg/− 2X/wk − 13wks1040 μg/kg 3.25 m	400	IV	SD/F/10–12mo	No	Md/1–2 M	Yes	Yes	91	56	No
Howie et al. (2015)	0.08 mg/kg once/wk − 13 wks1040 μg/kg 3.25 m	400	IV	SD/F/11 m	Yes #	Md/1–2 M	Yes	Yes	91	7, 14, 56	No
Silva et al. (2015)	3 cycles of 0.20 mg/kg once/wk+ 1 dose 5 wks later800 μg/kg 2 m	500	IV	WA /M /8w	No	Md /1 M	Yes2	No	42	28	Yes
Kim et al. (2015)	0.1 mg/kg once/wk − 15 wks1500 μg/kg 3.75 m	500	SC	SD /F/16w	Yes °	Md/1–3 M	Yes178% −OVX47% −Sham	Yes	42	56	Yes
Barba-Recreo et al. (2014)	0.1 mg /kg once/wk − 9 wks900 μg/kg 2.25 m	500	IV	WA /M /8w	No	Mx/1–3 M	Yes3	No	56	7	Yes

Author/Year	Dose	% of rat ZA Oncology dose	Route	Animal	Risk Factor	Surgical Site	MRONJ	Bone Exposure	Pre-XT (d)	Post-XT (d)	Post-XT treat
Kuroshima et al. (2014)	0.1 mg/kg every 2 d − 10 d150 μg/kg 0.33 m	570	SC	SD /#/6w	No	Mx/2 M	Yes	No	0	10	Yes
Jabbour et al. (2014)	0.13 mg/kg once/wk − 7 wks910 μg/kg 1.75 m	650	IP	SD /#/32w	DX	Md Mx/1 M	Yes	Yes	21	28	Yes
Ferreira et al. (2020)	0.6 mg/kg − 4 wks − 20wks3000 μg/kg 5 m	750	IP	WA/M/12w	No	Mx/1–3 M	Yes	No	45	105	Yes
Maahs et al. (2011)	0.6 mg/kg once/mo − 5 cycles3000 μg/kg 5 m	750	IP	WA/M/20w	No	Mx/1–3 M	Yes	No	45	105	Yes
de Almeida et al. (2018)	0.6 mg/kg once/mo − 3 cycles1800 μg/kg 3 m	750	#	WA/M/20w	No	Mx/2–3 M	Yes	Yes(100%)	60	15 30	No
Vasconcelos et al. (2012)	0.6 mg/kg− 1X/mo − 17wks3000 μg/kg 5 m	750	IP	WA/F/16w	No	Mx/1–3 M	Yes(100%)	Yes(100%)	60	42	Yes
Silveira et al. (2016)	0.6 mg/kg/mo −4mos 2400 μg/kg 4mos	750	IP	WA/F/17w	No	Mx/1–3 M	Yes	Yes	60	60	Yes
Kosach et al. (2020)	0.18 mg/kg− 1X/wk − 6wks1080 μg/kg 1.5 m	900	IV	WA/M/26w	No	Md/1 M	Yes	DNR	42	28	No

Rat ZA oncology dose is 0.08 mg/kg IV/month (Aguirre et al., 2022).

Column MRONJ relates to how authors interpret their results (*Yes* means treated rats developed osteonecrosis, *DNR* means do not report).

In Column Risk Factor,

means repeated trauma associated;

° means OVX associated.

In Column MRONJ and Bone Exposure, 1 means 78% of the samples present osteonecrosis; 2 means 14% of the samples present osteonecrosis; 3 means 25% of the samples present osteonecrosis.

Abbreviation: ZA, zoledronate; DX, dexamethasone, SC, subcutaneous; IP, intraperitoneal; IV, intravenous; IM, intramuscular; SD, *Sprague Dawley* rats; WA, *Wistar Albinus* rats; F, female; M, male; #, not reported; w, weeks; m, months; Mx, maxilla; Md, mandible; 1 M, fist molar; 2 M, second molar; 3 M, third molar; 1–3 M, first, second, and third molar; Pre-XT: pre-extraction treatment period; Post-XT: post-extraction period; Post-XT Treat: drug treatment applied during post-extraction period.

Table 3

Overall characteristics of studies evaluating Zoledronic Acid [Extremely Supraphysiologic Dose (≥1000% of rat ZA oncology dose)].

Author/Year	Dose	% of rat ZA oncology dose	Route	Animal	Risk Factor	Surgical Site	MRONJ	Bone Exposure	Pre-XT (d)	Post-XT (d)	Post-XT treat
Adachi et al. (2020)	0.066 mg/kg−3X/wk −4wks800 μg/kg 1 m	1000	SC	WA/F/4w	DX	Mx/1 M	Yes	Yes	14	14	Yes
Kaibuchi et al. (2016)	0.066 mg/kg−3X/wk −4wks800 μg/kg 1 m	1000	SC	SD/F/4w	DX	Mx/1 M	Yes	Yes	14	14	Yes
Sanda et al. (2022)	0.066 mg/kg−3X/wk −4wks800 μg/kg 1 m	1000	SC	WA/F/4w	NoDX	Mx/1 M	NoYes	NoYes	14	28	Yes
Yanik et al. (2016)	0.1 mg/kg− 2X/wk − 7wks1400 μg/kg 1.75 m	1000	IP	SD/F/12w	DX	Mx/1 M	Yes	Yes	49	21, 56, 70	No
Liu et al. (2021)	0.2 mg/kg once/wk − 8 wks1600μg/kg 2 m	1000	IP	SD/F/8w	Yes [°] DX	Md/1 M	Yes	Yes	56	56	No
Gao et al. (2021)	0.2 mg/kg−1X/wk − 16 wks3200 μg/kg 4 m	1000	IV	SD/M/6–8w	DX	Mx/1 M	Yes	Yes	56	556	Yes
Kolpakova et al. (2017)	0.36 mg/kg once + 0.18 mg/kg once/ week − 4 wks1080 μg/kg 1.25 m	1080	IV	WA/M/16	No	Md/1–2 M	Yes	No	0	56	Yes
Poubel et al. (2018a)	0.125 mg/kg twice/wk − 4wks 1000 μg/kg 1 m	1250	IP	WA/M/4w	No	Md/1 M	Yes	No	7	28	Yes
Cui et al. (2020)	0.125 mg/kg−2X/wk − 5wks1250 μg/kg 1.25 m	1250	IP	WA/M/8w	No	Mx/1 M	Yes	Yes	7	28	Yes
Statkievcz et al., 2018	0.1 mg/kg MWF − 7 wks2100 μg/kg 1.75 m	1500	IP	WA/F/80w	Yes $\theta\psi$	Md/1 M	Yes	Yes	21	28	Yes
Ervolino et al. (2022)	0.1 mg/kg MWF − 7 wks2100 μg/kg 1.75 m	1500	IP	WA/F/80w	Yes ψ	Md/1 M	Yes	Yes	21	28	Yes
Paulo et al. (2020)	0.1 mg/kg MWF − 7 wks2100 μg/kg 1.75 m	1500	IV	WA/F/17w	Yes θ	Md/1 M	Yes	Yes	28	14, 21	Yes
Barba-Recreo et al. (2014)	0.1 mg /kg MWF − 9 wks2700 μg/kg 2.25 m	1500	IP	WA/M/8w	No	Mx/1–3 M	Yes1	No	56	7	Yes
Barba-Recreo et al. (2015)	0.1 mg /kg MWF − 9 wks2700 μg/kg 2.25 m	1500	IP	WA/M/8w	No	Mx/1–3 M	Yes2	No	56	7	Yes
deSousa et al. (2022)	0.1 mg/kg-MWF 9wks2700 μg/kg 2.25 m	1500	IP	SD/F/12w	No	Mx/1–3 M	Yes(83%)	Yes(91%)	56	21	Yes
Dayisoylu et al. (2014)	0.1 mg/kg− 3X/wk − 8wks2400 μg/kg 2 m	1500	IP	SD/F/12w	No	Md/1 M	Yes	Yes	56	56	No
Dayisoylu et al. (2013)	0.1 mg/kg− 3X/wk − 8wks2400 μg/kg 2 m	1500	IP	SD/F/12w	No	Md/1 M	Yes(66%)	Yes(100%)	56	56	No

Author/Year	Dose	% of rat ZA oncology dose	Route	Animal	Risk Factor	Surgical Site	MRONJ	Bone Exposure	Pre-XT (d)	Post-XT (d)	Post-XT treat
Mergoni et al. (2019)	0.1 mg/kg−3X/wk −9wks2700μg/kg 2.25 m	1500	IP	WA/F/8w	DX	Mx/1 M	Yes	Yes	63	8	No
Jung et al. (2021)	0.1 mg/kg MWF − 14wks4200 μg/kg 3.5 m	1500	IP	SD/F/10–12w	DX	Mx/1–3 M	Yes*	Yes(50%)	77	21	Yes
Hadaya et al. (2019)	0.2 mg/kg twice/wk − 9 wks3600 Hg/kg 2.25m 0.2 mg/kg twice/wk − 9 wks3600 μg/kg 2.25 m	20002000	IP	W/A/M/8w	NoYes Δ	Md/1–2 M	NoYes	NoYes	3535	2828	YesYes
Soundia et al. (2018)	0.2 mg/kg twice/wk − 9 wks3600 μg/kg 2.25 m	2000	IP	W/A/M/8w	Yes Ψ	Mx/1–2 M	Yes	No	35	28	Yes
Silva et al. (2015)	3 cycles of 1 mg/kg once/wk+ 1 dose 5 wks later4000 μg/kg 2 m	2500	IV	WA/M/8w	No	Md/1 M	Yes	No	42	28	Yes
Ersan et al. (2014)	0.2 mg/kg−3X/wk −6wks3600μg/kg 1.5 m	3000	IP	SD/F/11w	No	Md/1 M	Yes	No	42	28	No
Bolette et al. (2019)	0.3 mg/kg 3X/wk − 4 wks3600 μg/kg 1 m	4500	IP	WA/M/8w	Yes Δ	Mx Md/1 M	Yes	Yes	28	56	No

Rat ZA oncology dose is 0.08 mg/kg IV/month (Aguirre et al., 2022).

Column MRONJ relates to how authors interpret their results (*Yes* means treated rats developed osteonecrosis).

In Column Risk Factor,

Θ means senescence animal associated;

Ψ means experimental periodontitis associated;

γ means diabetes associated;

Δ means periapical lesions associated;

° means OVX-associated.

In Column MRONJ and Bone Exposure, 1rmeans 80% of the sample present osteonecrosis; 2 means 50% of the sample present osteonecrosis; 3 means 76% of the sample present bone exposure.

Abbreviation: ZA, zoledronate; DX, dexamethasone; MWF Monday, Wednesday, Friday; SC, subcutaneous; IP, intraperitoneal; IV, intravenous; SD, *Sprague Dawley* rats; WA, *Wistar Albinus* rats; Mi, mice; OP, *Oryzomys palustris* rats; F, female; M, male; #, not reported; w, weeks; m, months; Mx, maxillae; Md, mandible; 1 M, fist molar; 2 M, second molar; 3 M, third molar; 1–3 M, first, second, and third molar; Pre-XT: pre-extraction treatment period; Post-XT: post-extraction period; Post-XT Treat: drug treatment applied during post-extraction period.

Table 4

Overall characteristics of the studies evaluating Alendronate.

Author/Year	Dose	% of rat ALN OP dose	Route	Animal	Risk Factor	Surgical Site	MRONJ	Bone Exposure	Pre-XT (d)	Post-XT (d)	Post-XT treat
Maahs et al. (2011)	0.05 mg/kg once/wk − 23 wks11.5 μg/kg 5.75 m	1.7	PO	WA/M/20 w	No	Mx/1–3 M	No	No	45	105	Yes
Isais et al., 2021	2.5 mg/kg/wk − 3 wks + 1 wk10000 μg/kg 2.5m5 mg/kg/wk − 3 wks + 1 wk20000 μg/kg 2.5m 7.5 mg/kg/wk − 3 wks + 1 wk30000 μg/kg 2.5 m	2.14.26.3	POPOPO	WA/M/9w	No	Md/1 M	No	No	424242	282828	YesYesYes
Isais et al., 2021	2.5 mg/kg/wk − 10 wks25000 μg/kg 2.5m5 mg/kg/wk − 10 wks50000 μg/kg 2.5m 7.5 mg/kg/wk − 10 wks75000 μg/kg 2.5 m	8.316.725	POPOPO	WA/M/9w	No	Md/1 M	No	No	424242	282828	YesYesYes
Aguirre et al. (2010)	0.015 mg/kg twice/wk − 3 wks90 μg/kg 0.75 m 0.150 mg/kg twice/wk − 3 wks900 μg/kg 0.75 m	1001000	SC	SD/F/10w	No	Md/1 M	No	No	2121	10, 21, 35, 70	Yes
Mustakim et al. (2022)	1 mg/kg/d − 5X/wk− 3wks15000 μg/kg 0.75m1 mg/kg/d − 5X/wk− 8wks40000 μg/kg 2	167167	PO	SD/M/7w	No	Md/1 M	NoNo	NoNo	2163	21 & 3521 & 35	NoNo
Berti-Couto et al., 2013	0.05 mg/kg−1X/wk −16wks800 μg/kg 4 m	167	SC	WA/F/20 w	NoDXDM	Mx/1–3 M	NoYes(28%)Yes(78%)	DNR	90	21	Yes
Conte-Neto et al. (2016)	1 mg/kg once/wk − 8 wks8000 μg/kg 2 m	3333	SC	WA/M/10 w	No	Md/1 M	Yes	Yes	60	28	Yes
Abtahi et al. (2012)	0.2 mg/kg daily − 2 wks2800μg/kg 0.5 m	4666	SC?	SD/M/10 w	NoDX	Mx/1 M	NoYes	NoYes	0	14	Yes
Movahedian-Attar et al., 2020	0.2 mg/kg-daily −14d2800 μg/kg 0.5 m	4666	SC	WA/M/9–11w	DX	Mx/1–2 M	Yes(85%)Con=23%	Yes	0	14	Yes
Altundal & Güvener (2004)	0.25 mg/kg daily − 2 wks3500μg/kg 0.5m 0.25 mg/kg daily − 4 wks7000 μg/kg 1 m	58335833	SC	WA/M/8w	No	Md/1 M	No	No	00	1428	Yes
Goncalves et al. 2023	1 mg/kg/d − 90d60000 μg/kg3 m	16700	SC	WA/M/10 w	No	Md/1 M	Yes	No	60	30	No
Conte-Neto et al., 2013	1 mg/kg daily − 63 days63000 ng/kg 2.25 m	2333323333	SC	WA /M/8 w	No	Md/1 M	Yes	Yes	60	328	Yes

Author/Year	Dose	% of rat ALN OP dose	Route	Animal	Risk Factor	Surgical Site	MRONJ	Bone Exposure	Pre-XT (d)	Post-XT (d)	Post-XT treat
	1 mg/kg daily − 88 days88000 μg/kg 3.12 m										

Rat ALN osteoporosis dose is 0.120 mg/kg SC/month (Seedor et al., 1999).

The column MRONJ relates to how authors interpret their results (*Yes* means treated rats developed osteonecrosis).

Abbreviations: ALN, alendronate; SC, subcutaneous; PO, orally by gavage; OP, osteoporosis; DX, dexamethasone; SD, *Sprague Dawley* rats; WA, *Wistar Albinus* rats; F, female; M, male; #, not reported; w, wks; Mx, maxillae; Md, mandible; 1 M, fist molar; 2 M, second molar; 3 M, third molar; 1–3 M, first, second, and third molar; Pre-XT: pre-extraction treatment period; Post-XT: post-extraction period; Post-XT Treat: drug treatment applied during post-extraction period.

Table 5

Overall characteristics of studies using Zoledronic Acid in mice.

Author/Year	Dose	% of mouse ZA oncology dose	Route	Animal	Risk Factor	Surgical Site	MRONJ	Bone Exposure	Pre-XT (d)	Post-XT (d)	Post-XT treat
Hokugo et al. (2019)	0.1 mg/kg once − 3 wks100 μg/kg 0.75 m	25	IV	F/7–10w	No	Mx/1 M	No	No	7	14	No
Hokugo et al. (2019)	0.3 mg/kg once − 3 wks300 μg/kg 0.75 m	74	IV	F/7–10w	No	Mx/1 M	No	No	7	14	No
Igarashi et al. (2023)	0.05 mg/kg−2X/wk −3wks300μg/kg 0.75 m	74	SC	F/6w	CYP	Mx/1 M	Yes	No	21	14	Yes
Kuroshima et al. (2021)	0.05 mg/kg/− 2X/wk − 7wks700 μg/kg 1.75 m	74	SC	F/8w	CYP	Mx/1 M	Yes1	Yes1	21	14, 28	Yes
Hayano et al. (2020)	0.05 mg/kg/− 2X/wk − 5wks500 μg/kg 1.250.05 mg/kg/− 2X/wk − 7wks700 μg/kg 1.75 m	74	SC	F/8w	No+CYP	Mx/1 M	NoYes[1]	NoYes[1]	21	1428	Yes
Kuroshima et al. (2018b)	0.05 mg/kg 2X/wk − 7 wks700 μg/kg 1.75 m	74	SC	F/8–12w	CYP	Mx/1 M	Yes1	No	21	28	Yes
Kuroshima et al. (2018a)	0.05 mg/kg 2X/wk − 7 wks700 μg/kg 1.75 m	74	IV	F/8–12w	CYP	Mx/1 M	Yes1	No	21	28	Yes
Kozutsumi et al. (2022)	0.05 mg/kg 2X/wk − 7 wks700 μg/kg 1.75 m	74	SC	F/8w	No	Mx /1 M	No	No	21	28	Yes
Kuroshima, Yamashita (2013)	0.05 mg/kg 2X/wk − 7 wks700 μg/kg 1.75 m	74	SC	F8–12w	NoDXMEL	Mx/1 M	Yes*	NoNoYes(81%)	21	28	Yes
Park et al. (2015)	0.540 mg/kg once − 5wks 540 μg/kg 1.25 m	80	IV	F/7wk	No	Mx/1 M	Yes[2]	Yes[3]	7	4, 7, 14, 28	No
Yu et al. (2020)	0.125 mg/kg 1X/wk − 10wks1250 μg/kg 2.5 m	93	IV	F/8–10w	DX	Mx/1 M	Yes(78%)	Yes(56%)	14	56,112	Yes
Sun et al. (2016)	0.5 mg/kg/−once500 μg/kg 1.33 m	70 120 220	IV	F/7w	No	Mx/1 M	Yes(14d, 28d)	Yes(14d, 28d)	10	3, 14, 28	Yes
Hokugo et al. (2019)	0.5 mg/kg once − 3 wks500 μg/kg 0.75 m	124	IV	F/7–10w	No	Mx/1 M	Yes	No	7	14	No
Kaneko et al. (2023)	0.1 mg/kg/− 2X/wk − 5wks1000 μg/kg 1.25 m	148	IV	F/8w	VAB	Mx/1 M	Yes	No	21	14	Yes
Córdova et al. (2016)	0.10 mg/kg/− 2X/wk − 12wks2400 μg/kg 3 m	148	IP	M/10w	No	Mx/1 M	Yes(21%)	Yes(21%)	21	2163	Yes
Hokugo et al. (2019)	0.7 mg/kg once − 3 wks700 μg/kg 0.75 m	173	IV	F/7–10w	No	Mx/1 M	Yes	No	7	14	No

Author/Year	Dose	% of mouse ZA oncology dose	Route	Animal	Risk Factor	Surgical Site	MRONJ	Bone Exposure	Pre-XT (d)	Post-XT (d)	Post-XT treat
Zhu et al. (2019)	0.125 mg/kg−2X/wk − 4wks1000 μg/kg 1 m	185	IV	M/8w	No	Mx/1 M	Yes	Yes(40%)	7	721	Yes
Shen et al. (2022)	0.125 mg/kg−2X/wk − 5wks1250 μg/kg 1.25 m	185	IV	M/8w	No	Mx/1 M	Yes	Yes(67%)	7	728	Yes
Zhang et al. (2013)	0.125 mg/kg−2X/wk − 3wks750 μg/kg 0.75 m	185	IV	F/8–10w	No	Mx/1 M	Yes	Yes	7	14	Yes
Williams et al. (2014)	0.125 mg/kg 2X/wk − 4 wks1000 μg/kg 1 m	185	IV	F/8w	No	Mx/1 M	Yes	Yes(60%)	7	21	Yes
Yoshioka et al. (2022)	0.125 mg/kg 2X/wk− 3 wks750 μg/kg 0.75 m	185	IV	F/5w	DX	Mx/1 M	Yes	No	7	14	Yes
Kim et al. (2017)	0.125 mg/kg 2X/wk − 3 wks750 μg/kg 0.75mo 0.125 mg/kg 2X/wk − 4 wks1000 μg/kg 1 m	185	IV	F/8w	No	Mx/1 M	Yes	Yes (20%)	77	1421	Yes
Zhang et al. (2015)	0.125 mg/kg 2X/wk − 3 wks750 μg/kg 0.75 m	185	IV	F/8–10w	Yes [γ]	Mx/1 M	Yes	No	7	14	Yes
Kikuiri et al. (2010)	0.125 mg/kg 2X/wk − 3 wks750 μg/kg 0.75m 0.125 mg/kg 2X/wk − 8 wks2000 μg/kg 0.75 m	185	IV	F/8–10w	DX	Mx/1 M	YesYes	Yes (50%) Yes (30%)	77	1449	Yes
Zhao et al. (2012)	0.125 mg/kg 2X/wk − 3 wks750 μg/kg 0.75 m	185	IV	C3H/HeJ F/8w	DX	Mx/1 M	Yes	Yes (45%)	7	14	Yes
Taniguchi et al., 2019	0.125 mg/kg/− 2X/wk − 3wks750 μg/kg 0.75 m	185	IV	F/6	No	Mx/1 M	Yes(11%)	Yes (78%)	14	7	Yes
Bi et al. (2010)	0.125 mg/kg 2X/wk − 6 wks1500 μg/kg 1.5m 0.125 mg/kg 2X/wk − 15 wks3750 μg/kg 3.75 m	185	IP	8–12w	DX	Mx Md/1 M	Yes	No	2121	2184	YesYes
Kim et al. (2018)	0.125 mg/kg twice/week − 7 wks 1750 μg/kg 1.75	185	IV	F/6w	Yes [Ψ]	Mx/1 M	Yes	Yes (20%)	28	21	Yes
Biguetti et al. (2019)	0.25 mg/kg once/wk − 7 wks1750 μg/kg 1.75 m	185	IP	129 Sv/F/60w	Yes [Θ]	Mx/In	Yes	No	28	7, 21	Yes
Mahmoud et al. (2021)	0.25 mg/kg once/wk − 4 wks1000 μg/kg 1 m	185	IP	129 Sv/F/60w	Yes [Θ]	Mx /In	Yes	Yes	28	7, 21	Yes
Song et al. (2016)	0.125 mg/kg 2X/wk − 6 wks1500 μg/kg 1.5 m	185	IV	F/6w	Yes [Δ]	Mx/1 M	Yes	Yes (20%)	28	21	Yes
Williams et al. (2020)	0.125 mg/kg/− 2X/wk − 7wks1750 μg/kg 1.75 m	185	IV	F/6	NoYes [Ψ]	Mx/2 M	NoYes	NoYes	28	21	Yes
Williams et al. (2022)	0.125 mg/kg 2X/wk − 6 wks1500 μg/kg 1.5m 0.125 mg/kg 2X/wk − 13 wks3250μg/kg 3.25 m	185	IV	F/8w	Yes [Ψ]	Mx/2 M	YesYes+ +	YesYes+ +	49x/ 2170	2121	Yes

Author/Year	Dose	% of mouse ZA oncology dose	Route	Animal	Risk Factor	Surgical Site	MRONJ	Bone Exposure	Pre-XT (d)	Post-XT (d)	Post-XT treat
Du et al. (2022)	0.125 mg/kg 2X/wk − 9 wks2250 μg/kg 2.25 m	185	IV	F/6w	Yes Δ	Mx/1 M	Yes	Yes (20%)	42	21	Yes
Wu et al. (2022)	0.125 mg/kg−2X/wk −7wks1750 μg/kg 1.75m 0.125 mg/kg−2X/wk −11wks2750 μg/kg 2.75	185	IP	F/4w	PGinv	Md/1 M	Yes (only with PGinv)	No	21	2856	Yes
Yoshioka et al. (2022)	0.125 mg/kg−2X/wk −3wks750 μg/kg 0.75 m	185	IV	F/5w	DX	Mx/1 M	Yes	Yes	7	14	Yes
Hokugo et al. (2019)	0.9 mg/kg once − 3 wks900 μg/kg 0.75 m	225	IV	F/7–10w	No	Mx/1 M	Yes	No	7	14	No
Chen et al. (2021)	0.25 mg/kg/− 2X/wk − 4wks2000 μg/kg 1 m	370	IP	F/4w	No	Mx/1 M	Yes	DNL	7	21	Yes
Soma et al. (2022)	0.5 mg/kg once/wk − 8 wks4000 μg/kg 2 m	370	SC	F/8w	No	Md/1–2 M	Yes	No	14	42	Yes
Soma et al. (2021)	0.5 mg/kg once/wk − 8 wks4000 μg/kg 2 m	370	IP	F/8w	No	Mx/1–2 M	Yes	No	14	42	Yes
Kozutsumi et al. (2022)	0.25 mg/kg 2X/wk − 7 wks3500 μg/kg 1.75 m	370	SC	F/8w	No	Mx /1 M	Yes	No	21	28	Yes
Zheng et al. (2023)	0.5 mg/kg−2X/wk −4wks4000 μg/kg 1 m	740	IP	F/7w	No	Mx/2 M	Yes	Yes	14	14	Yes
Kozutsumi et al. (2022)	0.5 mg/kg 2X/wk − 7 wks7000 μg/kg 1.75 m	740	SC	F/8w	No	Mx/1 M	Yes	Yes	21	28	Yes
Kobayashi et al. (2010)	0.25 mg/kg/d − 11d2750 μg/kg 0.35 m	1455	SC	M/6w	No	Mx/1 M	No	No	7	5	Yes
Zhao et al. (2023)	1 mg/kg/−MWF − 4wks12000 μg/kg 1 m	2222	IP	M/6–8w	ψSUNψ+SUN	Mx/2 M	YesNoYes	Yes (30%) Yes (10%) Yes (70%)	14	14	Yes
Zheng et al. (2023)	1 mg/kg/− daily − 4wks28000 μg/kg 1 m	5200	IP	F/6–8w	No	Mx/1 M	Yes	No	14	14	Yes

Mouse ZA oncology dose is 0.54 mg/kg IV/month (Park et al., 2015).

Mice were strain C57BL/6 unless otherwise noted.

Column MRONJ is related to how authors interpret their results (Yes means some animals developed osteonecrosis).

In the column Risk Factor,

θ means senescence animal associated;

ψ means periodontitis associated;

Δ means periapical lesions associated;

[γ] means diabetes associated. In the column MRONJ and Bone Exposure,

[1] means osteonecrosis was diagnosed only when ZA was associated with CYP;

[2] means 75% at 4wks only;

[3] means 67% at 4wks only.

Abbreviations: ZA, zoledronate; DX, dexamethasone, SC, subcutaneous; IP, intraperitoneal; IV, intravenous; SD, Sprague Dawley rats; WA, Wistar Albinus rats; F, female; M, male; CYP- cyclophosphamide; SUN- Suninitib; VAB- anti-VEGFA neutralizing antibody; PGinv- *Porphyromonas gingivalis*. #, not reported; wk, weeks; m, months; Mx, maxillae; Md, mandible; 1 M, fist molar; 2 M, second molar; 3 M, third molar; 1–3 M, first, second, and third molar; Pre-XT: pre-extraction treatment period; Post-XT: post-extraction period; Post-XT Treat: drug treatment applied during post-extraction period.