Ibandronate in metastatic bone disease: a review of preclinical data

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Bisphosphonates are widely used to prevent and treat skeletal complications of metastatic bone disease. There is increasing evidence that, besides inhibiting osteoclast activity and reducing bone resorption, bisphosphonates also have an anti-tumor effect. This paper reviews the preclinical data for ibandronate. Ibandronate increased the proportion of apoptotic tumor cells *in vitro* and *in vivo*, possibly following activation of caspase-like proteases. *In vitro*, ibandronate also prevented adhesion and spreading of tumor cells to bone, and tumor cell invasion. These inhibitory effects were additive when ibandronate was given with paclitaxel or docetaxel. In animal models of tumor-induced osteolysis, ibandronate significantly reduced the development of osteolytic lesions. Efficacy for the prevention and reduction of bone metastases was related to the timing of treatment; ibandronate treatment initiated prior to or shortly after tumor cell inoculation inhibited the growth of bone metastases and preserved skeletal integrity most effectively. As with other bisphosphonates, the influence of ibandronate on soft tissue metastases has been inconsistent. Overall, preclinical evidence supports the rationale for adjuvant treatment with ibandronate for patients at risk of metastatic bone disease. The renal safety profile of ibandronate supports its suitability for long-term adjuvant use, even with intermittent high dosing. Adjuvant clinical trials have been initiated. The ability of bisphosphonates to preserve skeletal integrity is also of benefit in other clinical settings. Recent studies in rat models demonstrate improved osseointegration of joint implants following ibandronate therapy, with potential application in patients with conditions such as degenerative arthritis or osteoporosis. *Anti-Cancer Drugs* 16:107–118 © 2005 Lippincott Williams & Wilkins.

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Introduction

Metastatic bone disease is common in patients with advanced malignancies, affecting up to 80% of patients with breast or prostate cancer [1]. It leads to serious skeletal complications that cause considerable morbidity, including bone pain, impaired mobility, pathological fractures, spinal cord compression and hypercalcemia [1–3].

The growth of metastases in the bone has been described as a 'vicious cycle' [4,5]. During osteoclast-mediated bone resorption, growth factors stored in the bone [e.g. insulin-like growth factor (IGF) and transforming growth factor-β (TGF-β)] are released. These growth factors promote the survival and proliferation of tumor cells within the bone. In turn, they stimulate tumor cells to synthesize peptides, such as parathyroid hormone-related protein, which increase osteoclast activity, resulting in a self-perpetuating cycle of osteolysis.

Bisphosphonates can disrupt this cycle as they are selectively taken up by the bone where they inhibit osteoclast activity, thereby reducing bone resorption [6]. Consequently, these agents are the standard of care for the prevention and treatment of skeletal-related complications from metastatic bone disease [7,8]. There is an accumulating body of evidence suggesting that bisphosphonates also have an anti-tumor effect [9–13] and make the bone marrow a less favorable place for tumor growth [14,15].

Bisphosphonates can be divided into two groups with different molecular mechanisms of action [16–18]. In the first group are etidronate and clodronate, which lack a nitrogen in their molecule. They appear to work by forming cytotoxic metabolites in osteoclasts or inhibiting protein tyrosine phosphatases. Those in the most potent, nitrogen-containing group (including ibandronate, pamidronate and zoledronate) inhibit the mevalonate pathway in osteoclasts. This prevents post-translation lipid modification (i.e. prenylation) of small GTPase signaling proteins required for osteoclast function and cytoskeletal integrity. Both mechanisms of action lead to apoptosis. Inhibition of the mevalonate pathway also ultimately activates proteolytic enzymes such as caspases, thought to be essential for apoptosis as well.

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Objectives

Ibandronate is a highly potent nitrogen-containing bisphosphonate, provided as i.v. and oral formulations, with proven efficacy in skeletal complications of bone metastases from breast cancer [19–21] and osteoporosis [22,23]. Previous reviews of ibandronate preclinical data have focused on its use in osteoporosis [24] or hypercalcemia of malignancy [25]. In this paper we review the preclinical data supporting ibandronate in metastatic bone disease, showing that it not only inhibits osteoclasts and prevents deterioration of bone structure, but can also prevent the development and progression of bone metastases. Since renal safety is an issue with some bisphosphonates, we also summarize preclinical data supporting the renal safety of ibandronate, based on toxicology studies. We also discuss the implications for the potential future of ibandronate as a long-term adjuvant therapy.

Pharmacology, pharmacokinetics and metabolism of ibandronate

In vivo, ibandronate is 2-, 10-, 50- and 500-fold more potent at inhibiting bone resorption than risedronate, alendronate, pamidronate and clodronate, respectively [26]. Ibandronate can therefore be used at lower doses than most bisphosphonates.

Consistent with other bisphosphonates [6], preclinical data show that ibandronate distributes rapidly and concentrates predominantly in calcified tissue. In rats, i.v. administration of 0.1 mg/kg [^{14}C]ibandronate showed that ibandronate is rapidly cleared from plasma and that renal excretion is the predominant route of elimination [24,27]. As with other bisphosphonates, ibandronate is not metabolized and so the radioactivity was entirely accounted for by the intact drug. Radioactivity was 17–38 times higher in calcified versus non-calcified tissue. A total of 40–50% of the dose was found in bone, with a terminal elimination half-life of 440–500 days for the trabecular and cortical sections of long bones.

Renal clearance accounts for 50–60% of the elimination of ibandronate from the plasma (54–112 ml/min) and is directly related to creatinine clearance [28]. The remaining ibandronate is absorbed into the bone [28] and slowly clears as it redistributes in the blood. As a result, plasma elimination is multiphasic, with a half-life of 10–60 h [28]. When administered repeatedly over 1 year, the concentration of ibandronate in rat bone was found to be linearly related to the systemic dose, suggesting linear kinetics in the dose range tested (0.2–25 μg/kg/day, resulting in a total cumulative dose of approximately 0.07–9.1 mg/kg) [29]. Studies in rats and monkeys indicate that the concentration of ibandronate in bone is related to the total cumulative dose administered over a given period and is independent of whether the dose was given daily or less frequently in a given time period [30,31].

Consistent with the metabolic stability of ibandronate, the rapid deposition in bone tissue and the unique renal elimination pathway of bisphosphonates, no pharmacokinetic drug interactions of clinical significance are likely [24,32].

Evidence for anti-tumor action from *in vitro* experiments

Inhibiting adhesion and spread to bone

Morphologic observations of patients with metastatic bone disease have shown cancer cells on the bone surface, often without evidence of resorbing osteoclasts, and there is evidence that breast cancer cells may directly destroy bone [14]. This suggests that attachment of cancer cells to the bone matrix may play an essential role in bone metastases development.

Ibandronate inhibited the adhesion and spread of MDA-MB-231 breast cancer cells to pre-treated slices of bovine cortical bone and mouse trabecular bone in a concentration-dependent manner, and was the most potent of six bisphosphonates [14]. In a similar study, adhesion of human MDA-MB-231 breast and PmPC3 prostate cells to mineralized and non-mineralized bone was inhibited by pre-treating the cells with ibandronate; ibandronate was the most potent of four bisphosphonates tested [15]. The bisphosphonates did not show any non-specific cytotoxic action in normal cells.

The mechanism behind the inhibition of tumor cell adhesion to bone matrix with bisphosphonates is unknown. It has been suggested that the modulation of cell adhesion molecules (e.g. cadherin, laminin and integrins) is involved. Supporting this, in human osteoclast-like cells, application of alendronate led to a 50% reduction in adhesion to extracellular matrices containing the integrin RGD sequence [33].

Effect on tumor cell invasion and metastasis

Boissier *et al.* [34] investigated the ability of five active bisphosphonates, including ibandronate, clodronate and zoledronate, to inhibit migration and invasion of breast and prostate cancer cells using an *in vitro* assay. Ibandronate inhibited invasion by up to about 75% concentration dependently. Of the bisphosphonates tested, only zoledronate was more potent than ibandronate.

Matrix metalloproteinases (MMPs) have also been associated with tumor invasiveness and are known to support the development of osteolytic metastasis, possibly by contributing to bone resorption [4]. Clodronate, ibandronate and zoledronate all inhibited with equal

potency the proteolytic activity of MMP-2, -9 and -12 *in vitro* [34], although none of these bisphosphonates interfered with the production of the MMPs or inhibited tumor cell migration at concentrations that affected tumor cell invasion.

Interestingly, Derenne *et al.* [35] observed that zoledronate inhibited up-regulation of MMP-1 (predominantly involved in bone resorption) by bone marrow stromal cells *in vitro*. MMP-2 (involved in bone resorption and the metastasizing process) production was stimulated by zoledronate and to a lesser extent pamidronate, by the same cells. Thus, combining bisphosphonate administration with an inhibitor of MMPs may help to avoid this unwanted stimulation of MMP-2 activity, and indeed another study found that ibandronate and the tissue inhibitor of MMP-2 (TIMP-2) had an additive effect in reducing osteolytic lesions *in vivo* [36]. In that study, ibandronate alone not only inhibited the development of new osteolytic lesions, but also prevented the progression of already existing lesions.

The potential for bisphosphonates to enhance the anti-tumor activity of chemotherapy is of considerable clinical interest, as combination therapy reflects the clinical setting more appropriately. The taxoids paclitaxel and docetaxel are increasingly used to manage early and advanced breast cancer, and the role of doxorubicin is well established. Consequently, ibandronate was investigated and found to have a significant additive effect in reducing tumor cell invasion *in vitro* when combined with either paclitaxel or docetaxel ($p < 0.0001$ and $p \leq 0.01$, respectively, for the combination compared with a taxoid alone [37] (Fig. 1). However, ibandronate did not enhance taxoid-induced apoptosis. Other, recent data on combining anti-tumor drugs with a bisphosphonate suggest that ibandronate enhances the growth inhibitory effects of the anti-estrogens tamoxifen and fulvestrant *in vitro* [38], which may also prove useful in clinical practice. Similar effects of *in vitro* combination with paclitaxel or tamoxifen on apoptosis of breast cancer cells have been reported for zoledronate [39,40].

Antiproliferative effects

The effect of clodronate, ibandronate, pamidronate and zoledronate were studied on the *in vitro* proliferation of three human breast cancer cell lines: MCF-7, T47D and MDA-MB-231 [41]. All four bisphosphonates irreversibly inhibited the growth of MCF-7 and T47D cells time and dose dependently. MDA-MB-231 cells were less responsive. Although zoledronate seemed to act faster than ibandronate, ibandronate was more potent after 24 h and expressed a longer-lasting action in prolonged culture for up to 6 days (Fig. 2). Ibandronate significantly increased the proportion of apoptotic MCF-7 and to a lesser extent, T47D cells compared with the control ($p < 0.05$ for each cell line), and also increased necrosis in T47D cells. Activity of the caspase-3 subfamily in the MCF-7 cell line increased about 2-fold with all four bisphosphonates, but it increased only slightly in T47D cells, consistent with the lower proportion of apoptotic cells after treatment with bisphosphonates in this cell line. Adding a caspase inhibitor (z-VAD-fmk) to cell culture media blocked bisphosphonate inhibition of MCF-7 cell proliferation, while z-VAD-fmk alone had no effect on cell growth. These results demonstrate that besides the described inhibition of the farnesyldiphosphatase by nitrogen-containing bisphosphonates, another independent mechanism for apoptosis induction is involved. Since clodronate (which belongs to the non-nitrogen-containing bisphosphonates) revealed similar results, this mechanism seems to be related to both types of bisphosphonate.

These results are consistent with those of Journe *et al.* [38], who found that ibandronate increased the duplication time of estrogen receptor (ER)-positive MCF-7 cells to 50 h compared with 20 h for untreated control cells. In further experiments, ibandronate abolished the mitogenic effect of 17β-estradiol on MCF-7 cells, without affecting expression of either the ER or the estrogen-inducible progesterone receptor, a result that argues for combination therapy with ibandronate and anti-estrogens.

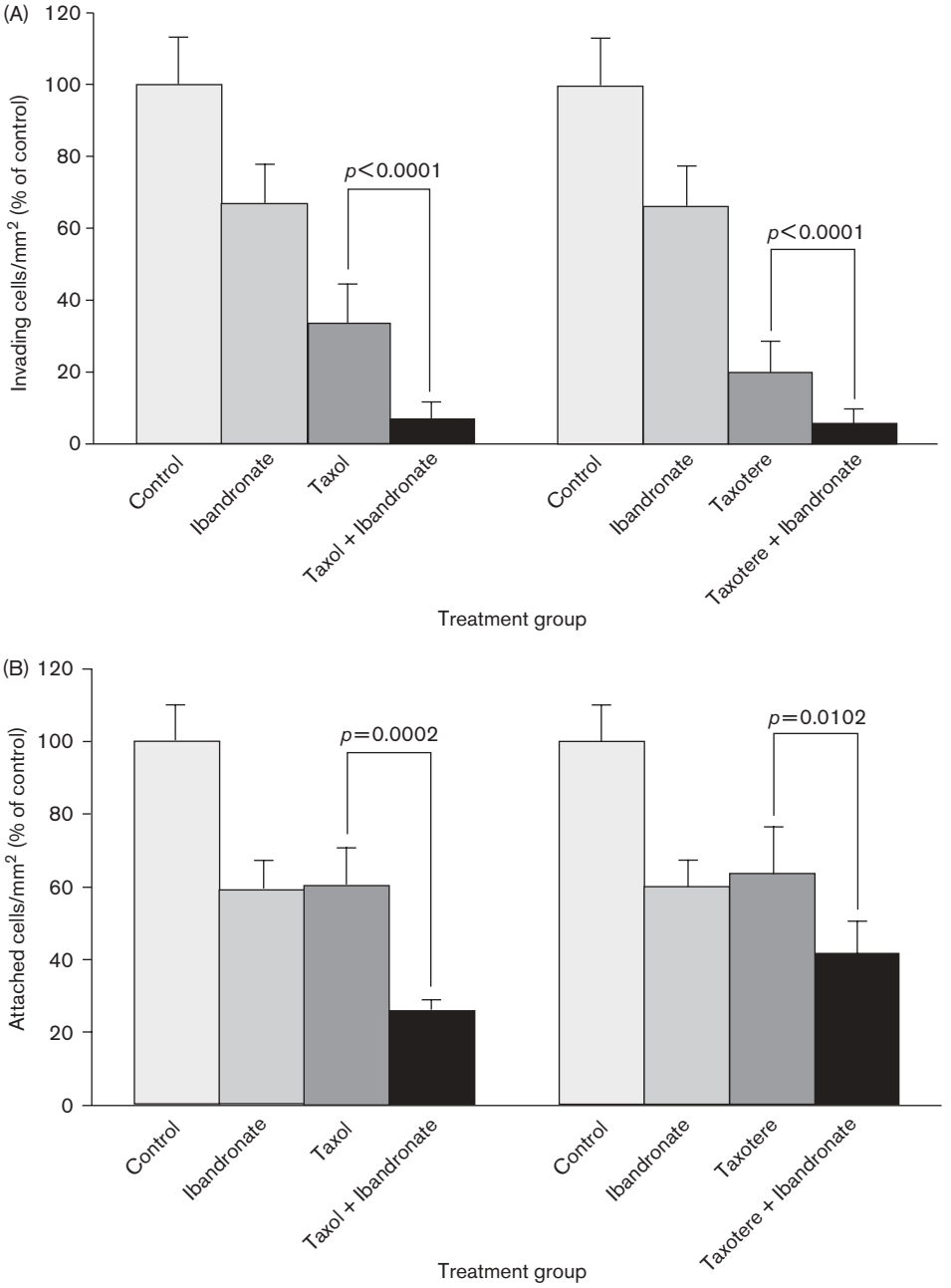
The effect of ibandronate on the apoptosis of MDA-231 breast cancer cells *in vitro* was studied by assessing intranucleosomal DNA fragmentation using gel electrophoresis [42]. Ibandronate at 100 μM induced evident apoptosis, but DNA fragmentation was almost completely inhibited by the caspase inhibitor, z-VAD-fmk. Further experiments showed that ibandronate significantly increased caspase-3 activity in MDA-231 cells, suggesting an involvement of this enzyme in ibandronate-induced apoptosis (Fig. 3). It is also possible that the recently postulated membrane-bound transport protein shown for ibandronate may help to explain its apoptotic effects [43].

Several groups have investigated the anti-proliferative effects of other bisphosphonates. Pamidronate and zoledronate induced apoptosis of myeloma cells and also breast cancer cell lines [4,44], although these effects did not lead to a reduction in non-osseous tumor burden [4,42,45]. Like ibandronate, clodronate, pamidronate and zoledronate also activate caspase-3 or caspase-3-like proteases thought to be involved in tumor cell apoptosis [41,42,46].

Anti-angiogenic effects

Angiogenesis, the formation of new blood vessels from pre-existing vessels, requires both proliferation of endothelial cells and their realignment to form new capillary

Fig. 1



Inhibition of MDA-MB-231 breast cancer cell invasion (A) and adhesion (B) with the combination of ibandronate and the taxoids paclitaxel and docetaxel. Adapted from [37].

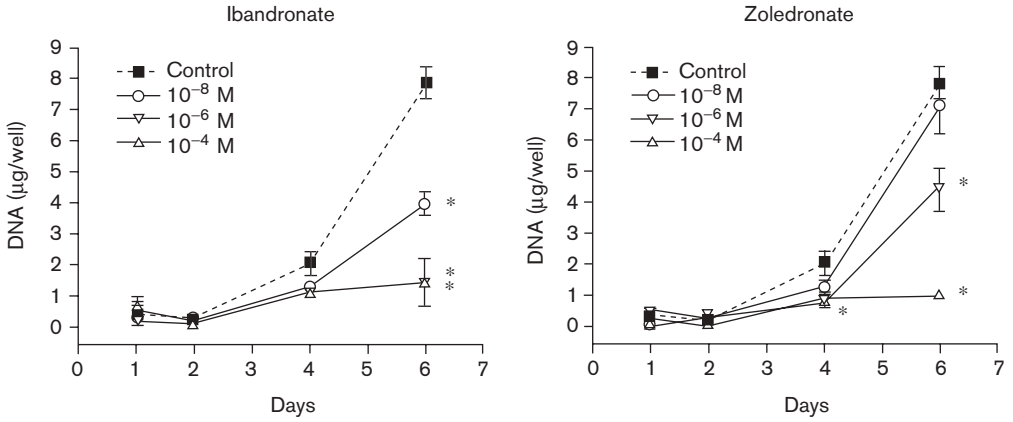
tubes, and is vital for tumor growth. A 3-day exposure of human umbilical vein endothelial cells to ibandronate or other bisphosphonates resulted in significant inhibition of cell proliferation compared with untreated controls ($p = 0.01$) [46]. Ibandronate also significantly inhibited capillary-like tube formation *in vitro* ($p < 0.0001$). There were similar results with clodronate, risedronate and zoledronate.

In castrated rats, in which a revascularization of the prostate gland was induced by testosterone, ibandronate inhibited this revascularization by about 50% [47]. These effects were also observed with zoledronate.

Interaction with growth factors

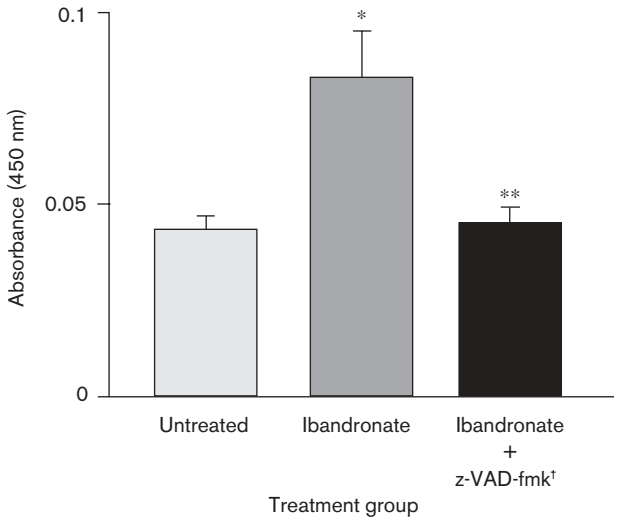
The bone micro-environment is rich in growth factors that may promote the growth of tumor cells.

Fig. 2



Long-term effects of ibandronate and zoledronate on MCF-7 cell proliferation. *$p<0.05$ versus control. Adapted from [41].

Fig. 3



Ibandronate-induced activation of caspase-3 in MDA-231 breast cancer cells *in vitro*. *$p<0.05$ versus untreated group; **$p<0.05$ versus ibandronate group; †caspase inhibitor.

Bisphosphonates appear to have the opposite effect on tumor cells, inhibiting growth and inducing apoptosis. Consequently, the interaction between bisphosphonates and growth factors through their effect on MCF-7 and T47D breast cancer cells was investigated *in vitro* [48]. Ibandronate inhibited the proliferative effects of IGF-I and IGF-II and fibroblast growth factor-2 (FGF-2) on MCF-7 cells, and of IGF-I and IGF-II, but not FGF-2, on T47D cells ($p < 0.05$ compared with growth factor alone). Ibandronate also antagonized FGF-2 activation of intracellular signaling pathways involved in cell survival. Similar effects on breast cancer cells were obtained with pamidronate, clodronate and zoledronate, suggesting that the anti-tumoral effects of bisphosphonates are not entirely explained by mevalonate pathway modulation by nitrogen-containing bisphosphonates or by their apoptosis-inducing effects via caspase secretion.

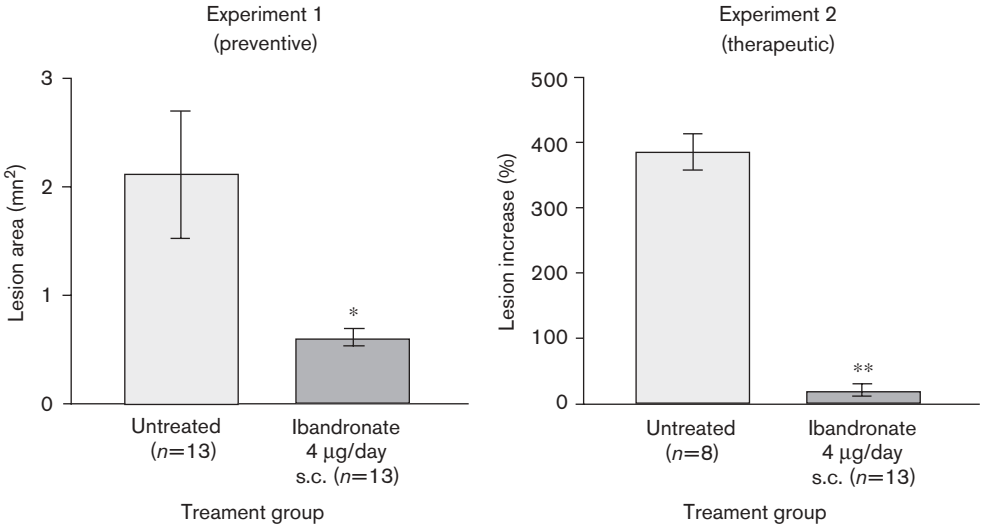
Evidence for anti-tumor action from animal models

Bone turnover and preservation of skeletal integrity

Accelerated bone turnover, characterized by increased osteoclast number and/or activity, is typical of tumor-induced bone disease and is accompanied by significant increases in urinary excretion of biochemical markers of bone turnover, such as collagen cross-link components [49,50]. There is some evidence that changes in these markers may predict skeletal-related events [51]. Besides other skeletal-related events, tumoral osteolysis increases the risk of bone fractures and much effort has gone into developing treatments to preserve skeletal integrity.

The Walker carcinosarcoma 256 rat model of tumor-induced osteolysis via parathyroid hormone-related protein (PTHrP) production was used to study the value of biochemical bone turnover markers in monitoring neoplastic bone disease [52]. Following tumor inoculation, the number of osteoclasts was highly correlated with urinary excretion of the biochemical markers for bone turnover, deoxypyridinoline (DPD, $r = 0.89$) and pyridinoline (PYD, $r = 0.91$) ($p < 0.001$ for both measures). Ibandronate significantly lowered the levels of excreted DPD and PYD compared with untreated, tumor-bearing controls and prevented a significant reduction in femoral bone mass. These results show that ibandronate inhibited tumor-induced bone resorption in this model, and the tested markers can be used to monitor the progression and influence of therapeutic intervention on disease.

Fig. 4



Effect of ibandronate on the development of new osteolytic bone metastases by human breast cancer MDA-MB-231. *$p<0.005$ versus untreated group; **$p<0.01$ versus untreated group. Adapted from [36].

The impact of ibandronate on bone quality was assessed in rats after intra-femoral injection of Walker carcinoma 256 cells [53,54]. Ibandronate significantly increased mean bone mineral density, bone mineral content and bone density compared with untreated tumor controls ($p < 0.001$ for each parameter), and limited loss of biomechanical bone strength [53]. In more experiments, ibandronate was most effective in preserving skeletal integrity when administered before plus immediately after tumor implantation, although preventative or interventional treatment alone also produced significant improvements in bone quality compared with untreated controls [54]. Further studies demonstrating normal or increased bone quality after long-term ibandronate administration in normal and osteoporotic animals, and in a model for fracture repair, is summarized in a review [24].

Inhibition of osteolytic bone lesions and bone metastases

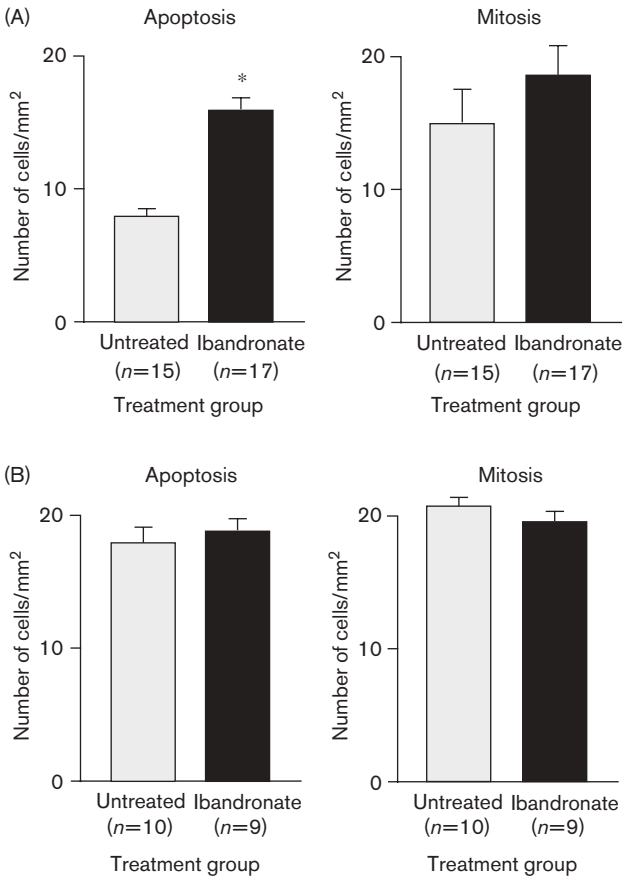
Breast cancer cell models

The inhibitory effect of ibandronate on tumor-induced osteoclastic bone resorption was investigated in nude mice after intra-cardiac injection with human breast cancer MDA-231 cells [36]. Also, cancer cells transfected with TIMP-2 to inhibit cancer invasiveness were tested either alone or in combination with ibandronate. In mice bearing tumors lacking TIMP expression, therapeutic intervention with ibandronate, given from day 17 to day 28 after injection of tumor cells, suppressed the progression of already existing osteolytic bone metastases. Pre-treatment with ibandronate for three weeks prior to tumor cell injection prevented both the formation and growth of new osteolytic bone metastases, arguing for early treatment of ibandronate in the clinical setting (Fig. 4). The combination of TIMP-transfected tumor cells and ibandronate further reduced the development of new osteolytic metastases significantly ($p < 0.01$) and, according to histological examination of the bone, also profoundly reduced metastatic tumor burden and osteoclastic bone destruction compared with either treatment alone.

In the same animal model, the MDA-231 human breast cancer cell line was used to investigate how ibandronate decreases tumor burden [42]. Ibandronate reduced osteoclastic bone resorption and increased apoptosis of osteoclasts. Quantitative radiologic assessment showed that ibandronate significantly inhibited the progression of osteolytic bone metastases compared with control ($p < 0.01$). A significant decrease in bone tumor burden detected by histomorphometric analysis was associated with significantly increased apoptosis of MDA-231 breast cancer cells ($p < 0.0001$ compared with control for both parameters) (Fig. 5A). However, when the tumor was inoculated orthotopically into the mammary fat pads, ibandronate was only active at inhibiting metastatic bone lesions and had no effect on orthotopic tumor formation (Fig. 5B). Ibandronate did not affect mitosis of tumor cells in both locations, suggesting that apoptosis of tumor cells in the bone was not due to cytotoxic effects.

These results are consistent with another study in nude rats, where injection of MDA-MB-231 breast cancer cells into the femoral artery induced multiple osteolytic lesions in the femora and tibiae [55]. Daily ibandronate

Fig. 5



Effect of ibandronate on apoptotic and mitotic MDA-231 breast cancer cells in bone (A) or mammary fat pads (B). *$p<0.0001$ versus untreated group. Adapted from [42].

Table 1 Development of osteolytic lesions in rats following injection of MDA-MB-231 human breast cancer cells into the femoral artery (adapted from [55])

	Control group ($n=11$)	Ibandronate group ($n=11$)
Mean osteolytic area ($mm^2 \pm SD$)		
day 18	4.72 ± 2.39	6.47 ± 4.00
day 26	15.78 ± 7.41[b]	11.05 ± 7.76
day 30	21.69 ± 10.57[b]	11.52 ± 9.20[a]
Mean osteolytic growth rate (mm^2 a.i. ± SD)		
day 18	4.72 ± 2.39	6.47 ± 4.00
day 26		
total group	5.01 ± 3.62	0.03 ± 3.45[a,b]
$<6\,mm^2$	5.11 ± 4.17	−0.85 ± 1.51[a]
$>6\,mm^2$	4.76 ± 2.13	1.10 ± 4.94
day 30		
total group	5.91 ± 3.95	0.47 ± 2.28[a,b]
$<6\,mm^2$	5.03 ± 3.22	−0.33 ± 2.08[a]
$>6\,mm^2$	8.28 ± 11.75	1.44 ± 2.32[a]

a.i. = analytical interval of 4 days; the measurement of <6 and $>6\,mm^2$ refers to the size of osteolytic lesions on day 18, when treatment with ibandronate was started.
[a]$p \leq 0.05$ versus control group.
[b]$p \leq 0.001$ versus day 18.

from day 18 (at which point osteolytic lesions were radiographically visible) until sacrifice at day 30 significantly inhibited growth of fully established metastases ($p < 0.05$ compared with control), and significantly reduced the mean osteolytic growth rate ($p < 0.01$) (Table 1). While growth rates were fairly constant in untreated animals, the effect of ibandronate on osteolytic growth appeared to depend on the size of the lesion at treatment initiation: lesions $< 6\,mm^2$ on day 18 had a negative growth rate on days 26 and 30, while those $> 6\,mm^2$ maintained a positive growth rate at all times (Table 1). In another experiment, ibandronate was given from 3 days before tumor inoculation until sacrifice of the animals at day 30. The proportion of animals developing lesions was substantially lower in the ibandronate group (maintained at 17%) than in the control group (95% at day 18, rising to 100% by day 30), indicating that ibandronate may be more beneficial when it is started as early as possible [55]. It is of note that in this model, osteolytic areas measured by radiography were closely correlated with metastatic areas as assessed by histology ($r = 0.82$, $p < 0.0001$) and thus radiographic analyses approximately reflected tumor burden in individual animals.

Doxorubicin is commonly used with other chemotherapy to treat breast cancer for primary care and for metastases. The use of doxorubicin alone caused a slight, but non-significant reduction in bone metastases using the intracardial MDA-231 human breast cancer cell model. When doxorubicin was combined with ibandronate, the effect was significantly greater than with either agent alone ($p < 0.05$) [45].

Myeloma models

Mice inoculated with 5TGM1 murine myeloma cells develop disease that closely mimics myeloma in humans, with monoclonal gammopathy, marrow replacement, osteolytic bone lesions, hind limb paralysis and occasional hypercalcemia. In this model, daily ibandronate significantly reduced the development of osteolytic lesions ($p \leq 0.05$) and prevented bone loss ($p \leq 0.05$) [56]. Tumor-bearing mice had a significantly stronger reduction in the mean height of the lumbar vertebrae, probably due to crush fractures, and trabecular and partial cortical bone destruction than mice given ibandronate. However, ibandronate did not affect the total tumor burden (as assessed by morphometric analyses or serum IgG2b concentrations), the percentage of bone marrow replaced by tumor cells, the growth of metastases in the spleen and liver, and hind limb paralysis or survival.

When IgGκ-secreting, human plasma cell leukemia, ARH-77 cells were injected into severe combined immunodeficient (SCID) mice, weight loss, paraplegia,

lytic bone lesions and hypercalcemia developed 25–40 days after inoculation [57]. Ibandronate was started 7 days before tumor cell inoculation (day −7), or on day 0, day 7 or day 14 after inoculation. Ibandronate given from day −7 significantly delayed paraplegia (32 days compared with 27 days for control mice, $p < 0.01$). The effect of ibandronate on formation of osteolytic lesions in mice also depended on the time when treatment began, with the most beneficial response when treatment began early rather than late: ibandronate administered from day −7, day 0 or day 7 significantly reduced the number of osteolytic lesions ($p < 0.05$), and ibandronate from day −7 and day 0 significantly reduced osteoclast-stimulatory activity compared with controls ($p < 0.05$).

Neuroblastoma model

In nude mice injected with CHLA0255 human neuroblastoma cells, ibandronate significantly delayed osteolytic lesions ($p \leq 0.02$), decreased the number of osteoclasts in the bone marrow ($p < 0.001$ compared with control) and increased bone mass [58]. Ibandronate also significantly increased apoptosis of tumor cells *in vivo*, correlating with the dose-dependent increase in apoptosis of neuroblastoma cells with ibandronate *in vitro*.

Osteosclerotic metastases

About one-third of breast carcinomas (below 30%), especially those that are estrogen receptor positive, develop osteosclerotic or mixed type (osteolytic and osteosclerotic) bone metastases. Ibandronate was tested in a MCF-7 human estrogen-dependent cell line that forms osteosclerotic bone metastases after intra-cardiac inoculation into female nude mice [5]. One group of mice was treated in the early phase after tumor inoculation for 1 week to inhibit early osteolysis, while another group was treated in the late phase during which osteosclerosis predominantly takes place. Both groups received the same total amount of ibandronate. Early treatment inhibited the development of osteosclerotic metastases, whereas late treatment failed to do so. These results suggest that inhibition of osteoclastic bone resorption reduces the subsequent development of osteosclerotic bone metastases [59]. They further suggest that ibandronate may also have therapeutic effects on osteosclerotic metastases in prostate cancer, when administered at appropriate stages of disease.

Effect on soft tissue metastases

The effect of bisphosphonates on visceral metastases needs to be established, since in the clinical situation, bisphosphonates are often used with patients who have visceral metastases as well as bone disease.

In a study specifically designed to look at the effect of ibandronate on metastases in the visceral organs, 4T1 mouse mammary tumor cells were orthotopically inoculated into the mammary fat pad of syngeneic BALB/c mice or MDA-MB-231 cells were injected intra-cardially in nude mice [45]. In the orthotopic model, lung and bone metastases developed within three weeks after inoculation, but metastases were not reproducibly observed in other organs. Although ibandronate significantly reduced the tumor burden in bone, it had no effect on metastatic burden in the lung either therapeutically (from day 18 after tumor cell inoculation) or preventatively (from day 0). When ibandronate was investigated in the intra-cardiac inoculation model without any co-medication, bone metastases were decreased, but metastases in adrenal glands were increased. However, combined treatment of ibandronate with doxorubicin more effectively suppressed both bone and adrenal metastases than both drugs alone. In particular, doxorubicin alone failed to inhibit bone metastases.

Following injection of ARH-77 cancer cells into the tail vein, Cruz *et al.* [57] found no evidence of macroscopic disease in the liver or soft tissues of the abdomen of mice bearing myeloma tumors. However, three of 12 mice given ibandronate had large tumor masses in the liver and/or attached to abdominal organs (despite absence of extensive marrow infiltration). The authors proposed that ibandronate might affect the homing of myeloma cells to the bone marrow microenvironment and favor the seeding of tumor cells in extra-osseous tissues.

Changes in visceral metastases in studies of other bisphosphonates have been inconsistent and hard to interpret. In orthotopic models, zoledronate had no effect on metastases to the lung or liver [4,5]. Although most investigators in other studies using other bisphosphonates found no significant effects, some reported either increases or decreases in soft tissue tumor burden [60–64]. Much of the variation in these findings appears to be related to the choice of animal model.

Generally, intra-cardiac or intravenous models seem inappropriate for the investigation of soft tissue metastases development, as after systemic bolus injection all cells are introduced into the arterial circulation at once, rapidly dispersing and colonizing in distant organs. Thus, these models lack the normal steps between primary tumor formation and entry of metastasizing cells into the circulation, making their relevance to the clinical situation questionable. Orthotopic tumor models that metastasize to other organs may replicate the clinical situation more closely and are therefore preferable for studying the effects of bisphosphonates on soft tissue metastases.

Renal safety of ibandronate

Besides preventing bone metastases and anti-tumor effects, preclinical studies of ibandronate have assessed

renal safety, following experimental evidence in animals or clinical observations showing that some bisphosphonates can damage the proximal convoluted tubules (PCT) to a degree that is undetectable by standard renal monitoring measures such as serum creatinine, serum urea or urinary excretion of enzymes or proteins [65–68]. When the acute renal effects of ibandronate (1 – 20 mg/kg single i.v. injection), zoledronate (1 – 10 mg/kg single i.v. injection) or clodronate (two 200 mg/kg i.p. injections on a single day) were assessed in the rat, there was tubular degeneration 4 days after dosing [69]. Although PCT degeneration and single cell necrosis were evident with all three bisphosphonates, there were qualitative differences in localization and type of lesion. Nephrotoxicity was dose dependent, but while the ratio between the lowest lethal dose and the minimum nephrotoxic dose was 25:1 for ibandronate, it was only 3.3:1 for zoledronate.

In a controlled, 25-week study of repeated dosing every three weeks in rats, there was subclinical renal damage to clinically relevant nephrotoxicity after a single dose of zoledronate (3 mg/kg), but not ibandronate (1 mg/kg) [68]. While the effect for ibandronate remained stable after repeated 3-weekly dosing, that for zoledronate worsened and even a 1 mg/kg dose that did not produce renal damage after a single administration produced borderline effects after repeated dosing. Since the risk of cumulative renal damage is related to the residual tissue concentration (i.e. the amount of bisphosphonate remaining in the kidney from the previous dose), the absence of toxic accumulation with ibandronate may be explained by its relatively short terminal tissue half-life (24 days) compared with that of zoledronate (150–200 days) [70]. Thus, intermittent doses of ibandronate every 3 weeks seem to give enough time for repair of any subclinical renal damage. There is no evidence so far that pharmacologically active doses of ibandronate produce a risk for renal damage in the clinical situation.

Ibandronate appears to be effective without adversely affecting renal function even under conditions of experimental renal failure. This has been demonstrated by a study investigating the effect of enterocystoplasty on renal bone disease in mildly uremic rats [71]. In this study, uremic rats exhibited a sustained 30% decrease in creatinine clearance following 5/6 nephrectomy. Ibandronate (10 μg/kg/day s.c.) prevented the relative decreases in bone mineral density of the tibia and femur that occurred following ileocystoplasty, without causing further deterioration in renal function. In another study, ibandronate increased bone volume and prevented bone abnormalities in rats with either normal or moderately impaired renal function while keeping the renal safety parameters in the range of controls [72].

Clinical implications: use of bisphosphonates as adjuvant therapy

Preclinical data support the use of bisphosphonate therapy, including ibandronate, in patients at risk of bone metastases. By preventing adhesion and the spread of tumor cells, as well as tumor cell invasion, bisphosphonates may stop tumor cells from becoming established in the bone [14,15,34].

Small tumors appear to be more susceptible to microenvironmental changes, such as reduction in growth factor concentrations, or to direct anti-tumor effects, suggesting that treatment should be started as soon as possible. Early preventative ibandronate therapy has been shown to be most effective against bone metastases in animal models [57,55], reducing tumor adhesion and the size of osteolytic lesions [55,57] and preserving skeletal integrity to a greater extent than delayed intervention [54]. Preventative treatment with other bisphosphonates, including pamidronate and clodronate, has also protected the skeleton against tumor-induced osteolysis in animal models [64,73,74].

Several clinical trials have assessed the role of adjuvant clodronate in primary operable breast cancer [9,75–77] or breast cancer recurring in soft tissues [78]. The results are conflicting, although there is convincing evidence that daily oral clodronate reduces the incidence of bone metastases in these patients and possibly increases survival. In two long-term follow-up studies, clodronate decreased the incidence of bone metastases, and improved survival in patients with no metastases at study entry for up to 10 years [79,80], while Saarto *et al.* [81] recently reported that overall survival rates were unaffected. A small trial assessing the benefits of adjuvant pamidronate therapy in breast cancer patients [82] found a significantly reduced incidence of bone metastases, but no effect on disease-free survival and overall survival.

It is possible that ibandronate, with its greater potency than clodronate and pamidronate, will offer enhanced protection for patients at risk of metastatic bone disease. The first positive results from phase III trials were published recently [19,21]. The renal safety profile of ibandronate in clinical use makes it particularly suitable for long-term therapy [83,84], while oral ibandronate would offer patients the option of once-daily dosing at home.

By optimizing the convenience of treatment, intermittent dosing schedules would also be of value to patients receiving bisphosphonates for metastatic bone disease or as adjuvant therapy. Intermittent dosing is viable because the total cumulative dose of bisphosphonate determines the response to treatment, independent of whether treatment is given daily or less frequently. The efficacy

of intermittent administration of subcutaneous and i.v. ibandronate has been demonstrated in animal models of post-menopausal osteoporosis, hypercalcemia and in intact animals [24], and clinical trials have since shown that both oral and i.v. ibandronate administered using extended dosing intervals are effective in normalizing bone turnover and increasing bone mineral density at all clinically relevant sites [22,23,85]. Clinical trials are required to further investigate optimal intermittent dosing schedules for adjuvant therapy in patients at risk of bone metastases from different tumor types. The absence of cumulative renal damage with intermittent dosing of ibandronate [68,70] could prove to be an important clinical benefit over alternative bisphosphonates in this indication, where patients may receive treatment for several years. Adjuvant trials of ibandronate in patients with breast cancer, multiple myeloma and prostate cancer are planned.

Preclinical evidence for improvement of implant osseointegration and prevention of osteonecrotic bone changes with ibandronate

Recent research suggests that the ability of bisphosphonates to preserve skeletal integrity can be exploited for patient benefit in other clinical settings. Uncemented total joint replacement is used in a growing number of indications, including ischemic osteonecrosis (e.g. due to polychemotherapy, corticosteroids or trauma), degenerative arthritis and osteopenia/osteoporosis. With greater mean life expectancy, the number of patients receiving artificial joints for osteoporosis and arthritis in particular is expected to grow. Emerging *in vivo* data suggests that ibandronate has a role in improving osseointegration and periprosthetic bone mineral density, essential factors in long-term joint implant stability, and in the prevention of femoral head deformity in experimental ischemic osteonecrosis.

In a rat model of experimental osteoporosis, administration of daily s.c. ibandronate (1 μg or 25 μg/kg) beginning on the day of surgery and continued for 27 days significantly increased the amount of bone attached to hydroxyapatite-coated titanium implants [86]. Bone mineral density of the lumbar vertebrae was also significantly improved ($p < 0.05$). Similar results were described in ovriectomized rats [87]. Another study by Eberhardt *et al.* [88] found that administration of a single dose of ibandronate (equivalent to 25 μg/kg × 27) was just as effective in improving osseointegration as daily application of ibandronate 25 μg/kg for 27 days. Administration of bisphosphonates as a single application would be particularly convenient for use in the clinical setting and warrants further investigation.

In a pig model for Legg–Calve–Perthes disease, femoral neck ligature produced ischemic necrosis that was accompanied by a reduction of femoral head deformity and trabecular bone mass. Both were prevented by ibandronate therapy [89].

Conclusion

The preclinical data presented here indicate that ibandronate reduces the burden of bone metastases, although the precise mechanisms of action are not fully elucidated. *In vitro* and *in vivo* data suggest that ibandronate may have a direct effect on tumor cells and may also be able to influence the complex processes of angiogenesis and tumor cell invasion. Several experiments indicate that ibandronate is most effective when treatment starts as early as possible. Adjuvant clinical studies with ibandronate are planned.

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