

# The antineoplastic role of bisphosphonates: from basic research to clinical evidence

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Received 19 December 2002; revised 7 March 2003; accepted 14 April 2003

Bisphosphonates are now well established as successful agents for the prevention and treatment of postmenopausal osteoporosis, corticosteroid-induced bone loss and Paget's disease. Bisphosphonates have also recently become important in the management of cancer-induced bone disease, and they now have a widely recognized role for patients with multiple myeloma and bone metastases secondary to breast cancer and prostate cancer. Recent studies suggest that, besides the strong antiosteoclastic activity, the efficacy of such compounds in the oncological setting could also be due also to direct antitumor effect, exerted at different levels. Here, after a brief analysis of the chemical structure, we will review the antineoplastic and biological properties of bisphosphonates. We will start from well established mechanisms of action and go on to discuss the latest evidence and hypotheses. In particular, we will review the antiresorptive properties in malignant osteolysis and the recent evidence of a direct antitumor effect. Furthermore, this review will analyze the influence of bisphosphonates on cancer growth factor release, their effect on cancer cell adhesion, invasion and viability, the proapoptotic potential on cancer cells, the antiangiogenic effect, and, finally, the immunomodulating properties of bisphosphonates on the  $\gamma\delta$  T cell population.

**Key words:** angiogenesis, antineoplastic, apoptosis, bisphosphonates, clinical and preclinical evidence

## Introduction

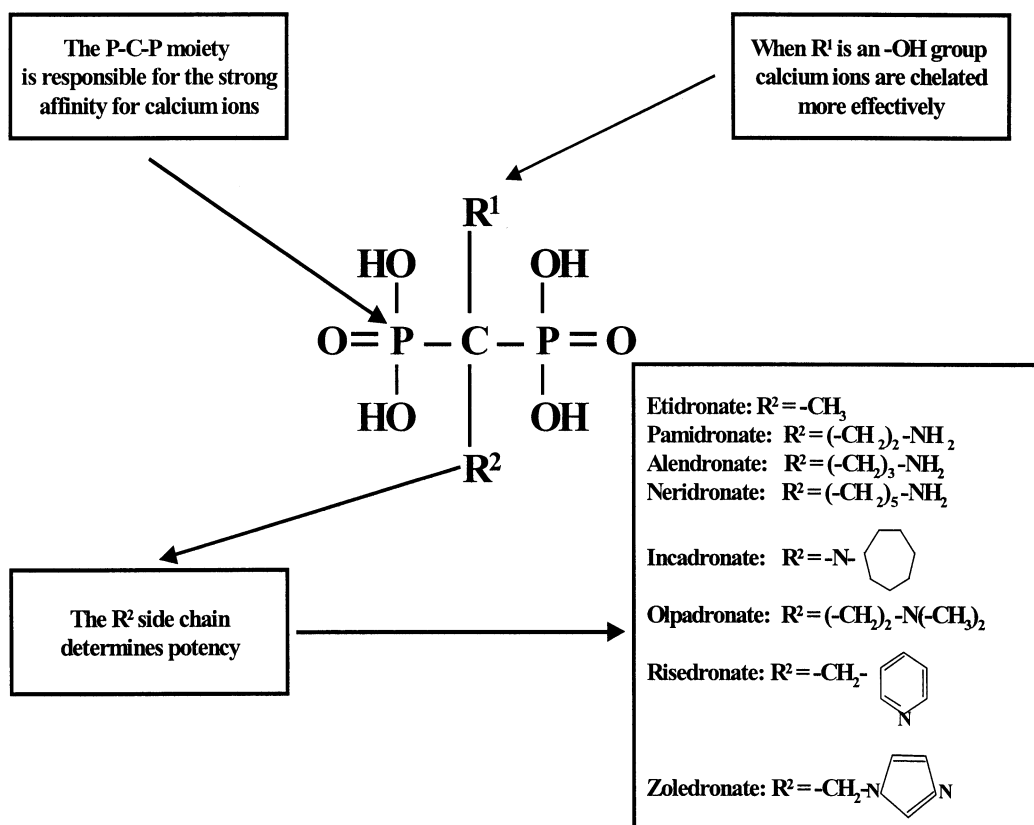
Bisphosphonates are analogs of endogenous pyrophosphates in which a carbon atom replaces the central oxygen atom. Their potential for strong inhibition of osteoclastic bone resorption has progressively extended the field of their clinical indications. Bisphosphonates are now well established as successful agents for the prevention and treatment of postmenopausal osteoporosis, since they have been shown to increase bone mass and diminish by half fracture rates at the spine, hip and other sites in postmenopausal osteoporosis [1, 2]. They are also utilized in corticosteroid-induced bone loss [3]. In Paget's disease, bisphosphonates can alleviate bone pain in the short term and prevent bone, joint, and neurological complications in the long term [4, 5]. Bisphosphonates have also recently become important in the management of cancer-induced bone disease, and they have now a widely recognized role for patients with multiple myeloma and bone metastases secondary to breast cancer [6–11]. Clinical studies have shown that, independent of the method of administration (intravenous or oral), bisphosphonates can reduce the overall amount of skeletal events in patients with myeloma and breast cancer by ~50% [7, 8]. Increased osteoclastic bone resorption is the central

mechanism underlying hypercalcemia of malignancy, and bisphosphonates have been shown to be extremely effective in the management of this disorder. In fact, bisphosphonates are the treatment of choice for this disorder [12]. Recent studies have suggested that, besides the strong antiosteoclastic activity, the efficacy of such compounds in the oncological setting could also be due to a direct antitumor effect. Here, after a brief analysis of the chemical structure, we will review the antineoplastic and biological properties of bisphosphonates, starting from well established mechanisms of action and finishing with the latest evidence and hypotheses.

## Chemical structure

Bisphosphonates are compounds with a chemical structure that closely resembles that of inorganic pyrophosphate (PP<sub>i</sub>). Whereas the two phosphate groups in PP<sub>i</sub> are linked by phosphoanhydride bonds, which are extremely unstable, the two phosphonate groups of bisphosphonates are linked to the central carbon atom by highly hydrolysis-resistant phosphoether bonds. The central carbon atom can form two additional covalent bonds, and the resulting side chains are usually indicated as R1 and R2 (Figure 1). The P-C-P moiety of bisphosphonates is responsible for their strong affinity for divalent metal ions, such as calcium ions, and for the skeleton. Furthermore, when the R1 side chain is a hydroxyl group, such compounds are able to chelate calcium ions more effectively, by

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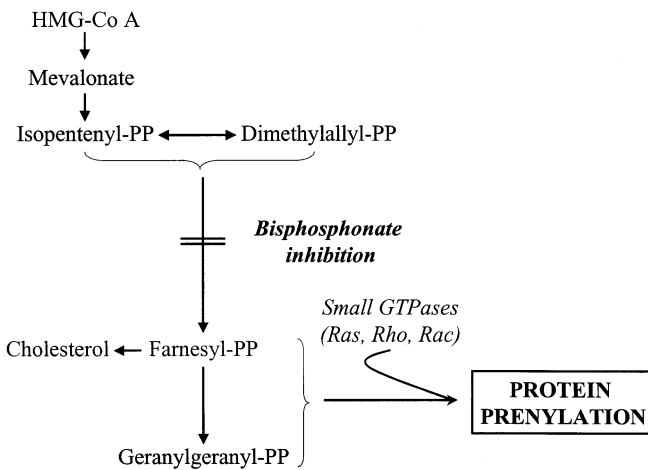
**Figure 1.** Bisphosphonate chemical structure. The two phosphonate groups are linked to the central carbon atom by phosphoester bonds. The carbon atom forms two additional covalent bonds, and the resulting side chains are referred to as R1 and R2.

tridentate rather than bidentate binding (Figure 1). Etidronate, the first bisphosphonate used to treat a human disease, was synthesized exactly 100 years ago [13]. It consists in a simple chemical structure in which the R1 side chain is a methyl group (-CH<sub>3</sub>) and the R2 side chain is a hydroxyl (-OH). When the length of the R2 side chain was increased from a simple methyl group to longer alkyl chains, significantly more potent compounds were obtained [14]. An up to 1000-fold increase in potency was achieved by the introduction of a primary amino group (-NH<sub>2</sub>) at the extremity of the R2 alkyl chain, to form the amino-bisphosphonates (e.g. alendronate, pamidronate and neridronate) [15]. Amino-bisphosphonates with a secondary amino group (e.g. incadronate) and a tertiary amino group (e.g. olpadronate) are even more effective, and potency reaches the peak when the tertiary nitrogen is included within a ring structure in the R2 side chain (as in risedronate and zoledronic acid) [16, 17] (Figure 1).

### Antiresorptive properties in malignant osteolysis

Numerous clinical studies have shown that bisphosphonates can reduce the occurrence of pathological fractures, bone pain, hypercalcemic episodes, and the need for radiation therapy and surgery in patients with osteolytic bone metastases [6–11]. Such compounds are particularly indicated in the palliative setting, for the reduced side-effects and the improvement in quality of life deriv-

ing from the reduction of skeletal events. Bisphosphonates have been clearly demonstrated to reduce tumor osteolysis and bone destruction and to prolong survival in animal tumor models [18–20]. Such efficacy is first of all related to the inhibitory activity on osteoclast resorption. Bisphosphonates affect osteoclast-mediated bone resorption in a variety of ways, which include effects on osteoclast formation, resorptive activity and viability [21–23]. Osteoclasts are the bone cells most likely to be exposed to high concentrations of drug; experimental studies support the hypothesis that they are able to internalize bisphosphonates by endocytosis [24]. After cellular uptake, bisphosphonate-treated osteoclasts show important changes in morphology, both *in vitro* [23, 25] and *in vivo* [26–28]. These include the lack of the ruffled border [29], the disruption of cytoskeleton and the loss of actin rings [30, 31]. These structural alterations lead to a decreased osteoclast function and are by themselves sufficient to prevent bone resorption [32]. Furthermore, it has been reported that bisphosphonates can induce osteoclast apoptosis, both *in vitro* and *in vivo* [22, 33]. Another mechanism by which bisphosphonates exert their antiresorptive activity is the inhibition of osteoclast differentiation. In fact, such compounds have been shown to inhibit the formation of osteoclast-like cells in a dose-dependent manner in long-term cultures of human bone marrow [21]. These results suggest that the anti-proliferative activity on osteoclasts could play a major role in bisphosphonate efficacy. Finally, it has been shown that bisphosphonates may also act through the modulation of the osteoclast-



**Figure 2.** Bisphosphonate level of action on the mevalonate pathway. Bisphosphonates inhibit the formation of farnesyl diphosphate (PP) and geranylgeranyl-PP, which are required for the prenylation of small GTPases that regulate a variety of important cell processes. HMG-Co A, 3-hydroxy-3-methylglutaryl-Co A; GTPases, guanosine triphosphatases.

osteoblast interrelations. When pure osteoblastic cell populations were pretreated with alendronate and ibandronate and then co-cultured with osteoclasts, a decrease in osteoclast-mediated resorption was observed [34]. It was subsequently shown that bisphosphonate-treated osteoblasts produce an inhibitor of osteoclast resorption, which has not yet been well characterized but is known to be a labile factor of ~3–4 kDa [35].

### Molecular level of action

The molecular events by which bisphosphonates lead to osteoclast inactivation and diminish osteoclast maturation are not fully understood. To date, there is no evidence of active receptors or binding sites on the osteoclast membrane. However, it is well known that bisphosphonates can be divided in two different classes (amino-bisphosphonates and non-amino-bisphosphonates), not only because of the molecular structures (as discussed above), but also the intracellular mechanism of action. Early studies suggested that non-amino-bisphosphonates, such as etidronate and clodronate, can affect a large number of metabolic processes, including glycolysis, fatty acid oxidation and lactate production [36, 37]. Non-amino-bisphosphonates can be metabolized to methylene-containing analogs of ATP, which are extremely resistant to hydrolysis [38, 39]. These metabolic reactions are likely to be catalyzed by members of the family of type 2 class of aminoacyl-tRNA synthetases [40], which play a pivotal role in protein synthesis. Since osteoclasts are able to internalize bisphosphonates, consequently formed non-hydrolyzable ATP analogs can accumulate intracellularly and inhibit metabolic enzymes, such as phosphatases and pyrophosphatases [41, 42], which play important roles in cellular growth, differentiation and activity. On the other hand, the more potent amino-bisphosphonates (such as alendronate, risedronate, pamidronate, ibandronate and zoledronic acid) are not metabolized to ATP analogs, and show a completely different mechanism of action. These compounds are able to inhibit the mevalonate pathway [43, 44] (Figure 2), a bio-

synthetic pathway on which depends the synthesis of cholesterol and isoprenoid lipids, such as isopentenyl diphosphate (IPP), farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP). FPP and GGPP are required for the prenylation of small GTPases, such as Ras, Rho and Rac [45], which are signaling proteins that regulate a variety of cellular processes. The lack of GTPase prenylation, derived from inhibition of the mevalonate pathway, is responsible for their inadequate function [46]. The function of these GTPases has been shown to be important for osteoclast morphology and activity [47]. Therefore, inhibiting farnesyl pyrophosphate synthase or other enzymes of the mevalonate pathway [43, 44], amino-bisphosphonates can deprive osteoclasts of important regulators of intracellular dynamics, leading to poor cell functioning or programmed cell death. This mechanism of action is confirmed by the ability of geranylgeraniol (a cell-permeable form of GGPP) and, to a lesser extent, farnesol (a cell-permeable form of FPP) to protect osteoclasts from the inhibitory properties of amino-bisphosphonates [48, 49].

### Direct antitumor effects

Bisphosphonate efficacy for the treatment of bone metastases was initially thought to depend only on the antiosteoclast activity of such compounds. Antiresorptive properties were considered to be sufficient by themselves to explain their ability to reduce skeletal morbidity in patients with lytic bone disease. Bisphosphonates were shown to inhibit establishment and growth of osteoblastic bone metastases from prostate cancer; the efficacy was attributed to the observation that abnormal osteoblastic bone formation within metastases is preceded by osteoclastic activation [50]. However, several lines of evidence suggesting a direct antitumor effect of bisphosphonates have progressively accumulated. Sasaki et al. [51] demonstrated a reduced tumor burden in nude mice pretreated with risedronate and then injected with human breast carcinoma cells. In the same animal model, risedronate was shown to also reduce the number, extent and size of bone metastases when given after tumor cell inoculation [52]. In humans, trials with adjuvant clodronate in primary breast carcinoma reported conflicting results. Diel et al. [9] described a reduction in bone and, surprisingly, visceral metastases, and an improvement in survival in patients with bone marrow disseminated tumor cells. On the other hand, Saarto et al. [53] reported an increase in the incidence of bone and visceral metastases and a poorer survival in node-positive breast cancer patients. Even though further investigation of bisphosphonates in the adjuvant setting is still required, such observations led to the suggestion that bisphosphonates might have some kind of direct effect on cancer cells. Currently, several studies are being published with the aim of clarifying the mechanisms by which this effect is achieved.

### Effect on cancer growth factor release

It was postulated previously that bisphosphonates could have an antitumor activity by altering the release of growth factors in the bone microenvironment. Although calcified bone matrix shows relatively low cellularity and metabolic activity, it stores many different osteoblast-derived growth factors [54]. These growth

**Table 1.** Experimental evidence of bisphosphonate's direct antitumor activity

Reference	Compound(s)	Model	Effect(s)
Sasaki et al. [51]	Risedronate	Nude mice injected with a human breast carcinoma cell line	Delay and reduction of skeletal metastases
Van der Pluijm et al. [64]	Pamidronate, olpadronate, alendronate, ibandronate	Human breast carcinoma cell line cultures	Inhibition of tumor cell adhesion and spreading to bone
Boissier et al. [63]	Pamidronate, clodronate, ibandronate, NE-10244	Human prostate and breast carcinoma cell line cultures	Inhibition of tumor cell adhesion to unmineralized and mineralized bone extracellular matrices
Shipman et al. [73]	Incadronate	Human multiple myeloma cell line cultures	Induction of apoptosis
Magnetto et al. [68]	Ibandronate	Human breast carcinoma cell line cultures	Enhancement of taxoid antitumor activity against tumor cell invasion and adhesion to bone
Senaratne et al. [66]	Zoledronic acid, pamidronate, clodronate, EB 1053	Human breast carcinoma cell line cultures	Decrease of tumor cell number and viability, and induction of apoptosis
Fromigue et al. 2000 [72]	Zoledronic acid, pamidronate, clodronate, ibandronate	Human breast carcinoma cell line cultures	Induction of apoptosis and necrosis
Boissier et al. [65]	Zoledronic acid, clodronate, ibandronate, NE-10244	Human prostate and breast carcinoma cell line cultures	Inhibition of tumor cell invasion
Kunzmann et al. [87]	Pamidronate, alendronate, ibandronate	Human peripheral blood mononuclear cell cultures	Increase in the count of $\gamma\delta$ T cells
	Pamidronate	Human peripheral blood $\gamma\delta$ T cell cultures	Activation against lymphoma and myeloma cell lines
	Pamidronate	Bone marrow cultures of multiple myeloma patients	Reduction of plasma cell survival
Wood et al. [80]	Zoledronic acid	Human endothelial cell cultures	Inhibition of proliferation, and modulation of adhesion and migration
		Cultured aortic rings and chicken egg chorioallantoic membrane assay	Reduction of vessel sprouting
		Murine growth factor implant model	Reduction of blood content and weight of tissue growing around the implant
Jadjev et al. [70]	Zoledronic acid	Human breast cancer cell line cultures	Induction of apoptosis even by short-term exposure and synergic effect with paclitaxel
Lee et al. [78]	Pamidronate	Human prostate carcinoma cell lines	Induction of apoptosis
	Zoledronic acid		Dramatic reduction of tumor cell proliferation
Mackie et al. [76]	Zoledronic acid, pamidronate	Rat osteosarcoma cell line cultures	Inhibition of tumor cell proliferation and induction of apoptosis
Riebeling et al. [77]	Pamidronate	Human melanoma cell line cultures	Inhibition of tumor cell growth and induction of apoptosis
Fournier et al. [81]	Zoledronic acid	Prostate gland in castrated rats	<i>In vivo</i> antiangiogenic properties, inhibition of testosterone-induced revascularization of the prostate gland

factors, such as transforming growth factor- $\beta$  (TGF- $\beta$ ) and insulin-like growth factor-I (IGF-I), are released into the bone marrow as a consequence of osteoblast resorption, and represent the essential nutrients for cancer cells localized in bone [55]. Furthermore, although still controversial, it has been shown that cancer cells are unable to degrade bone matrix by themselves [56], meaning that the release of such essential nutrients is strictly osteoclast resorption related. Moreover, cultured human breast cancer cells respond to TGF- $\beta$  by releasing PTHrP, a major stimulator of osteoclast activity in breast cancer osteolysis [57, 58]. Thus, there is a vicious circle, where osteoclasts and cancer cells interact through the mediation of several soluble factors in the bone microenvironment. Bisphosphonates may interrupt this cycle by decreasing

osteoclast activity, thereby inhibiting the release of TGF- $\beta$ , IGF-I and other peptides from bone matrix. Cancer cells result deprived of essential nutrients and reduce the release of osteoclast stimulating factors.

### Effect on cancer cell adhesion, invasion and viability

As reviewed elsewhere [59], the process leading to bone metastases involves cancer cell migration, adhesion to cortical bone and finally invasion of extracellular bone matrix. It has been shown previously that extracellular matrix-bound bisphosphonates may inhibit the adhesion of osteoclast precursors and inhibit their subsequent differentiation into mature resorbing osteoclasts [60, 61]. Other studies have suggested that such compounds may

**Table 2.** Evidence of bisphosphonate antitumor activity in cancer patients

Reference	Compound(s)	Model	Effect(s)
Diel et al. [9]	Adjuvant clodronate	Breast carcinoma patients with disseminated tumor cells in the bone marrow	Reduction in osseous and visceral metastases, and improvement in survival
Kunzmann et al. [88]	Pamidronate	Patients with acute-phase reaction after first infusion	Increase in the count of $\gamma\delta$ T cells
Santini et al. [82]	Pamidronate	Bone metastatic cancer patients	Reduction of VEGF circulating levels in humans
Powles et al. [92]	Adjuvant clodronate	Breast cancer patients	Reduction in skeletal metastases
Saad et al. [93]	Zoledronic acid	Prostate cancer patients	Reduction in skeletal-related events

VEGF, vascular endothelial growth factor.

also inhibit the attachment of mature osteoclasts to cortical bone slices and to specific extracellular matrix proteins, such as bone sialoprotein-derived peptide [62]. Recently, a similar effect was observed on cancer cell lines. Bisphosphonate pretreatment of prostate and breast carcinoma cell lines has been shown to inhibit tumor cell adhesion to unmineralized and mineralized bone extracellular matrices in a dose-dependent way [63]. Van der Pluijm et al. [64] showed that pretreatment of bovine cortical bone slices and cryostat sections of trabecular bone from neonatal mouse tail with amino-bisphosphonates (pamidronate, olpadronate, alendronate and ibandronate) can inhibit the adhesion of breast cancer cells in a dose-dependent manner. In contrast, no effect was obtained with the non-amino-bisphosphonates clodronate and etidronate [64]. The order of potency of the four amino-bisphosphonates corresponded to their ranking in bone resorption assays. Moreover, Boissier et al. [65] demonstrated that bisphosphonate pretreatment inhibits breast and prostate carcinoma cell invasion in a dose-dependent manner. Although no activity was observed on cancer cell matrix metalloproteinases (MMPs) production, bisphosphonates were shown to inhibit MMP activity through zinc chelation [65]. Senaratne et al. [66] investigated the *in vitro* effects of bisphosphonates zoledronic acid, pamidronate, clodronate and EB 1053 on growth, viability and induction of apoptosis in three human breast cancer cell lines. All four bisphosphonates significantly reduced cell viability in all three cell lines. In addition, in different murine models bisphosphonate markedly inhibited the progression of established osteolytic lesions and the expansion of breast and prostate cancer cells within bone [67]. Finally, Magnetto et al. [68] reported the ability of ibandronate to enhance the antitumor activity of taxoids against breast cancer cell invasion and adhesion to bone.

### Proapoptotic effect on cancer cells

Recently, numerous studies have shown that bisphosphonates can induce apoptosis in *in vitro* models. These results are consistent with earlier reports that bisphosphonates can inhibit cell proliferation and induce apoptosis in osteoclasts [33] and in J774 macrophage-like cells [69]. In the work previously mentioned by Senaratne et al. [66], the investigators demonstrated that all the bisphosphonates studied decreased breast cancer cell number and viability, but also induced apoptosis in a dose-dependent manner. This study has shown that there is no equivalence between

bisphosphonate potency in bone resorption and in the induction of apoptosis: zoledronic acid was the most effective compound. The proapoptotic efficacy of zoledronic acid on breast cancer cells was confirmed in two subsequent studies [70, 71]. In the first, Jadjev et al. [70] showed that acute exposure to zoledronic acid (2, 6 and 12 h), more accurately reflecting the *in vivo* condition than long-term exposure (72 h), was sufficient to determine an antitumor effect in breast cancer cells. A synergic action with paclitaxel was also observed. Moreover, zoledronic acid-induced apoptosis was inhibited by the addition of intermediates of the mevalonate pathway (completely inhibited by geranylgeraniol and partially by farnesol), suggesting that amino-bisphosphonate activity on breast cancer cells is strictly related to the inhibition of enzymes of the same pathway [70]. The second study aimed to identify the signaling pathways involved in zoledronic acid-induced apoptosis [71]. Zoledronate treatment was shown to induce the failure of Ras protein membrane localization, the release of mitochondrial cytochrome *c* into the cytosol and the subsequent activation of caspase-3 proteases [71]. Such events were inhibited by the addition of farnesol, and by forced expression of bcl-2. The authors suggested that the reduced Ras protein prenylation, with impaired membrane localization and functioning, represents the initial event inducing apoptosis [71]. This apoptosis is associated with the release of cytochrome *c* into the cytosol and the subsequent activation of the caspase cascade. Fromiguet et al. [72] demonstrated that the apoptosis of breast cancer cells induced by clodronate, pamidronate, ibandronate and zoledronic acid was almost completely reversed by the z-VAD-fmk caspase inhibitor. This suggests a role of caspase activation in bisphosphonate-induced apoptosis. When analyzing non-amino-bisphosphonate-induced apoptosis, mechanisms other than the lack of Ras-protein prenylation need to be considered. In fact, caspase activation should be induced by toxic ATP analogs accumulating intracellularly. Bisphosphonate activity was also observed in other cancer cell lines. Shipman et al. [73] demonstrated that the amino-bisphosphonate incadronate may induce apoptosis of human multiple myeloma cells *in vitro*. This effect is mediated by the inhibition of the mevalonate pathway [74], and is completely abrogated by forced expression of bcl-2 [75]. Moreover, pamidronate and clodronate were shown to inhibit cell proliferation and to induce apoptosis in the UMR 106-01 clonal rat osteosarcoma cell line in a dose- and time-dependent fashion [76]. Recently, Riebeling et al. [77] reported that pamidronate can induce apoptosis and inhibit

proliferation of human melanoma cells *in vitro* in a dose-dependent manner. In contrast, clodronate did not show any effect on the same melanoma cell line. Apoptosis was associated with caspase-3 activation and was strongly reduced by the addition of geranylgeraniol to the culture medium. On the other hand, p53 or bcl-2 overexpression did not abolish pamidronate-induced apoptosis [77]. Finally, Lee et al. [78] reported that pamidronate and zoledronic acid significantly reduce the growth of prostate cancer cell lines *in vitro*. Pamidronate treatment was shown to induce significant amounts of cell death, while only zoledronic acid exerted a dramatic effect on cell proliferation [78].

### Antiangiogenic effect

Recent evidence suggests that part of the antitumor activity of bisphosphonates may be attributed to an antiangiogenic effect. The studies by Wood et al. [79] with the amino-bisphosphonate zoledronate are the most important in describing this effect. The authors reported that the cell proliferation induced by fetal calf serum, basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) is inhibited by zoledronic acid on human umbilical vein endothelial cells (HUVEC) *in vitro*. Zoledronic acid inhibition on HUVEC migration was dependent on the dose administered. Zoledronic acid was also shown by the same investigators to reduce vessel sprouting in cultured aortic rings and in the chicken egg chorioallantoic membrane assay. Moreover, in a subcutaneous growth factor implant model in mice, zoledronate treatment strongly inhibited the angiogenic response induced by bFGF and VEGF [80]. The INSERM research group has recently demonstrated that zoledronic acid clearly inhibits the angiogenesis both in bone and in prostate tissues in a murine model [81]. Finally, for the first time in humans, our research group showed a significant decrease of circulating levels of VEGF in bone metastatic cancer patients receiving a single dose of pamidronate [82]. A significant decrease of VEGF was already evident the first day after single pamidronate infusion (90 mg), and the effect was still present on day 7. A shorter-lasting increase of interferon (IFN)- $\gamma$  circulating levels was also observed, while no significant modifications in interleukin (IL)-8 levels were found. With these encouraging results, further investigations on the antiangiogenic effects of bisphosphonates both *in vitro* and *in vivo* are urgently needed.

### Effect on $\gamma\delta$ T cell activation and proliferation

$\gamma\delta$  T cells represent a minor subset of human peripheral T cells (1–10%), differing from  $\alpha\beta$  T cells in that they have a limited combinatorial diversity of T-cell receptor (TCR) and an human leukocyte antigen-unrestricted antigen recognition site. In adults, most of these  $\gamma\delta$  T cells present a V $\gamma$ 9/V $\delta$ 2 TCR [83], and are able to recognize a broad spectrum of non-peptide compounds of low molecular weight (100–600 Da) with an essential phosphate residue.  $\gamma\delta$  T cells are suggested to play a surveillance role for infected and transformed cells [84], and they have already been shown to recognize and lyse certain hematopoietic tumor cells (such as the Burkitt's lymphoma cell line Daudi, and myeloma cell lines PPM1 8226 and U266) *in vitro* [85–87]. Kunzmann et al.

[87, 88] demonstrated for the first time that amino-bisphosphonates are potent activators of human  $\gamma\delta$  T cells both *in vitro* and *in vivo*. In the first study, the amino-bisphosphonates alendronate, ibandronate and pamidronate have been shown to induce a dose-dependent activation and expansion of  $\gamma\delta$  T cells in primary peripheral blood mononuclear cell cultures of healthy donors at clinically relevant concentrations, while non-amino-bisphosphonates clodronate and etidronate were demonstrated to be inactive [87]. The activation of  $\gamma\delta$  T cells was associated with CD25 and CD69 expression and increased secretion of IFN- $\gamma$ , while  $\gamma\delta$  T cell proliferation was obtained only when low doses of exogenous IL-2 were added to the culture medium. In the same study, pamidronate-treated bone marrow cultures of patients with multiple myeloma showed a reduced survival of plasma cells [87]. Such reduction in survival was observed particularly when the bone marrow  $\gamma\delta$  T cells were activated, suggesting that pamidronate can induce a  $\gamma\delta$  T cell activity against the plasma cells through a direct cell contact-dependent lysis or by the secretion of inhibitory cytokines such as IFN- $\gamma$  [89]. *In vivo*, pamidronate treatment has been shown to significantly increase the number of peripheral blood  $\gamma\delta$  T cells [90]. Amino-bisphosphonate activity on  $\gamma\delta$  T cells (V $\gamma$ 9/V $\delta$ 2 subset) has been demonstrated to be strictly dependent on the presence of monocyte lineage cells [90]. It was suggested that monocytes act as antigen presenting cells, presenting amino-bisphosphonates to  $\gamma\delta$  T cells, and then determining their activation [90]. In conclusion, the amino-bisphosphonates activate the  $\gamma\delta$  T cell population, which shows potential cytotoxic activity toward a broad spectrum of tumors [89–91]. This represents another intriguing aspect of the antitumor activity of such compounds, which deserves further investigation.

### Conclusions

The literature clearly shows the potential and impressive antineoplastic properties of bisphosphonates (Tables 1 and 2), and sheds new light on the biological applications of such compounds in the clinical setting. Bisphosphonates interact with osteoclasts, osteoblasts, tumor cells, cytokine and growth-factor production, leading to the interruption of bone destruction. For these reasons they may represent a new class of drug with antitumor power.

Several ongoing preclinical trials are evaluating the possibility of associating zoledronic acid with: (i) tyrosine kinase inhibitors, such as imatinib; (ii) aromatase inhibitors, such as letrozole; and (iii) antineoplastic agents, such as paclitaxel or docetaxel, with the aim of obtaining a synergic effect in experimental models.

Other fields of research concern the immunomodulating properties of bisphosphonates on  $\gamma\delta$  T cells, their proapoptotic and antiangiogenic potentials and their use as radiation sensitizers. All these findings indicate that it is possible to extend the potential use of bisphosphonates, and in particular of zoledronic acid, to other diseases that involve angiogenesis or an immunological component.

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