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Direct and indirect antitumor activity of zoledronic acid

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Zoledronic acid (Zol) is the most potent aminobisphosphonate currently available to treat bone disease in cancer patients.[1] Zol specifically targets the mevalonate (MVA) pathway of osteclasts precursors and mature osteoclasts. By inhibiting the farnesyl pyrophosphate (FPP) synthase, Zol prevents the generation of FPP and geranylgeranylpyrophosphate (GGPP) that are essential compounds to prenylate proteins like Ras and Rho among others.[2] The accumulation of unprenylated proteins in osteoclast precursors and mature osteclasts prevents their differentiation and activation and ultimately leads to cell death by apoptosis. By acting upstream on the same pathway targeted by farnesyl transferase (FTase) and geranylgeranyltransferase (GGTase) inhibitors (FTI, GGTI), Zol can be considered as a drug impacting on farnesylation-dependent survival and differentiation pathways.

Unlike conventional FTI, Zol is not used to directly inhibit cancer cell growth, but it is used in supportive care to prevent or treat bone disease. Zol is effective and well tolerated, provided that appropriate precautions are taken and clinical and laboratory monitoring is performed to prevent side effects like renal toxicity or osteonecrosis of the jaw (ONJ). Accumulating evidences, however, indicate that Zol may have direct and indirect antitumor activity as well. Zol can exert direct cytotoxicity against a variety of tumor cell lines, including leukemia, and myeloma cell lines.[3,4] The antitumor activity of Zol as single agent *in vitro* is observed at doses in the range of 50-100 μM which cannot be achieved *in vivo* by conventional doses when Zol is administered as anti-osteoclastic agent (4 mg every 3-4 weeks). However, Zol enhances the cytotoxic activity of conventional drugs,[3] steroids,[4] and tyrosine kinase inhibitors like Gleevec.[5] Timing is probably crucial to fully exploit the antitumor activity of Zol in association with cytotoxic drugs. Using clinically relevant concentrations of Zol and Doxorubucin (Dox), synergistic effects on apoptosis was achieved in cancer cell lines only when Dox was used first and Zol followed 24 hours later.[6] Replacing Zol with non nitrogen-containing bisphosphonates (nBPs) like clodronate did not induce increased apoptosis. As expected, induction of apoptosis was mainly via inhibition of the MVA pathway, as addition of the MVA pathway intermediary geranylgeraniol inhibited the induction of apoptosis by Dox followed by Zol. Given the specific effect of Zol on the MVA pathway, it is worth of investigation whether Zol can have synergistic activity with other agents targeting the farnesylation-dependent survival pathways. Indeed, Caraglia *et al.*, have recently shown that pamidronate or Zol synergistically enhance the growth inhibition and apoptosis of epidermoid cancer cells induced by the FTI R115777 (tipifarnib).[7] The synergistic activity between aminobisphosphonates (nBPs) and tipifarnib allowed to produce tumor cell growth inhibition and apoptosis at *in vivo* achievable concentrations (0.1 μM).

Several mice models have shown that Zol has antitumor activity *in vivo*.[5,8,9] Accumulating evidence indicates that the *in vivo* antitumor activity of Zol is not only mediated by its direct effect on tumor cells, but it is also due to indirect effects mediated by stromal cells located in the tumor bed. In particular, Zol has activity on endothelial cells and macrophages that are important contributors to tumor cell survival and disease progression through different mchanisms.[8-12] The role of tumor microenvironment as a cofactor of tumor progression is very well recognized in Multiple Myeloma (MM) which has become a reference disease for translational research and the development of innovative treatment paradigms. MM is a plasma cell neoplasia that remains fatal despite the extensive use of autologous transplantation. A better understanding of the mechanisms involved in plasma cell survival and growth has led to the development of novel agents targeting the tumor microenvironement

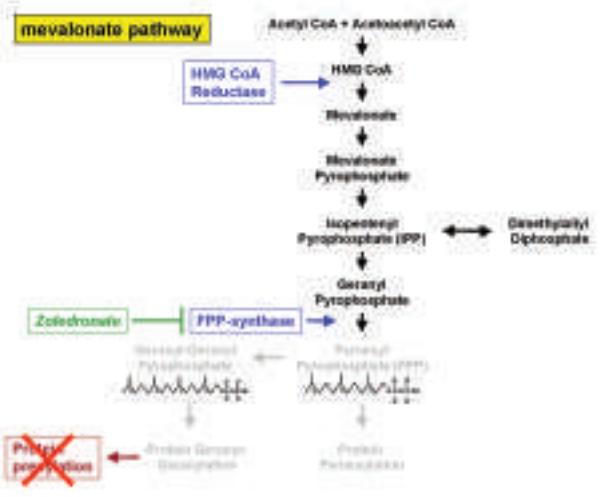

Figure 1.

beside tumor cells.[13] It is well established that myeloma cells closely interact with many other cell types located in the bone marrow as well as with the extracellular matrix. All these interactions activate signaling pathways in both myeloma cells and stromal cells leading to the paracrine and autocrine production of growth factors, activation of survival and antiapoptotic genes, enhanced expression of adhesion molecules, enhanced transducing activity of surface receptors. and increased drug resistance. Activation of osteclasts and neoangiogenesis are tumor-related events that contribute to the pathogenesis of the disease.

Zol has antiangiogenic properties *in vitro* and *in vivo*. *In vitro*, Zol inhibits the proliferation of human endothelial cells stimulated with fetal calf serum FCS), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF), and modulates endothelial cell adhesion and migration.[10,11] When administered systemically to mice, Zol potently inhibits the angiogenesis in several mice models, including the 5T2MM model of myeloma,[8] In the latter, Zol decreased paraprotein concentration, decreased tumor burden, reduced angiogenesis, and prolonged survival compared with controls. A possible mechanism of the antiangiogenic properties of Zol is the ability to inhibit MMP-9, a proangiogenic protease involved in the mobilization of VEGF.[9] MMP9 is expressed in tumor cells or tumor-associated macrophages, concomitantly with the angiogenic switch. In the course of a prevention trial, Zol inhibited angiogenesis and limited the progression of premalignant precursors to invasive carcinomas of the cervix in a mouse model involving the human papillomavirus type-16 oncogenes. These mice develop cervical cancers by lesional stages analogous to those in humans, suggesting that Zol is worth of investigation in an adjuvant setting or in premalignant states. Indeed, a trial has been proposed by the Italian Working Party on MM to determine whether Zol can prevent or delay the progression of smoldering myeloma to symptomatic disease.

Beside its ability to target tumor cells, endothelial cells, and tumor associated macrophages, Zol has immunomodulatory properties.[14] nBPs like Pam and Zol share the chemical features of nonpeptide com-

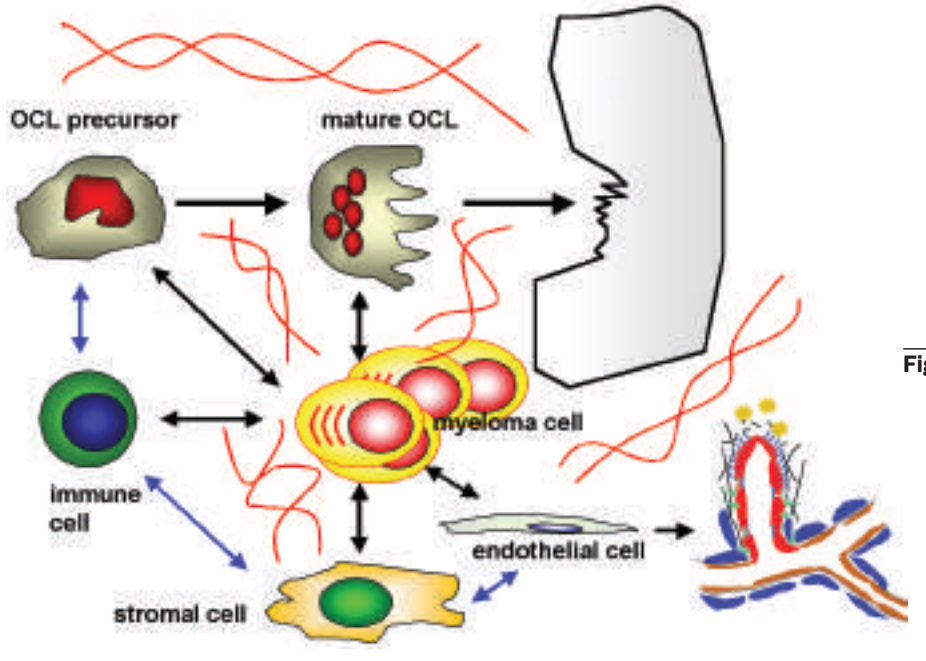

Figure 2.

pounds naturally recognized by Vγ9/Vδ2 T cells. These cells recognize families of unprocessed nonpeptide compounds of low molecular weight (100-600 d) with conserved patterns, including microbial metabolites such as pyrophosphomonoesters and alkyl amines. Certain hemopoietic tumor cell lines, such as the Daudi Burkitt's lymphoma and the RPMI 8226 myeloma line are also recognized and lysed by Vγ9/Vδ2 T cells. We have recently shown that Zol can activate Vγ9/Vδ2 T cells and induce antitumor activity in MM patients.[15] Notably, immunomodulation is observed at significantly lower concentrations than those required for its antiangiogenic, antimetalloproteinase or proapoptotic activity. We have also found that Zol sentisizes MM cell lines and primary myeloma cells to the cytotoxic activity of Vγ9/Vδ2 T cells. These effects are mediated by the MVA pathway, since Zol-treated tumor cells accumulate phosphorylated mevalonate metabolites that are similar to the phosphorylated nonpeptidic ligands of Vγ9/Vδ2 T cells. This accumulation is due to the increased expression in tumor cells of hydroxy-methylglutaryl-CoA reductase (HMGR), the rate-limiting enzyme in the MVA pathway, and the concomitant FPP inhibition by Zol. Mouse models have confirmed that the antitumor activity of Zol *in vivo* is partly mediated by Vγ9/Vδ2 T cells[16,17] and studies are under progress in patients with hematological malignancies in which Vγ9/Vδ2 T cells are stimulated with nPBs and IL-2 to induce antitumor activity.[18] We have recently carried out a tumor vaccination study in MM with encouraging results. However, the immune system of MM patients, who were vaccinated in first remission after autologous transplantation, was too much deteriorated by treatment to be efficiently activated by the vaccines. In particular, conventional T cells (i.e., T cells with α/β TCR) were functionally impaired and showed limited TCR diversity. Since it is possible to boost adaptive immunity by stimulating Vγ9/Vδ2 T cells, which are better preserved after transplantation, Zol is currently under investigation as a tool to increase the baseline levels of immune responsiveness in MM patients and make them more receptive to tumor-specific vaccination. In conclusion, Zol has some unique properties which can be exploited in the multimodality treatment paradigms which are increasingly used to treat MM patients and other cancer patients. Thalidomide, lenalidomide, bortezomib and a variety of new drugs are under investigation inMM patients. The ability to interfere with tumor-host interactions gives an added value to these drugs.[19] Even better clinical achievements can be obtained when the new drugs are associated with conventional drugs like alkylating agents or Dox. Zol has the capacity to target stromal cells in MM and other cancers and it can easily be combined with cytotoxic drugs and the new drugs. Recently, FTI have also been shown to have activity in MM. Tipifarnib as single agent has *in vitro* and *in vivo* activity against myeloma cells[20,21] and a recent study by Zhu et al. has shown that tipifarnib can synergistically be combined with paclixatel or docetaxel to inhibit the growth of myeloma cells *in vitro* and in the SCID-hu bone model of myeloma growth,[22] These data provide the basis for combination therapy clinical trials in MM patients and other cancer patients targeting all the components involved in tumor cell survival and growth. Zol is worth of investigation in these combination trials due to its antitumor, antiangiogenic, and immunomodulatory properties.

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