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Unconventional properties of zoledronic acid

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Zoledronic acid (Zol) is the most potent aminobisphosphonate (ABP) currently available to treat bone disease in cancer patients.[1] Zol specifically targets the mevalonate (MEV) 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.

Accumulating evidence, however, indicates that targeting the MEV pathway in cells other than osteoclasts confers unique properties to Zol. In particular, targeting the MEV pathway of antigen-presenting cells, such as peripheral blood monocytes, yields to the accumulation of phosphorylated MEV metabolites that activate Vγ9/Vδ2 T cells.[3,4] The latter are unconventional T cells with effector and regulatory functions. They account for 5–10% of $CD3^+$ peripheral blood T cells and recognize families of unprocessed nonpeptide compounds of low molecular weight (100-600 Da) with conserved patterns, including natural phosphoesters of microbial origin that are similar to the phosphorylated metabolites of the MEV pathway. Vγ9/Vδ2 T cells also recognize induced self ligands like stress-inducible MHC class I-related MICA/MICB molecules, and/or other ligands that are recognized through the Vγ9/Vδ2 TCR or through coreceptors like the NK receptor member D of the lectin-like receptor family (NKG2D).[5,6] The ability to recognize self-induced ligands is the basis of their prominent role in tumor surveillance and rejection, since these ligands are not expressed by most normal cells, but are upregulated on transformed, infected or stressed cells

Recent data indicate that induced self and foreign pattern recognitions of Vγ9/Vδ2 T cells, like other innate immune players, not only directly induce effector responses of innate immunity, but are also important to initiate and enhance antigen-specific immune responses mediated by conventional T cells and antibodies,[7] as shown in Figure 1.

We have recently shown that Zol can activate Vγ9/Vδ2 T cells in MM and CLL patients by targeting the MEV pathway of autologous monocytes.[4] Upon incubation for 7 days with Zol and low doses IL-2, Vγ9/Vγ2 T cells release large quantities of IFN-gamma and exert cytotoxic activity against autologous tumor cells. Since DC are more potent than monocytes in activating adaptive and innate immunity, we have investigated whether targeting the MEV pathway in DC may further improve their immunodulatory properties and ability to activate Vγ9/Vγ2 T cells. To this end, we have generated highly purified immature (iDC) and mature DC (mDC) from peripheral blood monocytes and exposed to short-term incubation with low doses of Zol. We have shown that Zol does not affect the viability or endocytic ability of iDC and mDC, but rather improves their immunostimulatory phenotype by increasing the expression of costimulatory molecules like CD80 and CD86. On a per cell basis, Zol-treated iDC stimulated better than monocytes the activation and proliferation of autologous Vγ9/Vδ2 T cells. More importantly, Zol treatment improved the ability of iDC, pulsed with influenza-derived peptides, to induce antigen-specific cytotoxic responses against HLA-A2 restricted influenza peptide-loaded T2 cells. Thus, these data indicate that short term DC incubation with Zol improves their ability to activate both innate and adaptive immunity, paving the way to future studies investigating the role of Zol as an immunoadjuvant in the antitumor vaccination setting. This strategy is currently under *in vivo* investigation in a transgenic NPM-ALK mouse model. The aim of these studies is to determine whether immunization of mice with DNA vaccines coding for the appropriate NPM-ALK sequences in the presence

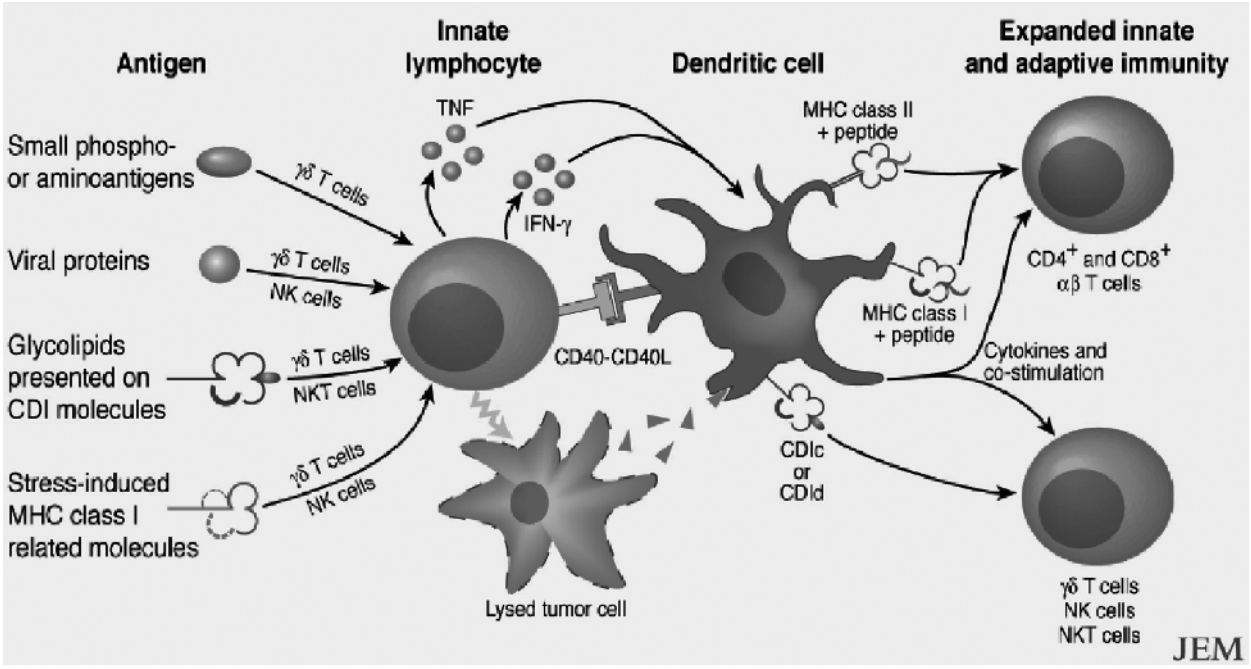

Figure 1.

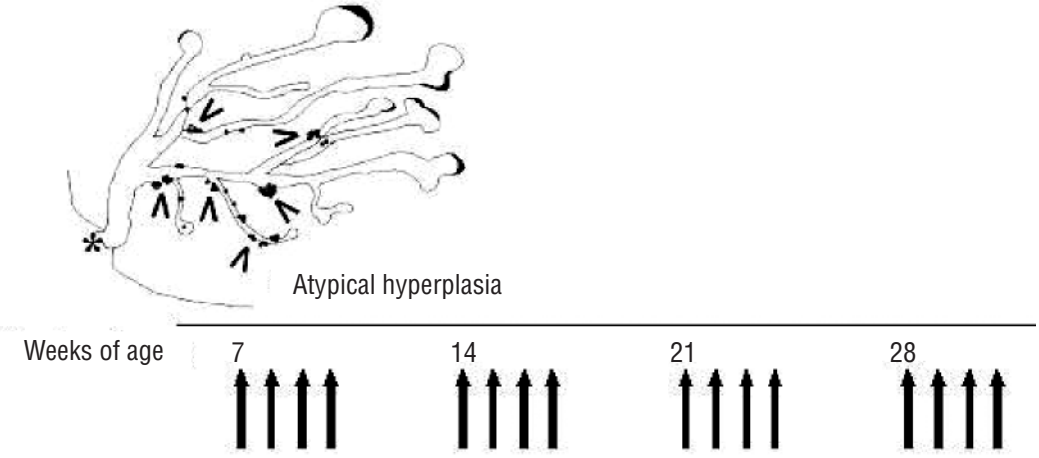

Figure 2.

of Zol can prevent or delay the spontaneous generation of myeloma and lymphoma.

Zol has also been shown to have antiangiogenic properties *in vitro* and *in vivo* and to exert direct antitumor activity *in vitro* against a variety of tumor cell lines. However, it is very unlikely that the *in vivo* antitumor activity observed in mice models is due to a direct effect on tumor cells, since the latter is observed *in vitro* at concentrations hardly achievable *in vivo*. On the contrary, the *in vivo* antitumor activity is more likely to be dependent on the immunomodulatory and antiangiogenic properties of Zol that are induced at very much lower concentrations. 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.

We have recently shown that Zol has the ability to impair spontaneous mammary carcinogenesis in a transgenic BALB-neuT mouse model. The step-wise pattern of mammary tumor progression in these mice closely mimics that of breast carcinoma in women, thus providing a realistic model for assessing the efficacy of Zol against early stages of tumorigenesis.[10] BALB-neuT mice were treated with 16 administration of 100 μg/Kg of Zol divided into four courses of a single weekly injections for four weeks followed by a three weeks rest. Zol administration was started when mice were 7 weeks old and therefore when all the 10 mammary glands display a widespread atypical hyperplasia. Zol was administered intravenously (i.v.) or into the mammary pad (i.mam.), as shown in Figure 2.

While these experiments are still currently ongoing and under evaluation, preliminary data indicate that a significant tumor growth impairment does occur in Zol-treated mice. Zol is as effective as IL-12 in impairing the mammary carcinogenesis, further supporting the immunomodulatory and antiangiogenic properties as key factors in Zol antitumor activity.

In conclusion, Zol is endowed with unconventional properties targeting all the components involved in tumor cell survival and growth. Thus, Zol is worth of investigation in combination therapy clinical trials due to its antitumor, antiangiogenic, and immunomodulatory properties.

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