Zoledronic acid impairs mammary carcinogenesis in BALB/c mice transgenic for the Her-2/neu oncogene

Zoledronic acid (Zol) is the most potent aminobisphosphonate clinically available. Preclinical in vivo data suggest that it modulates the development of bone disease, decreases tumor burden with a direct anti-cancer activity, and reduces the migration and the metastatic invasion of cancer cells. Zol efficacy in anti-cancer adjuvant therapy also rests on its anti-angiogenic properties and ability to expand γ/δ T cells, both in vitro and in vivo. The published data thus far available concern murine models of transplanted tumors. However, a recent report shows that Zol impairs tumor-associated angiogenesis in a transgenic model of cervical carcinoma.

Present work was made to assess Zol ability to impair spontaneous mammary carcinogenesis in a transgenic mouse model. Virgin BALB/c female mice transgenic for the activated rat Her-2/neu oncogene (BALB-neuT mice) provide one of the most aggressive and consistent model of autochthonous mammary carcinogenesis. 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 progressive stages of the mammary carcinogenesis. In 4-week-old BALB-neuT mice epithelial nodular neoformations (side buds) stemming from the main and secondary mammary ducts are becoming evident around the nipple. Histologically, these buds are the foci of atypical hyperplasia in carcinomatous progression. A thick network of microvessels expressing αvβ3 integrin associated with these lesions shows a close correlation between the increased epithelial cell proliferation of hyperplastic lesions and the activation of angiogenesis. At 8 weeks, most side buds have progressed to in situ carcinomas, while an increasing number of hyperplastic foci are evident all over the gland. Between the 10th and the 20th week, the in situ carcinomas become invasive and metastasize to the bone marrow and lungs. By week 18 mammary tumors become palpable. This progression is similar in all the mammary glands. The cells of the hyperplastic, neoplastic and metastatic lesions constantly highly over express Her-2/neu protein product (p185neu) in both their cytoplasm and their membrane. The inexorability of the development of a palpable mammary tumor by week 15 by all BALB-neuT mice allows an assessment of the protection afforded as the extension of the disease-free survival and the percentage of tumor-free mice as time progresses. Moreover, as a tumor is palpable in all ten mammary gland around week 33, the tumor multiplicity can also be assessed and the increase in the size of each lesion can be measured. The gene expression signatures of carcinomas progressing in BALB-neuT mice are also similar to those of human Her-2/neu positive tumors.

Previous data have shown that the early stages of this aggressive neoplastic progression are hampered by the stimulation of innate immunity following α-galactosylceramide15 and IL-12 administration. A strong anti-tumor protection is also provided when adaptive immunity is elicited by anti-p185neu DNA vaccines.

To assess Zol efficacy, BALB-neuT mice were treated with 16 administrations of 100 µg/Kg of Zol divided into four courses of a single weekly injections for four weeks followed by a three week rest. Zol administration was started when mice were 7 weeks old and therefore when all the 10 mammary glands display a widespread atypical hyperplasia (Figure 1). Zol was administered intravenously (i.v.) or into the mammary pad (i.mam.). While these experiments are still currently ongoing and under evaluation, a few provisional data can be reported. Mice are evaluated for: 1) tumor onset, 2) tumor multiplicity and 3) overall survival. Preliminary data show that a similar significant tumor growth impairment was evident in mice
receiving Zol administered i.v. or i.m. In both cases, Zol was as effective as IL-12 in impairing the mammary carcinogenesis, thus suggesting a possible immuno-mediated mechanism in addition to its direct anti-tumor and anti-angiogenic effects. We are currently investigating the mechanism of Zol mediated inhibition of mammary carcinogenesis, focusing on Zol capacity to modulate the innate and adaptive anti-tumor immune response of BALB-neuT mice.

References


