[haematologica reports]
2006;2(3):37-38

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

COSCIA M[1]
QUAGLINO E[2]
CURCIO C[2]
PANTALEONI F[1]
FORNI G[2]
MASSAIA M[1]

[1]Division of Hematology, Department of Medicine and Experimental Oncology, Laboratory of Hematology Oncology, Center for Experimental Research and Medical Studies, San Giovanni Battista Hospital, University of Turin, Torino, Italy;
[2]Department of Clinical and Biological Sciences, S. Luigi Gonzaga Hospital, University of Turin, Orbassano, Turin

Zoledronic acids (Zol) is the most potent aminobisphosphonate clinically available.[1] Preclinical *in vivo* data suggest that it modulates the development of bone disease, decreases tumor burden[2] with a direct anti-cancer activity,[3–5] and reduces the migration and the metastatic invasion of cancer cells.[6] Zol efficacy in anti-cancer adjuvant therapy also rests on its anti-angiogenic properties[2,7] and ability to expand gamma/delta T cells, both *in vitro* and *in vivo*.[8–10] The published data thus far available concern murine models of transplanted tumors.[2,4] However, a recent report shows that Zol impairs tumor-associated angiogenesis in a transgenic model of cervical carcinoma.[7]

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.[11] 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.[12] Histologically, these buds are the foci of atypical hyperplasia in carcinomatous progression. A thick network of microvessels expressing $\alpha v\beta 3$ 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 (p185^{neu}) in both their cytoplasm and their membrane.[11,13] 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.[14]

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[16,17] administration. A strong anti-tumor protection is also provided when adaptive immunity is elicited by anti-p185neu DNA vaccines.[18,19]

To asses 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

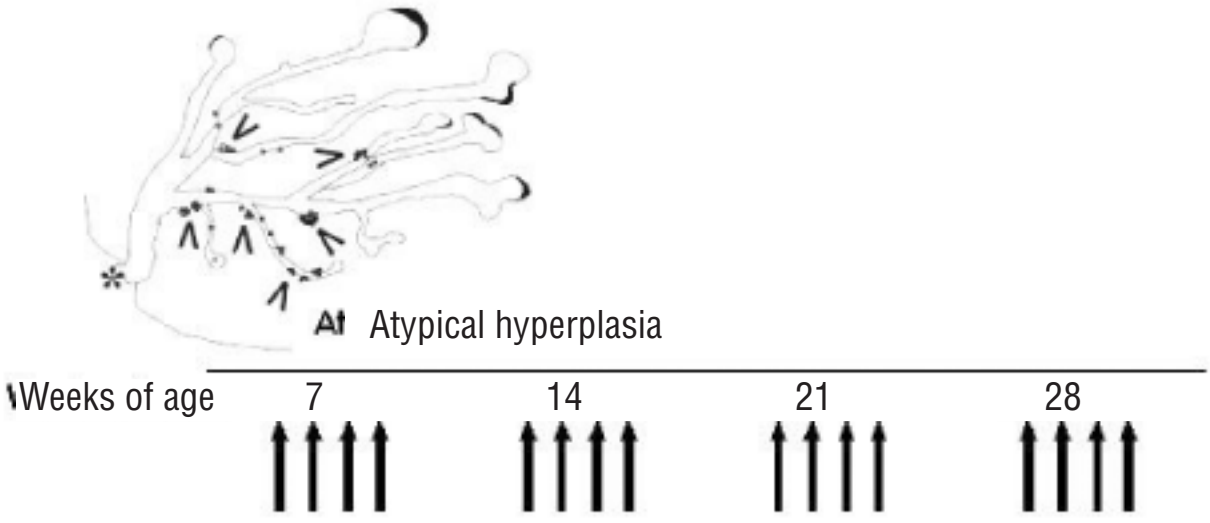

Figure 1. Zol treatment schedule. 100 µg/Kg of Zol were injected i.v. or i.mam. at the indicated time points (black arrows). The arrows shows when mice received 100 µg/Kg Zol i.v. or i.mam. Zol administration started when all the 10 mammary glands already display a widespread atypical hyperplasia.

receiving Zol administered i.v. or i.mam. 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

1. Rosen LS, Gordon D, Kaminski M, Howell A, Belch A, Mackey J, et al. Long-term efficacy and safety of zoledronic acid compared with pamidronate disodium in the treatment of skeletal complications in patients with advanced multiple myeloma or breast carcinoma: a randomized, double-blind, multicenter, comparative trial. Cancer 2003, 98:1735-44.
2. Croucher PI, De Henrik L, Perry MJ, Hijzen A, Shipman CM, Lippitt J, et al. Zoledronic acid treatment of 5T2MM-bearing mice inhibits the development of myeoma bone disease: evidence for decreased osteolisys, tumor burden and angiogenesis, and increased surival. J Bone Miner Res 2003;18 :482-92
3. Yaccoby S, Pearse RN, Jhonson CL, Barlogie B, Choi Y, Epstein J. Myeloma ineracts with the bone marrow microenvironment to induce osteoclastogenesis and is dependent on osteoclast activity. Br j of Heamatol 2002;116:278-90.
4. Kuroda J, Kimura S, Segawa H, Kobayashi Y, Yoshikawa T, Urasaki Y, et al. The third-generation bisphosphonate zoledronate synergistically augments the anti-Ph^+ leukemia activity of imatinib mesylate. Blood 2003;102:2229-35.
5. Kabelitz D, Wesch D, Pitters E, Zoller M. Characterization of tumor reactivity of human V gamma 9V delta 2 gamma delta T cells *in vitro* and in SCID mice *in vivo*. J Immunol 2004, 173:6767-76.
6. Hiraga T, Williams PJ, Ueda A, Tamura D, Yoneda T. Zoledronic acid inhibits visceral metastases in the 4T1/luc mouse breast cancer model. Clin Cancer Res 2004;0:4559-67.
7. Giraudo E, Inoue M, Hanahan D. An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. J Clin Invest 2004;114:623-33.
8. Kunzmann V, Bauer E, Feurle J, Weissinger F, Tony HP, Wilhelm M. Stimulation of gammadelta T cells by aminobisphosphonates and induction of antiplasma cell activity in multiple myeloma. Blood 2000, 96:384-92
9. Dieli F, Gebbia N, Poccia F, Caccamo N, Montesano C, Fulfaro F, et al. Induction of gammadelta T-lymphocyte effector functions by bisphosphonate zoledronic acid in cancer patients *in vivo*. Blood 2003;102:2310-1.
10. Mariani S, Muraro M, Pantaleoni F, Fiore F, Nuschak B, Peola S, et al. Effector gammadelta T cells and tumor cells as immune targets of zoledronic acid in multiple myeloma. Leukemia 2005;19:664-70.
11. Boggio K, Nicoletti G, Di Carlo E, Cavallo F, Landuzzi L, Melani C et al. Interleukin-12 mediated prevention of spontaneous mammary adenocarcinomas in two lines of Her-2/neu transgenic mice. J Exp Med 1998;188: 589-96.
12. Di Carlo E, Diodoro MG, Boggio K, Modesti A, Modesti M, Nanni P et al. Analysis of mammary carcinoma onset and progression in Her-2/neu oncogene transgenic mice reveals a lobular origin. Lab Invest 1999; 79:1261-69.
13. Pannellini T, Forni G, Musiani P. Immunobiology of Her-2/neu transgenic mice. Breast Dis 2004;20:33-42.
14. Astolfi A, Landuzzi L, Nicoletti G, De Giovanni C, Croci S, Paladini A, et al. Gene expression analysis of immune-mediated arrest of tumorigenesis in a transgenic mouse model of HER-2/neu-positive basal-like mammary carcinoma. Am J Pathol 2005;166:1205-16.
15. Hayakawa Y, Rovero S, Forni G, Smyth M. α–Galactosylceramide (KRN7000) suppression of chemical- and oncogene-dependent carcinogenesis. Proc Natl Acad Sci USA 2003;100:9464-69.
16. Boggio K, Di Carlo E, Rovero S, Cavallo F, Quaglino E, Lollini P-L et al. Ability of systemic IL-12 to hamper progressive stages of mammary carcinogenesis in Her2/neu transgenic mice. Cancer Res 2000;60:359-64.
17. Cifaldi L, Quaglino E, Di Carlo E, Musiani P, Spadaro M, Lollini P-L et al. A light, nontoxic IL-12 protocol inhibits Her2/neu mammary carcinogenesis in BALB/c transgenic mice with established hyperplasia. Cancer Res 2001;61:2809-12.
18. Quaglino E, Rolla S, Iezzi M, Spadaro M, Musiani P, De Giovanni C et al. Concordant morphological and gene expression data shows that vaccination halts Her-2/neu preneoplastic lesions. J Clin Invest 2004;113:709-17.
19. Quaglino E, Iezzi M, Mastini C, Amici A, Pericle F, Di Carlo E et al. Electroporated DNA vaccine clears away multifocal mammary carcinomas in Her-2/neu transgenic mice. Cancer Res 2004;64:2858-64.