

Common adjuvant breast cancer therapies do not inhibit cancer vaccine induced T cell immunity

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Received: 24 November 2007 / Accepted: 16 January 2008
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Abstracts Cancer vaccines may have the most potential for clinical impact when used in the adjuvant setting when tumor burden is at its lowest. Application of cancer vaccines in the adjuvant setting, however, requires integration of immunization with more standard cytotoxic or cytostatic therapies. Common adjuvant therapies for breast cancer patients, i.e. trastuzumab, bisphosphonates and hormonal agents are often administered over several years requiring concurrent administration of these drugs with active immunization. We questioned whether these common adjuvant therapies would impact a patient's ability to develop tumor specific immunity with vaccination. Immune parameters from 36 subjects were evaluated. We determined these adjuvant therapies have no impact on the ability to develop an immune response specific for HER-2/neu peptides ($P > 0.1$) nor do they have an impact on the magnitude of T cell immunity developed with concurrent vaccination ($P > 0.1$). This is the first report to show that the use of trastuzumab, bisphosphonates and hormonal therapy concurrent with cancer vaccine administration have no impact on either the generation or the magnitude of vaccine induced immunity.

Keywords HER-2/neu · vaccine · T cell · Lymphocyte · Adjuvant · Immunity · Bisphosphonates · Tamoxifen · Aromatase inhibitors · Trastuzumab

Abbreviations

APC	Antigen presenting cell
DC	Dendritic cell
NK	Natural killer cell
S.I.	Stimulation Index

Introduction

Breast cancer is ideally suited for immunomodulation in the minimal residual disease state [1] as breast cancer is immunogenic [2] and time to relapse can take place over years allowing for the generation of an adaptive immune response [3]. Eliciting cancer specific immunity may be more clinically effective when tumor burden is at its lowest [1]. Large tumors are associated with the elaboration of T regulatory cells and secretion of specific cytokines, such as TGF-beta and IL-6, that are associated with immune suppression [4]. For this reason, immune based cancer therapies, such as cancer vaccines, are being administered in the adjuvant setting concurrent with or directly following standard cytoreductive therapies that have served to debulk the tumor. As many common adjuvant therapies are administered chronically, over several years, breast cancer vaccines must be delivered at the same time as these agents, however, the impact of adjuvant treatments on a patient's ability to develop tumor specific immunity with active immunization is unknown.

We questioned whether concurrent administration of a HER-2/neu vaccine with common adjuvant therapies used in the treatment of breast cancer; trastuzumab, bisphosphonates, and hormonal agents impacted the ability of advanced stage breast cancer patients to develop T cell immunity to components of a HER-2/neu peptide vaccine. Both the incidence as well as the magnitude of tumor

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specific immunity induced by vaccination was evaluated in patients who were and were not currently receiving these agents. Data suggest concurrent administration of common adjuvant breast cancer therapies with a tumor antigen specific vaccine did not limit the development of immunity.

Methods and materials

Patient population

Immune parameter data is presented on 34 subjects, 17 subjects received a HER-2/neu peptide based vaccine in the context of a phase I trial [5] and 19 subjects received the same vaccine while receiving concurrent trastuzumab monotherapy in the context of a Phase I/II trial [6]. Data was analyzed based on the subject's usage of trastuzumab, bisphosphonates and hormonal therapies. All subjects were treated on protocols approved by the University of Washington Human Subjects Division and the United States Food and Drug Administration. All subjects were required at entry to have stage III or IV breast cancer with no evidence of or stable disease, to have been off and be able to remain off chemotherapy or other immune modulatory therapies for a minimum of 30 days before enrollment, and to have had adequate hematopoietic, hepatic and renal function. Enrollment on the trial of vaccination concurrent with trastuzumab was limited to patients with stage IV disease [6]. All subjects analyzed received a minimum of 3 vaccines, which is sufficient to generate an immune response in the majority of subjects [7]. Based on receiving or not receiving trastuzumab, bisphosphonates and hormonal therapies, the proportion of subjects receiving 3–5 vs 6 vaccines was not significantly different ($P = 0.695, 0.648, 1.000$, respectively), the mean age was not significantly different ($P = 0.808, 0.378, 0.448$), and time from last chemotherapy administration was not significantly different ($P = 0.153, 0.499, 0.051$). Based on receiving or

not receiving trastuzumab, bisphosphonates and hormonal therapies a significant difference in stage is found based on trastuzumab usage ($P = 0.016$); there is no significant difference based on usage of bisphosphonates and hormonal therapies ($P = 0.302, 0.628$). As previously stated, the difference in stage dependent on trastuzumab usage is due to that trial being restricted to enroll only stage IV patients [6] (Table 1).

Evaluation of complete blood count

Complete blood counts were performed by the Research Testing Services, a division of the Department of Laboratory Medicine, at the University of Washington Medical Center. The Department maintains a licensure with the College of American Pathologists. Blood was drawn at baseline, at each vaccination visit, and one month following the final vaccination. The average value over the course of active immunization was used for analysis. At time of entry to study, mean leukocyte count was not statistically different dependent on use of trastuzumab, bisphosphonates and/or hormonal therapies ($P = 0.587, 0.725, 0.257$) nor was lymphocyte count ($P = 0.850, 0.709, 0.916$).

Detection of peripheral blood T cell responses

HER-2/neu-specific T cell responses were assessed by tritiated thymidine incorporation as previously described [8]. Antigens used in the assay consisted of peptides 13–15 amino acids in length which are designed to stimulate Class II or CD4⁺ T cell responses. The maximal T response achieved was used for evaluation. Results were reported as a standard stimulation index (S.I.), which is defined as the mean of all 24 experimental wells divided by the mean of the control wells (no antigen). An S.I. >2 was considered evidence of a positive immunized response based on analysis of a reference population [5]. If subjects had an S.I.

Table 1 Patient population

	Trastuzumab		Bisphosphonates		Hormonal Rx		
	Yes	No	Yes	No	Yes	No	
Subjects	19	17	9	27	14	22	
Mean Age, years (Range)	52 (34–76)	53 (40–86)	51 (43–60)	53 (34–86)	51 (34–63)	53 (40–86)	
Number Vaccines Received	3–5	5	3	1	7	3	5
	6	14	14	8	20	11	17
Months since chemotherapy	7	13	7	11	16	6	
Stage III/IV	0/19	5/12	0/9	5/22	1/13	4/18	

>2 at baseline, i.e. pre-existent immunity to HER-2/neu [9], a post-vaccination response was defined as positive if it was a minimum of 2 times baseline. Subjects were considered to have developed an immune response if they had a positive evaluation to at least one peptide in the immunizing mixture.

Statistical methods

Subjects were divided into groups based on the use of trastuzumab, bisphosphonates and hormonal therapies where hormonal therapies included tamoxifen and aromatase inhibitors. The differences in continuous variables between treatment groups were evaluated by a Mann-Whitney test or an unpaired *t*-test with Welch's correction where appropriate. The differences in categorical variables between groups were evaluated by the Fisher's exact test. All tests were 2-sided. Multivariate analysis of HER-2/neu specific maximal S.I. was performed using multiple regression, and comparison of maximal S.I. between groups receiving 3–5 or 6 vaccinations was performed by unpaired *t*-test. All analysis was executed using GraphPad Prism 3.02 (GraphPad Software, San Diego California) except the multivariate analysis which was performed using SPSS 13.0 (SPSS, Chicago Illinois).

Results

Common adjuvant breast cancer therapies have minimal impact on immune cell levels

The administration of trastuzumab, bisphosphonates and/or hormonal therapies did not have an impact on total white blood cell counts. Figure 1(a) shows the average white blood cell count for each individual over the course of the study while receiving or not receiving trastuzumab, bisphosphonates and hormonal therapies. The average white blood cell count was 4.88 ± 0.22 and 4.94 ± 0.48 for those receiving and not receiving trastuzumab, respectively ($P = 0.904$); 4.21 ± 0.32 and 5.14 ± 0.31 for those receiving and not receiving bisphosphonates ($P = 0.112$); and 4.72 ± 0.26 and 5.03 ± 0.38 for those receiving and not receiving hormonal therapy ($P = 0.496$). Trastuzumab and hormonal therapies did not have an impact on lymphocyte count. Figure 1(b) shows the average lymphocyte count for individuals currently receiving or not receiving trastuzumab, bisphosphonates and/or hormonal therapies. The average lymphocyte count was 1.26 ± 0.10 and 1.54 ± 0.22 for those receiving and not receiving trastuzumab ($P = 0.24$) and 1.38 ± 0.12

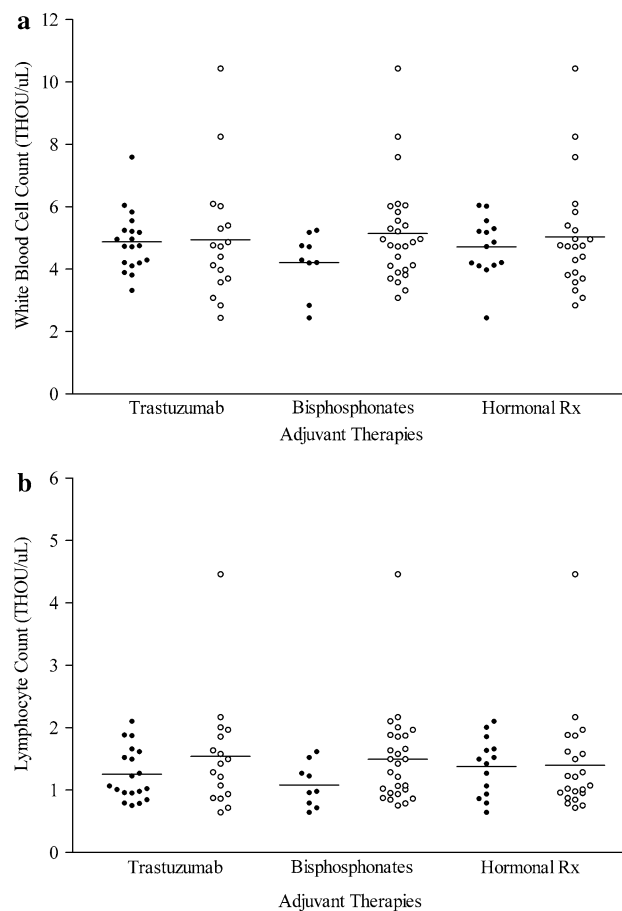


Fig. 1 Common adjuvant breast cancer therapies have minimal impact on immune cell levels. **(a)** The average white blood cell count over the course of active vaccination is plotted for each of the 36 subjects receiving (●) or not receiving (○) trastuzumab, bisphosphonates and/or hormonal therapies. The solid line represents the mean of each population. **(b)** The average lymphocyte count over the course of active vaccination is plotted for each of the 36 subjects receiving (●) or not receiving (○) trastuzumab, bisphosphonates and/or hormonal therapies. The solid line represents the mean of each population

and 1.40 ± 0.17 for those receiving and not receiving hormonal therapy ($P = 0.925$). Only bisphosphonate therapy had a significant impact on lymphocyte count at 1.08 ± 0.12 and 1.49 ± 0.18 for those receiving and not receiving bisphosphonates ($P = 0.033$). The subjects receiving and not receiving bisphosphonate therapy were similar in age ($P = 0.378$), stage ($P = 0.302$) and time from chemotherapy ($P = 0.370$) at entry to study.

The average neutrophil, monocyte, eosinophil and basophil counts for individuals currently receiving or not receiving trastuzumab, bisphosphonates and/or hormonal therapies were also evaluated. Trastuzumab use did not have a significant impact on neutrophil, monocyte, eosinophil or basophil counts ($P = 0.434, 0.181, 0.507, 0.500$, respectively). Bisphosphonate use did not have a significant impact

on neutrophil, monocyte, eosinophil or basophil counts ($P = 0.191, 0.489, 0.912, 0.644$, respectively). Hormonal therapy did not have a significant impact on neutrophil, monocyte, eosinophil or basophil counts ($P = 0.716, 0.105, 0.131, 0.051$, respectively).

Common adjuvant breast cancer therapies have no impact on the ability to develop an immune response specific for HER-2/neu peptides

Figure 2 depicts the percentage of subjects who demonstrated an immune response to HER-2/neu peptides within one month after the final vaccination. Subjects receiving and not receiving trastuzumab developed a HER-2/neu specific immune response during active vaccination 79% and 88% of the time ($P = 0.662$). Subjects receiving and not receiving bisphosphonates developed a HER-2/neu specific immune response during active vaccination 88% and 82% of the time ($P = 1.000$). Subjects receiving and not receiving hormonal therapy developed a HER-2/neu specific immune response during active vaccination 93% and 77% of the time ($P = 0.371$).

Common adjuvant breast cancer therapies have no impact on the magnitude of HER-2/neu peptide specific T cell immunity developed during active immunization

The maximum S.I. obtained during active vaccination categorized by trastuzumab, bisphosphonate and hormonal therapy is shown in Fig. 3. The average S.I. is 5.17 ± 0.97 and 7.38 ± 1.95 for subjects receiving and not receiving

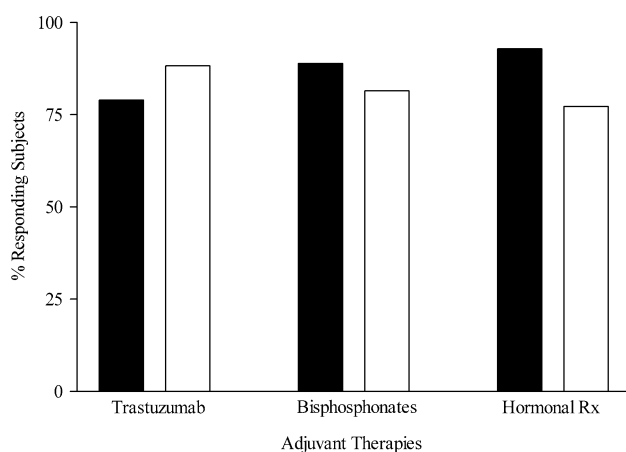


Fig. 2 Common adjuvant breast cancer therapies have no impact on the ability to develop an immune response specific for HER-2/neu peptides. The percentage of subjects who developed HER-2/neu specific T cell immunity is categorized by those receiving (■) or not receiving (□) trastuzumab, bisphosphonates and/or hormonal therapies

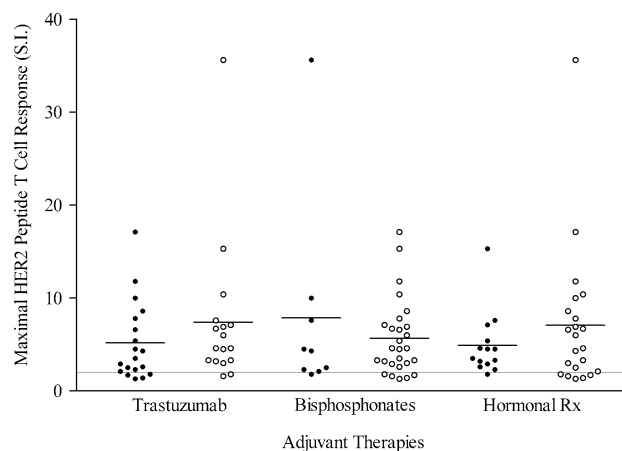


Fig. 3 Common adjuvant breast cancer therapies have no impact on the magnitude of HER-2/neu peptide specific T cell immunity developed during active immunization. The maximum S.I. to the HER-2/neu peptide is plotted for each of the 36 subjects receiving (●) or not receiving (○) trastuzumab, bisphosphonates and hormonal therapies. The solid line represents the mean of each population. The dotted line depicts S.I. of 2.0

trastuzumab respectively ($P = 0.319$); 7.86 ± 3.59 and 5.67 ± 0.79 for subjects receiving and not receiving bisphosphonates ($P = 0.568$); and 4.90 ± 0.92 and 7.05 ± 1.61 for subjects receiving and not receiving hormonal therapy ($P = 0.256$). Even when the analysis was performed only for subjects completing all 6 vaccines the maximum S.I. was not significantly different between groups ($P = 0.279, 0.842, 0.219$). Multivariate analysis demonstrates treatment did not significantly predict that maximum S.I. ($P = 0.299$).

Discussion

Data presented here are the first to demonstrate that the administration of trastuzumab, bisphosphonates, or hormonal therapy, concurrent with a cancer vaccine, has minimal to no impact on the generation of a tumor antigen specific immune response. Furthermore, the magnitude of immunity achieved during active immunization is not impacted by concurrent administration of these agents. An understanding of how concurrent standard therapies impact the immunologic efficacy of cancer vaccines is particularly important as vaccines are increasingly being evaluated in the adjuvant setting.

It has been demonstrated that cytotoxic chemotherapy can have an adverse impact on the development of immunity after administration of a vaccine. Most classic chemotherapy agents have been shown to induce non-selective cell death by interfering with DNA, RNA and protein synthesis resulting in myelosuppression [10]. After

the cessation of chemotherapy, the lymphocyte population is first reconstituted by B and NK cells, which may return to baseline in as soon as 3 months after treatment. T cells return to baseline levels approximately 6 months after the completion of chemotherapy. Even then the CD45RA⁺ CD4⁺ helper-naïve cells remain decreased and only recover to 90% of baseline at 12 months [11]. Qualitative abnormalities may persist in T cells for 1 to 2 years after discontinuation of therapy [12]. Studies have shown that chemotherapy can down regulate costimulatory molecules on dendritic cells, such as CD80, CD86 and ICAM-1, inhibiting T cell activation [13]. These abnormalities are clinically evident by the increased infection rate after the completion of chemotherapy by organisms such as varicella zoster and herpes simplex virus [12]. All these factors may prevent successful immunization.

We questioned whether non-cytotoxic adjuvant treatments such as trastuzumab, bisphosphonates and/or hormonal therapy influence the development of an immune response after a HER-2/neu peptide based vaccine. These adjuvant therapies, though mild in toxicity, do have side effects which may impact the ability to mount an immune response after vaccination. Trastuzumab is a fully humanized anti-HER-2/neu monoclonal antibody that binds to the extracellular domain of the HER-2/neu protein [14]. Although depressed blood counts have been reported after the use of the drug, trastuzumab therapy causes less leukopenia, neutropenia and lymphopenia than standard cytotoxic chemotherapy [15]. Data presented here demonstrates trastuzumab therapy does not affect the generation or the magnitude of a HER-2/neu peptide specific T cell response after vaccination.

Bisphosphonates may also impact the development of immunity. There is evidence that bisphosphonate use may alter the function of myeloid cells. Zoledronic acid has been shown to inhibit TNF-alpha production by monocytes, dendritic cells, and macrophages; phagocytosis by macrophages and immature dendritic cells; and effect the differentiation of monocytes into dendritic cells [16]. Furthermore, dendritic cell maturation markers CD83, the costimulatory molecules CD80 and CD86 and DC-SIGN, could be down regulated with increasing concentration of zoledronic acid [17]. These bisphosphonate induced alterations could lead to the functional impairment of cytotoxic T cells. Despite the decrease in lymphocytes seen in our subject population receiving bisphosphonates, the generation of immunity and the magnitude of the response was the same in subjects receiving and not receiving this class of agents.

Estrogen has been found to promote the differentiation of dendritic cells from bone marrow precursors and alteration of systemic estrogen levels in vivo affects the number and function of antigen presenting cells. Estrogen receptor

antagonists may inhibit dendritic cell differentiation, and tamoxifen, when present during a 7-day culture period, could reduce the number of differentiated dendritic cells [18]. Indeed, estrogen receptor antagonists have been used clinically to modulate pro- and anti-inflammatory cytokines in both mice and women with breast cancer [19, 20]. Estrogen receptor antagonists decreased autoimmune symptoms in a murine model of systemic lupus erythematosus and in two women who had autoimmune dermatomyositis [21, 22]. The immune effect seen in our subject population indicates the ability to respond to a HER-2/neu peptide based vaccine remains intact after hormonal modulation.

Despite the known detrimental effects of adjuvant breast cancer therapies on the immune system, emerging data suggests there may be immune stimulatory effects as well. Subjects pretreated with cyclophosphamide have been shown to have a two to four fold higher titer of antibody response to a Muc-1 vaccine compared to untreated patients [23]. Monoclonal antibodies, trastuzumab in particular, have been shown to mediate some of their therapeutic activity via antibody-dependent cell-mediated cytotoxicity [24, 25]. Recent studies have shown that aminobisphosphonates can expand gamma delta T cells by up to 50-fold. These specialized T cells are capable of recognizing various pathogens including tumor cells. Finally, although hormonal therapy can dampen the immune response, aromatase inhibitors used *in vitro* rendered tumor cells more sensitive to lysis by human peripheral blood mononuclear cells via monocyte-mediated antibody-dependent cellular cytotoxicity [26] and may increase NK cell activity [27]. Although our work suggests common adjuvant breast cancer therapies do not inhibit the development of cellular immunity, numbers of subjects were too small to determine whether these therapies could augment the vaccinated immune response.

For cancer vaccines to be used as adjuvant breast cancer therapy, most likely the approach will have to be applied concurrently with common adjuvant therapies. Data presented here would suggest that it would be possible to combine biologic with standard therapies without impacting the potency of the vaccine. While there was no evidence of a negative effect on immunity, further evaluation is needed to see if any of these treatments will yield a beneficial immunologic effect in the setting of vaccination against tumor antigens.

Acknowledgements This work was supported by grants from the NIH, NCI; R01 CA75163, U54 CA090818, the Cancer Research Treatment Foundation, and Athena Water. Patient care was conducted through the Clinical Research Center Facility at the University of Washington that is supported through NIH grant MO1-RR-00037. We are grateful for the excellent technical assistance supplied by Mr. Chris Bolander as well as assistance in manuscript preparation

provided by Ms. Sally Zebrick. Our heartfelt thanks to all the patients who agreed to participate in this study.

References

1. Finn OJ (2003) Cancer vaccines: between the idea and the reality. *Nat Rev Immunol* 3(8):630–641
2. Disis ML, Shiota FM, Cheever MA (1998) Human HER-2/neu protein immunization circumvents tolerance to rat neu: a vaccine strategy for ‘self’ tumour antigens. *Immunology* 93(2):192–199
3. Day RS, Shackney SE, Peters WP (2005) The analysis of relapse-free survival curves: implications for evaluating intensive systemic adjuvant treatment regimens for breast cancer. *Br J Cancer* 92(1):47–54
4. Nicolini A, Carpi A, Rossi G (2006) Cytokines in breast cancer. *Cytokine Growth Factor Rev* 17(5):325–337
5. Disis ML, Gooley TA, Rinn K et al (2002) Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide-based vaccines. *J Clin Oncol* 20(11):2624–2632
6. Webster DJ, Waisman J, Macleod B et al (2006) A phase I/II study of a HER2/neu (HER2) peptide vaccine plus concurrent trastuzumab. *J Clin Oncol* 24(18S):2528
7. Salazar LG, Coveler AL, Swensen RE et al (2007) Kinetics of tumor-specific T-cell response development after active immunization in patients with HER-2/neu overexpressing cancers. *Clin Immunol* 125(3):275–280
8. Disis ML, Grabstein KH, Sleath PR et al (1999) Generation of immunity to the HER-2/neu oncogenic protein in patients with breast and ovarian cancer using a peptide-based vaccine. *Clin Cancer Res* 5(6):1289–1297
9. Disis ML, Knutson KL, Schiffman K et al (2000) Pre-existent immunity to the HER-2/neu oncogenic protein in patients with HER-2/neu overexpressing breast and ovarian cancer. *Breast Cancer Res Treat* 62(3):245–252
10. Sommer AL, Wachel BK, Smith JA (2006) Evaluation of vaccine dosing in patients with solid tumors receiving myelosuppressive chemotherapy. *J Oncol Pharm Pract* 12(3):143–154
11. Sfrikakis PP, Gourgoulis GM, Mouloupoulos LA et al (2005) Age-related thymic activity in adults following chemotherapy-induced lymphopenia. *Eur J Clin Invest* 35(6):380–387
12. Morrison VA, Rai KR, Peterson BL et al (2001) Impact of therapy With chlorambucil, fludarabine, or fludarabine plus chlorambucil on infections in patients with chronic lymphocytic leukemia: intergroup Study Cancer and Leukemia Group B 9011. *J Clin Oncol* 19(16):3611–3621
13. Zhou H, Zou P, Chen ZC et al (2007) A novel vicious cycle cascade in tumor chemotherapy. *Med Hypotheses* 69(6):1230–1233
14. Sliwkowski MX, Lofgren JA, Lewis GD et al (1999) Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Semin Oncol* 26(4 Suppl 12):60–70
15. Marty M, Cognetti F, Maraninchi D et al (2005) Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *J Clin Oncol* 23(19):4265–4274
16. Wolf AM, Rumpold H, Tilg H et al (2006) The effect of zoledronic acid on the function and differentiation of myeloid cells. *Haematologica* 91(9):1165–1171
17. Bringmann A, Schmidt SM, Weck MM et al (2007) Zoledronic acid inhibits the function of Toll-like receptor 4 ligand activated monocyte-derived dendritic cells. *Leukemia* 21(4):732–738
18. Paharkova-Vatchkova V, Maldonado R, Kovats S (2004) Estrogen preferentially promotes the differentiation of CD11c+ CD11b(intermediate) dendritic cells from bone marrow precursors. *J Immunol* 172(3):1426–1436
19. Nalbandian G, Paharkova-Vatchkova V, Mao A et al (2005) The selective estrogen receptor modulators, tamoxifen and raloxifene, impair dendritic cell differentiation and activation. *J Immunol* 175(4):2666–2675
20. Premkumar VG, Yuvaraj S, Vijayarathay K et al (2007) Serum cytokine levels of interleukin-1beta, -6, -8, tumour necrosis factor-alpha and vascular endothelial growth factor in breast cancer patients treated with tamoxifen and supplemented with co-enzyme Q(10), riboflavin and niacin. *Basic Clin Pharmacol Toxicol* 100(6):387–391
21. Sereda D, Werth VP (2006) Improvement in dermatomyositis rash associated with the use of antiestrogen medication. *Arch Dermatol* 142(1):70–72
22. Dayan M, Zinger H, Kalush F et al (1997) The beneficial effects of treatment with tamoxifen and anti-oestradiol antibody on experimental systemic lupus erythematosus are associated with cytokine modulations. *Immunology* 90(1):101–108
23. Yang E, Hu XF, Xing PX (2007) Advances of MUC1 as a target for breast cancer immunotherapy. *Histol Histopathol* 22(8):905–922
24. Clynes RA, Towers TL, Presta LG et al (2000) Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nat Med* 6(4):443–446
25. Arnould L, Gelly M, Penault-Llorca F et al (2006) Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? *Br J Cancer* 94(2):259–267
26. Braun DP, Crist KA, Shaheen F et al (2005) Aromatase inhibitors increase the sensitivity of human tumor cells to monocyte-mediated, antibody-dependent cellular cytotoxicity. *Am J Surg* 190(4):570–571
27. Berry J, Green BJ, Matheson DS (1987) Modulation of natural killer cell activity in stage I postmenopausal breast cancer patients on low-dose aminoglutethimide. *Cancer Immunol Immunother* 24(1):72–75