Potential Use of Vaccines in the Primary Prevention of Breast Cancer in High-Risk Patients

Matteo Lazzeroni · Davide Serrano

Division of Cancer Prevention and Genetics, European Institute of Oncology, Milan, Italy

Keywords

Vaccines · Breast cancer prevention

Summary

Cancer vaccines are an emerging therapeutic and prophylactic modality that may play a more important role in cancer prevention and treatment in the future. Therapeutic cancer vaccines are designed to generate a targeted, immune-mediated antitumor response. Successful prophylactic vaccines are those against oncogenic viral infections, such as the human papillomavirus and cervical cancer. However, a tough challenge for the majority of tumor vaccines is the self-nature of tumor antigens. Ongoing studies are investigating methods to enhance vaccine strategies including immune-modulating agents. The present review analyzes the potential use of vaccines in the primary prevention of breast cancer, focusing on the recent extension of vaccine target selection to self-proteins that are overexpressed during the early stages of tumor development but whose expression no longer occurs as we age, a feature that may avoid clinically significant autoimmune sequelae.

Schlüsselwörter

Vakzine · Prävention von Mammakarzinomen

Zusammenfassung

Tumorstoffe sind eine aufstrebende therapeutische und prophylaktische Modalität, die zukünftig eine durchaus bedeutendere Rolle bei der Prävention und Behandlung von Tumoren spielen könnte. Therapeutische Tumorstoffe haben die Aufgabe, eine gezielte, immunvermittelte Antitumor-Antwort zu erzeugen. Beispiele für erfolgreiche prophylaktische Vakzine sind die gegen onkogene Virusinfektionen, z.B. humane Papillomavirus-Infektion, und Zervixkarzinome gerichteten Impfstoffe. Eine schwierige Herausforderung für die Mehrzahl der Tumorstoffe ist jedoch die «Eigen»-Natur von Tumorantigenen. Derzeit laufende Studien untersuchen Methoden zur Stärkung von Impfstrategien, einschließlich immunmodulierender Wirkstoffe. Die vorliegende Übersichtsarbeit analysiert den potentiellen Einsatz von Vakzinen zur primären Prävention von Mammakarzinomen mit Fokus auf der jüngsten Ausweitung der Impfstoff-Ziel-Selektion auf Eigenproteine, die während der frühen Phasen der Tumorentwicklung überexprimiert werden, deren Expression mit zunehmendem Alter jedoch endet – ein Merkmal, das klinisch signifikante Autoimmun-Folgekrankheiten verhindern könnte.

Introduction

The idea of controlling cancer by stimulating the immune system dates back to more than a century ago when the first bacterial extracts were used in order to stimulate tumor-specific immune responses [1]. As of today, we know that optimizing cancer vaccine formulations and schedules as well as

selecting patients most likely to benefit from cancer vaccines both help in achieving clinical efficacy. However, despite considerable efforts having been directed towards this goal, the success to date is limited. A major concern is that the antigenic determinant of a vaccine should be ideally tumor-specific, but perhaps most experimental cancer vaccines have targeted tumor-associated antigens that are not critical to

tumor survival, allowing cancer cells to escape immunity and thus metastasize. Secondly, the majority of cancer vaccine trials focused on later stages of disease, often on immunosuppressed patients unlikely to respond. This strategy is equivalent to vaccinating against pathogens after the appearance of symptoms.

The history of vaccination strategies demonstrates that the use of prophylactic vaccination before the progression of the disease is preferable. Childhood vaccination programs show that prophylactic vaccination is a proven way to reduce the incidence of diseases [2]. So far, no similar vaccination programs exist to protect people from clinically detectable cancer. The recent development of a prophylactic vaccine for cervical cancer is a promising example, although this setting is peculiar because the identified pathogen which confers the cancer risk has also a specific antigen to be targeted. The lack of such an etiological agent for breast cancer is a real challenge in the development of an effective prophylactic vaccine. Women with an increased risk of developing breast cancer might benefit the most from a prophylactic vaccine, in terms of both the risk/benefit ratio and acceptability. Several approaches are available for identifying women with a higher than average risk of breast cancer. These include an assessment of family history with genetic testing, a review of clinical and epidemiological variables including prior breast biopsies, and the evaluation of mammographic density.

The present review analyzes the potential use of vaccines in the primary prevention of breast cancer, focusing on the recent extension of vaccine target selection to self-proteins that are overexpressed during the early stages of tumor development but not in the normal tissue, and antigens expressed early in life but no longer expressed in adulthood. These strategies are necessary to guarantee enough specificity and to avoid clinically significant autoimmune sequelae.

Therapeutic Breast Cancer Vaccination against Tumor-Restricted Antigens

A great number of strategies employ the approach of vaccinating after tumors clinically appear [3]. Restricting immunotherapy to a precise tumor antigenic target allows specific responsiveness and avoids collateral tissue damage and general side effects. On the other hand, such tumor specificity presents an increased likelihood that the selected tumor antigen may not elicit a clinically effective immune response. Considerable efforts have been directed towards this issue, with limited success to date.

A major problem with vaccine development is that the antigenic determinant ideally should be tumor-specific. Furthermore, the effectiveness of therapeutic cancer vaccines is limited by the well-documented immune suppression commonly occurring in cancer patients. Tumors create a micro-environment that fosters immune tolerance through a variety

of mechanisms that include inhibition of antigen presentation and elaboration of immunosuppressive factors [4]. Thus, the induction of effective immunity before invasive tumors emerge might be an ideal setting to test vaccines before the tumors become able to inhibit the immunity directed against them.

With respect to breast cancer and potential intervention with a cancer vaccine, ductal intraepithelial neoplasia (DIN) is an ideal stage during which to test cancer vaccination. DIN is an early pre-invasive malignancy of the breast and represents an intermediary between normal breast tissue and invasive breast cancer in which tumor burden is quite low. Sharma et al. [5] recently analyzed in a presurgical window of opportunity trial the effects of a vaccine targeting HER-2/neu expression in women with a recent diagnosis of DIN. HER-2/neu overexpression plays in fact a critical role in breast cancer development, and a vaccine targeting HER-2/neu expression in DIN may initiate immunity against invasive cancer. A HER-2/neu dendritic cell vaccine was administered to 27 patients with HER-2/neu-overexpressing DIN. The HER-2/neu vaccine was administered once weekly for 4 weeks before surgical resection, and pre- and post-vaccination analysis was conducted to assess clinical results. At surgery, 5 of 27 (18.5%) vaccinated subjects had no evidence of remaining disease, whereas among 22 subjects with residual DIN, HER-2/neu expression was down-regulated in 11 (50%). When comparing estrogen receptor (ER)-negative with ER-positive DIN lesions, vaccination was more effective in hormone-independent DIN. After vaccination, no residual DIN was found in 40% of ER-negative subjects compared with 5.9% in ER-positive subjects. Sustained HER-2/neu expression was found in 10% of ER-negative subjects compared with 47.1% in ER-positive subjects ($p = 0.04$). Post-vaccination phenotypes were significantly different between ER-positive and ER-negative subjects ($p = 0.01$), with 7 of 16 initially presenting with ER-positive+HER-2/neu-positive phenotypes turning into ER-positive+HER-2/neu-negative, and 3 of 6 with ER-negative+HER-2/neu-positive phenotypes changing to ER-negative+HER-2/neu-negative. Results showed that vaccination against HER-2/neu was safe and well tolerated and induced a decline and/or eradication of HER-2/neu expression. However, some important considerations about HER-2 vaccination of DIN patients are mandatory: i) the emergence of a HER-2/neu-negative phenotype after vaccination is also a warning that a single target may not be sufficient to eliminate or prevent disease in all individuals; ii) HER-2 has been targeted for vaccination in many trials despite its limited frequency of overexpression and above all its substantial irregular intratumor distribution [6–8]; iii) it is essential to remember that while with regard to invasive breast cancer HER-2 molecular profiling to stratify molecular subtypes for prognosis and treatment guidance has been proven to be effective [9, 10], reports about HER-2 status as a risk factors in DIN patients are relatively few in number and controversial [11–16].

Cancer testis (CT) antigens are expressed in various types of malignant tumors but are absent in normal adult tissues with the exception of testicular germ cells. To date, more than 100 CT antigens and antigen families have been identified [17]. Several therapeutic cancer vaccine trials of CT antigens have demonstrated the ability to induce cellular and humoral immune responses with a good safety profile and without evidence of clinical autoimmunity [18, 19]. While their function is not entirely clear, CT antigens appear to be involved in proliferation [20], stem cell function [21, 22], and carcinogenesis of at least some tumors [23]. While studies have reported varying frequencies of CT antigens in breast cancer [24–28], a high prevalence of CT antigen expression has recently been demonstrated in triple-negative (TN) breast cancers [29, 30]. TN tumors, which lack estrogen, progesterone, and HER-2, are more commonly associated with BRCA1 mutations than other breast cancer phenotypes [31]. BRCA1 and BRCA2 are tumor suppressor genes that are involved in DNA repair. Germline mutations of these genes confer a high lifetime risk for a number of malignant tumors, in particular breast and ovarian cancers. In 2011, Curigliano et al. [30] investigated the expression of the 2 CT antigens, NY-ESO-1 and the melanoma antigen family A (MAGE-A), by immunohistochemistry in 100 selected invasive breast cancers including 50 ER-positive+HER-2/neu-negative and 50 TN tumors. A significantly higher expression of MAGE-A and NY-ESO-1 was detected in TN breast cancers compared with ER-positive tumors ($p = 0.04$). MAGE-A expression was detected in 13 (26%) TN cancers compared with 5 (10%) ER-positive tumors ($p = 0.07$). NY-ESO-1 expression was confirmed in 9 (18%) TN tumor samples compared with 2 (4%) ER-positive tumors. More recently, the high expression of CT antigens mapping to chromosome X (CT-X) genes, including MAGE-A, has been correlated with worse survival in a multivariate analysis of 394 TN breast cancers [32]. Furthermore, Adams et al. [33] reported a high incidence of CT antigen

expression (61.5%) in invasive and in situ ductal breast cancers as well as their absence in benign breast tissue of BRCA mutation carriers, thus identifying potential target antigens for preventive cancer vaccines. In particular, MAGE-A was expressed in 13/26 (50%) cancers. In contrast, none of the CT antigens were expressed in adjacent or contralateral normal breast epithelium. Thus, women with deleterious BRCA germline mutations represent a group for whom preventive vaccination may be useful. In addition, CT antigens are frequently expressed in other cancers that develop in BRCA mutation carriers, such as melanoma [34] and ovarian carcinoma [35], which may be useful when using these targets in immunoprevention. However, the process of ‘immunoediting’ [36] could be an obstacle to effective prevention with CT antigen vaccines. Under the selective pressure of vaccine-induced immune response, tumors may downmodulate the antigen resulting in antigen loss variants. Furthermore, since intracellular CT antigens are processed and presented by major histocompatibility complex (MHC) molecules for immune recognition, tumor downmodulation of MHC-I or the peptide-processing machinery could also hamper effective immunoprevention [33].

Viral Targets for Prophylactic Cancer Vaccination

Viral targeted vaccines have already been shown to protect against cancers that arise as sequelae of chronic viral infection. Recent data have correlated breast cancer to viral infections, such as Epstein-Barr virus [37], mouse mammary tumor virus (MMTV) [38], and human papillomavirus (HPV) [39]. The role of HPV as a potential cause of human breast cancer has recently received more attention because of the possible prevention of breast cancer using an HPV vaccine, which is now used in the primary prevention of human cervical cancer even if the mechanism by which the virus reaches the breast

Table 1. Main criteria for a prophylactic (breast) cancer vaccination

Therapeutic breast cancer vaccination against tumor-restricted antigens [3]	Viral targets for prophylactic cancer vaccination [52]	Self-targets for prophylactic breast cancer vaccination [67]
<ul style="list-style-type: none"> – A sufficient number of immune cells with highly avid recognition of tumor antigens must be generated in vivo. – The immune cells must traffic to and infiltrate the tumor stroma. – The immune cells must be activated at the tumor site to manifest appropriate effector mechanisms such as direct lysis or cytokine secretion capable of causing tumor destruction. 	<ul style="list-style-type: none"> – Presence of viral genetic material in the cancer whilst being absent in normal tissue. – The ability of the virus to transform or immortalize normal cells in culture. – Virus-specific antibodies being more frequently detected in patients when compared to control subjects. – The presence of the virus, virus-specific antibodies, or both preceding the development of the cancer. – Induction of specific malignant phenotypes and characteristic histological changes. – Evidence of oncogenicity of the virus in laboratory animals. 	<ul style="list-style-type: none"> – The antigen must be constitutively overexpressed in the majority of targeted tumors. – The expression of the target antigen in normal tissue must be conditional. – The condition determining expression of the target antigen in normal tissue must be readily avoidable.

tissue has not yet been elucidated [40, 41]. A number of recent studies have demonstrated that approximately 29% of human breast cancers are positive for high-risk HPV subtypes, especially subtypes 16, 18, and 33 [42–46]. However, several studies did not detect HPV in human breast cancer and normal breast tissue [47–51]. Possible reasons for the inconsistency in published reports regarding the prevalence of HPV in human breast cancer may be attributed to variable sampling, tissue processing protocols, assay methods, designed primers, sample size, and HPV prevalence in different populations [52]. However, HPV meets virtually all of the criteria developed to test the validity of the evidence for specific viruses to cause specific cancers (table 1) with the important exceptions of evidence of a positive interventional effect and consistent immunologic evidence [53].

Self-Targets for Prophylactic Breast Cancer Vaccination

The target antigen to test for prophylactic breast cancer vaccination should be constitutively overexpressed in the majority of targeted tumors. Furthermore, its expression in normal tissue should be conditional, and the condition determining expression of the target antigen in normal tissue should be readily avoidable [54].

One such example is the glycoprotein mucin 1 (MUC-1) which is expressed by normal mammary epithelial cells. In cancer cells, however, the glycosylation pattern of MUC-1 is altered – rendering it antigenic [55]. However, clinical trials with MUC-1 showed that, with respect to its expression levels, MUC-1 is a relatively poor immunogen in human beings. *Bacillus Calmette-Guerin* (BCG) is used widely in human vaccines. Furthermore, it can potentially offer unique advantages for developing a safe and effective multi-vaccine vehicle. Due to these properties, the development of MUC-1-based recombinant BCG (rBCG) vaccines for breast cancer immunotherapy has gained great momentum in recent years [56].

Yuan et al. [57] analyzed if an immunotherapy targeting multiple variable-number tandem repeat (VNTR) regions might induce anti-MUC-1 immune responses. In this study, they constructed 2 rBCG vaccines that combine the expression of multiple tandem repeats of MUC-1 and colony-stimulating factor (CSF), since granulocyte-macrophage (GM)-CSF has been shown to increase the percentage and activity of antigen-presenting cells. The effect of these 2 novel breast cancer vaccines (rBCG-MVNTR4-CSF and rBCG-MVNTR8-CSF) on the growth of breast tumors in SCID mice inoculated with human peripheral blood lymphocytes was evaluated. The incidence of tumors in animals injected with rBCG-MVNTR4-CSF (25% on day 35, 63% on day 70) or rBCG-MVNTR8-CSF (25% on day 35, 38% on day 70) was significantly lower than that in control mice (100%). Furthermore, a significant induction of MUC-1-specific cytotoxic T lympho-

cyte response was shown in mice receiving the MUC-1 vaccine, suggesting that the rBCG-MVNTR4-CSF and rBCG-MVNTR8-CSF vaccines might be good candidates for breast tumor immunotherapy [57]. However, despite several promising MUC-1-based vaccines having been developed so far, these have only been tested in the preclinical setting [56].

An intriguing hypothesis postulated recently by Jaini et al. [54] is that a full-strength autoimmune attack sufficient to induce organ-specific failure may provide protection and therapy against tumors derived from the targeted organ. They selected α -lactalbumin as their target vaccine autoantigen because it is a breast-specific differentiation protein expressed in high amounts in the majority of human breast carcinomas [58–60] and in mammary epithelial cells only during lactation [61–64]. 2 transgenic mouse breast cancer models were used: MMTV-neu transgenic mice which express the non-activated neu under regulation of the long terminal repeat of MMTV and show a 50% incidence of spontaneous mammary tumors by 205 days of age [65], and MMTV-PyVT transgenic mice that express the polyomavirus middle T antigen (PyVT) under MMTV regulation and develop very rapidly growing mammary tumors palpable by 5 weeks of age [66]. They found that vaccination of 2-month-old MMTV-neu transgenic mice with α -lactalbumin completely prevented the appearance of autochthonous breast tumors at 10 months of age; in contrast 100% of sham vaccinated control mice developed breast tumors [54]. Prophylactic α -lactalbumin vaccination was not associated with detectable inflammation of normal breast tissue or any other tissues examined. The authors concluded that α -lactalbumin vaccination may provide safe and effective protection against the development of breast cancer for women in their post-childbearing, premenopausal years, when lactation is readily avoidable and risk for developing breast cancer is high [67].

An important concern is whether prophylactic α -lactalbumin vaccination of normal healthy cancer-free women may result in autoimmune complications due to possible foci expressing α -lactalbumin in the normal breast tissue. Watson and Gusterson [75] addressed this issue on the basis of previous results published by Bailey et al. in 1982 [68]. However, no quantification of the frequency of such foci was reported, and the appearance of these foci in the breasts of non-lactating women was significantly associated with the use of contraceptive hormones available in the late 1970s. It has been suggested that this occurrence might be avoided by prescreening candidate women for high serum prolactin levels required for induction of α -lactalbumin expression [69] and by excluding women using contraceptive hormone therapy associated with expression of lactational foci in the normal breast [68]. In addition, this potential problem may be circumvented by targeting the vaccine to women with sufficient involution of their breast parenchyma to preclude expression of lactation-dependent proteins, such as older women at high risk of developing breast cancer.

Conclusion and Future Directions

Great progress has been made in the field of tumor immunology in the past decade, but optimism about the clinical application of currently available cancer vaccine approaches is based more on surrogate endpoints than on clinical tumor regression [3]. Women with a history of HER-2/neu-positive DIN might be the target population in which to test a vaccine targeting HER-2/neu, and TN DIN patients and healthy women with a pathogenic germline mutation of the BRCA1 gene might benefit from a vaccine targeting 1 or more CT antigens such as MAGE-A and NY-ESO-1. These vaccines may be tested in phase IIB trials to evaluate both systemic and local immunization. The primary endpoint might be the measurement of peak antibody titers against MAGE-A after vaccinations and the spectrum of CD4+ and CD8+ T cells against various new and known MAGE-A epitopes. Furthermore, cytokine levels could be measured in nipple aspirate fluid to evaluate a possible immune response in the target organ. Secondary analysis might focus on breast epithelial proliferation and other biomarkers of breast cancer risk. Consented women could undergo a random fine-needle aspiration (FNA), and those with a high number of epithelial cells (4,000 or more) could be randomized to vaccine intervention vs. no treatment, followed by repeat FNA. Cells could be examined for Ki-67 labeling index and atypia. Prophylactic HPV vaccines for cervical cancer may also reduce the development of breast cancer in women. Long-term follow-up of women who receive this vaccine at a young age may determine the validity of this hypothesis.

The possibility to develop a vaccination program designed to provide protection against adult cancers by extending vaccine target selection to self-proteins that are overexpressed during the early stages of tumor development but serve functionally as non-self viral-like substitutes is really appealing, but the concern regarding inflammatory consequences in vaccinating healthy women even if at high risk is real and should be solved in further preclinical and above all in phase I safety clinical trials. Furthermore, it must be noted that restricted targeting may provide a limited immunologic pressure pre-

cluding the inhibition of tumors that do not express the target antigen during their early development. With this idea in mind, an optimal prophylactic breast cancer vaccination may require a multivalent vaccine targeting several lactation-dependent self-proteins, peptides with tumor expression features and conditional expression profiles similar to α -lactalbumin, in order to enhance the protective effect of the prophylactic breast cancer vaccine in the absence of any increased autoimmune risk.

In conclusion, antigen formulation is essential for a vaccine to be effective. Proteins, small or long peptides, DNA, and polypeptides have all been tested. However, to identify the best self-antigen is not the only challenge that cancer vaccine researchers have to face up to. It is clear now that a major issue is how the antigen is presented to T lymphocytes to determine the immune response or the tolerance [70, 71]. In this context, the right delivery system is fundamental to overcome immune tolerance of self-antigens. Latest strategies involve micro- or nano-particles loaded with the antigen to interact directly with the professional antigen-presenting cells, in particular dendritic cells [71, 72]. Cancer chemoprevention is defined as the use of natural, synthetic, or biologic-chemical agents to reverse, suppress, or prevent carcinogenic progression to invasive cancer. The success of several recent clinical trials in preventing cancer in high-risk populations suggests that chemoprevention is a rational and appealing strategy [73, 74]. Prophylactic cancer vaccination or 'immunoprevention' could hopefully help chemoprevention to redefine the concept of preventing cancer as far more than simply early detection.

Acknowledgement

We thank Margherita Omesso for writing assistance.

Disclosure Statement

The authors deny any sponsorship or funding arrangement and have no conflicts of interest to declare.

References

- 1 Coley WB: The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases. 1893. *Clin Orthop Relat Res* 1991;3:11.
- 2 Roush SW, Murphy TV: Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. *JAMA* 2007;298:2155–2163.
- 3 Rosenberg SA, Yang JC, Restifo NP: Cancer immunotherapy: moving beyond current vaccines. *Nat Med* 2004;10:909–915.
- 4 Rabinovich GA, Gabrilovich D, Sotomayor EM: Immunosuppressive strategies that are mediated by tumor cells. *Annu Rev Immunol* 2007;25:267–296.
- 5 Sharma A, Koldovsky U, Xu S, Mick R, Roses R, Fitzpatrick E, Weinstein S, Nisenbaum H, Levine BL, Fox K, Zhang P, Koski G, Czerniecki BJ: HER-2 pulsed dendritic cell vaccine can eliminate HER-2 expression and impact ductal carcinoma in situ. *Cancer* 2012;Epub ahead of print.
- 6 Pertschuk LP, Axiotis CA, Feldman JG, Kim YD, Karavattayhayil SJ, Braithwaite L: Marked intratumoral heterogeneity of the proto-oncogene Her-2/neu determined by three different detection systems. *Breast J* 1999;5:369–374.
- 7 Brunelli M, Manfrin E, Martignoni G, Miller K, Remo A, Reghellin D, Bersani S, Gobbo S, Eccher A, Chilosi M, Bonetti F: Genotypic intratumoral heterogeneity in breast carcinoma with HER2/neu amplification: evaluation according to ASCO/CAP criteria. *Am J Clin Pathol* 2009;131:678–682.
- 8 Lee S, Jung W, Hong SW, Koo JS: Evaluation of intratumoral HER-2 heterogeneity by fluorescence in situ hybridization in invasive breast cancer: a single institution study. *J Korean Med Sci* 2011;26:1001–1006.
- 9 Perou CM, Jeffrey SS, van de RM, Rees CA, Eisen MB, Ross DT, Pergamenschikov A, Williams CF, Zhu SX, Lee JC, Lashkari D, Shalon D, Brown PO, Botstein D: Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proc Natl Acad Sci U S A* 1999;96:9212–9217.

- 10 Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de RM, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein LP, Borresen-Dale AL: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 2001;98:10869–10874.
- 11 Provenzano E, Hopper JL, Giles GG, Marr G, Venter DJ, Armes JE: Biological markers that predict clinical recurrence in ductal carcinoma in situ of the breast. *Eur J Cancer* 2003;39:622–630.
- 12 Cornfield DB, Palazzo JP, Schwartz GF, Goonewardene SA, Kovatich AJ, Chervoneva I, Hyslop T, Schwarting R: The prognostic significance of multiple morphologic features and biologic markers in ductal carcinoma in situ of the breast: a study of a large cohort of patients treated with surgery alone. *Cancer* 2004;100:2317–2327.
- 13 Ringberg A, Anagnostaki L, Anderson H, Idvall I, Ferno M: Cell biological factors in ductal carcinoma in situ (DCIS) of the breast-relationship to ipsilateral local recurrence and histopathological characteristics. *Eur J Cancer* 2001;37:1514–1522.
- 14 Horimoto Y, Tokuda E, Arakawa A, Kosaka T, Saito M, Kasumi F: Significance of HER2 protein examination in ductal carcinoma in situ. *J Surg Res* 2011;167:e205–e210.
- 15 Liao N, Zhang GC, Liu YH, Li XR, Yao M, Xu FP, Li L, Wu YL: HER2-positive status is an independent predictor for coexisting invasion of ductal carcinoma in situ of the breast presenting extensive DCIS component. *Pathol Res Pract* 2011;207:1–7.
- 16 Guerrieri-Gonzaga A, Botteri E, Rotmensz N, Bassi F, Intra M, Serrano D, Renne G, Luini A, Cazzaniga M, Goldhirsch A, Colleoni M, Viale G, Ivaldi G, Bagnardi V, Lazzaroni M, Decensi A, Veronesi U, Bonanni B: Ductal intraepithelial neoplasia: postsurgical outcome for 1,267 women cared for in one single institution over 10 years. *Oncologist* 2009;14:201–212.
- 17 Simpson AJ, Caballero OL, Jungbluth A, Chen YT, Old LJ: Cancer/testis antigens, gametogenesis and cancer. *Nat Rev Cancer* 2005;5:615–625.
- 18 Atanackovic D, Altorki NK, Cao Y, Ritter E, Ferrara CA, Ritter G, Hoffman EW, Bokemeyer C, Old LJ, Gnjatic S: Booster vaccination of cancer patients with MAGE-A3 protein reveals long-term immunological memory or tolerance depending on priming. *Proc Natl Acad Sci U S A* 2008;105:1650–1655.
- 19 Valmori D, Souleimanian NE, Tosello V, Bhardwaj N, Adams S, O'Neill D, Pavlick A, Escalon JB, Cruz CM, Angiulli A, Angiulli F, Mears G, Vogel SM, Pan L, Jungbluth AA, Hoffmann EW, Venhaus R, Ritter G, Old LJ, Ayyoub M: Vaccination with NY-ESO-1 protein and CpG in Montanide induces integrated antibody/Th1 responses and CD8 T cells through cross-priming. *Proc Natl Acad Sci U S A* 2007;104:8947–8952.
- 20 Jungbluth AA, Ely S, DiLiberto M, Niesvizky R, Williamson B, Frosina D, Chen YT, Bhardwaj N, Chen-Kiang S, Old LJ, Cho HJ: The cancer-testis antigens CT7 (MAGE-C1) and MAGE-A3/6 are commonly expressed in multiple myeloma and correlate with plasma-cell proliferation. *Blood* 2005;106:167–174.
- 21 Cronwright G, Le BK, Gothelstrom C, Darcy P, Ehnman M, Brodin B: Cancer/testis antigen expression in human mesenchymal stem cells: down-regulation of SSX impairs cell migration and matrix metalloproteinase 2 expression. *Cancer Res* 2005;65:2207–2215.
- 22 Yawata T, Nakai E, Park KC, Chihara T, Kumazawa A, Toyonaga S, Masahira T, Nakabayashi H, Kaji T, Shimizu K: Enhanced expression of cancer testis antigen genes in glioma stem cells. *Mol Carcinog* 2010;49:532–544.
- 23 Ajiro M, Katagiri T, Ueda K, Nakagawa H, Fukukawa C, Lin ML, Park JH, Nishide T, Daigo Y, Nakamura Y: Involvement of RQCD1 overexpression, a novel cancer-testis antigen, in the Akt pathway in breast cancer cells. *Int J Oncol* 2009;35:673–681.
- 24 Theurillat JP, Ingold F, Frei C, Zippelius A, Varga Z, Seifert B, Chen YT, Jager D, Knuth A, Moch H: NY-ESO-1 protein expression in primary breast carcinoma and metastases: correlation with CD8+ T-cell and CD79a+ plasmacytic/B-cell infiltration. *Int J Cancer* 2007;120:2411–2417.
- 25 Bandic D, Juretic A, Sarcevic B, Separovic V, Kujundzic-Tiljak M, Hudolin T, Spagnoli GC, Covic D, Samija M: Expression and possible prognostic role of MAGE-A4, NY-ESO-1, and HER-2 antigens in women with relapsing invasive ductal breast cancer: retrospective immunohistochemical study. *Croat Med J* 2006;47:32–41.
- 26 Mischo A, Kubuschok B, Ertan K, Preuss KD, Romeike B, Regitz E, Schormann C, de BD, Wadle A, Neumann F, Schmidt W, Renner C, Pfreundschuh M: Prospective study on the expression of cancer testis genes and antibody responses in 100 consecutive patients with primary breast cancer. *Int J Cancer* 2006;118:696–703.
- 27 Sugita Y, Wada H, Fujita S, Nakata T, Sato S, Noguchi Y, Jungbluth AA, Yamaguchi M, Chen YT, Stockert E, Gnjatic S, Williamson B, Scanlan MJ, Ono T, Sakita I, Yasui M, Miyoshi Y, Tamaki Y, Matsuura N, Noguchi S, Old LJ, Nakayama E, Monden M: NY-ESO-1 expression and immunogenicity in malignant and benign breast tumors. *Cancer Res* 2004;64:2199–2204.
- 28 Taylor M, Bolton LM, Johnson P, Elliott T, Murray N: Breast cancer is a promising target for vaccination using cancer-testis antigens known to elicit immune responses. *Breast Cancer Res* 2007;9:R46.
- 29 Grigoriadis A, Caballero OL, Hoek KS, da SL, Chen YT, Shin SJ, Jungbluth AA, Miller LD, Clouston D, Cebon J, Old LJ, Lakhani SR, Simpson AJ, Neville AM: CT-X antigen expression in human breast cancer. *Proc Natl Acad Sci U S A* 2009;106:13493–13498.
- 30 Curigliano G, Viale G, Ghioni M, Jungbluth AA, Bagnardi V, Spagnoli GC, Neville AM, Nole F, Rotmensz N, Goldhirsch A: Cancer-testis antigen expression in triple-negative breast cancer. *Ann Oncol* 2011;22:98–103.
- 31 Anders CK, Carey LA: Biology, metastatic patterns, and treatment of patients with triple-negative breast cancer. *Clin Breast Cancer* 2009;9(suppl 2):S73–S81.
- 32 Karn T, Puszta L, Ruckhaberle E, Liedtke C, Muller V, Schmidt M, Metzler D, Wang J, Coombes KR, Gatz R, Hanker L, Solbach C, Ahr A, Holtrich U, Rody A, Kaufmann M: Melanoma antigen family A identified by the bimodality index defines a subset of triple negative breast cancers as candidates for immune response augmentation. *Eur J Cancer* 2012;48:12–23.
- 33 Adams S, Greeder L, Reich E, Shao Y, Fosina D, Hanson N, Tassello J, Singh B, Spagnoli GC, Demaria S, Jungbluth AA: Expression of cancer testis antigens in human BRCA-associated breast cancers: potential targets for immunoprevention? *Cancer Immunol Immunother* 2011;60:999–1007.
- 34 Velazquez EF, Jungbluth AA, Yancovitz M, Gnjatic S, Adams S, O'Neill D, Zavilevich K, Albukh T, Christos P, Mazumdar M, Pavlick A, Polsky D, Shapiro R, Berman R, Spira J, Busam K, Osman I, Bhardwaj N: Expression of the cancer/testis antigen NY-ESO-1 in primary and metastatic malignant melanoma (MM) – correlation with prognostic factors. *Cancer Immunol* 2007;7:11.
- 35 Odunsi K, Jungbluth AA, Stockert E, Qian F, Gnjatic S, Tammela J, Intengan M, Beck A, Keitz B, Santiago D, Williamson B, Scanlan MJ, Ritter G, Chen YT, Driscoll D, Sood A, Lele S, Old LJ: NY-ESO-1 and LAGE-1 cancer-testis antigens are potential targets for immunotherapy in epithelial ovarian cancer. *Cancer Res* 2003;63:6076–6083.
- 36 Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD: Cancer immunoeediting: from immunosurveillance to tumor escape. *Nat Immunol* 2002;3:991–998.
- 37 He JR, Tang LY, Yu DD, Su FX, Song EW, Lin Y, Wang SM, Lai GC, Chen WQ, Ren ZF: Epstein-Barr virus and breast cancer: serological study in a high-incidence area of nasopharyngeal carcinoma. *Cancer Lett* 2011;309:128–136.
- 38 Melana SM, Nepomnaschy I, Hasa J, Djougarian A, Djougarian A, Holland JF, Pogo BG: Detection of human mammary tumor virus proteins in human breast cancer cells. *J Virol Methods* 2010;163:157–161.
- 39 Altundag K, Baptista MZ: Human papilloma virus and breast cancer. *Br J Cancer* 2006;94:338.
- 40 Bosch FX: Human papillomavirus: science and technologies for the elimination of cervical cancer. *Expert Opin Pharmacother* 2011;12:2189–2204.
- 41 Nieto K, Gissmann L, Schadlich L: Human papillomavirus-specific immune therapy: failure and hope. *Antivir Ther* 2010;15:951–957.
- 42 Heng B, Glenn WK, Ye Y, Tran B, Delprado W, Lutze-Mann L, Whitaker NJ, Lawson JS: Human papilloma virus is associated with breast cancer. *Br J Cancer* 2009;101:1345–1350.
- 43 Akil N, Yasmeen A, Kassab A, Ghabreau L, Darnel AD, Al Moustafa AE: High-risk human papillomavirus infections in breast cancer in Syrian women and their association with Id-1 expression: a tissue microarray study. *Br J Cancer* 2008;99:404–407.
- 44 Sarkola M, Rintala M, Grenman S, Syrjanen S: Human papillomavirus DNA detected in breast milk. *Pediatr Infect Dis J* 2008;27:557–558.
- 45 Kulka J, Kovalszky I, Svastics E, Berta M, Fule T: Lymphoepithelioma-like carcinoma of the breast: not Epstein-Barr virus-, but human papilloma virus-positive. *Hum Pathol* 2008;39:298–301.
- 46 Yasmeen A, Bismar TA, Kandouz M, Foulkes WD, Desprez PY, Al Moustafa AE: E6/E7 of HPV type 16 promotes cell invasion and metastasis of human breast cancer cells. *Cell Cycle* 2007;6:2038–2042.
- 47 Mendizabal-Ruiz AP, Morales JA, Ramirez-Jirano LJ, Padilla-Rosas M, Moran-Moguel MC, Montoya-Fuentes H: Low frequency of human papillomavirus DNA in breast cancer tissue. *Breast Cancer Res Treat* 2009;114:189–194.

- 48 Khan NA, Castillo A, Koriyama C, Kijima Y, Umekita Y, Ohi Y, Higashi M, Sagara Y, Yoshinaka H, Tsuji T, Natsugoe S, Douchi T, Eizuru Y, Akiba S: Human papillomavirus detected in female breast carcinomas in Japan. *Br J Cancer* 2008;99:408–414.
- 49 De CP, Thioux M, Lebigot I, Sigal-Zafrani B, Salmon R, Sastre-Garau X: No evidence of human papillomavirus DNA sequences in invasive breast carcinoma. *Breast Cancer Res Treat* 2008;109:55–58.
- 50 Choi YL, Cho EY, Kim JH, Nam SJ, Oh YL, Song SY, Yang JH, Kim DS: Detection of human papillomavirus DNA by DNA chip in breast carcinomas of Korean women. *Tumour Biol* 2007;28:327–332.
- 51 Lindel K, Forster A, Altermatt HJ, Greiner R, Gruber G: Breast cancer and human papillomavirus (HPV) infection: no evidence of a viral etiology in a group of Swiss women. *Breast* 2007;16:172–177.
- 52 Wang T, Chang P, Wang L, Yao Q, Guo W, Chen J, Yan T, Cao C: The role of human papillomavirus infection in breast cancer. *Med Oncol* 2012;29:48–55.
- 53 Vonka V: Causality in medicine: the case of tumours and viruses. *Philos Trans R Soc Lond B Biol Sci* 2000;355:1831–1841.
- 54 Jaini R, Kesaraju P, Johnson JM, Altuntas CZ, Jane-Wit D, Tuohy VK: An autoimmune-mediated strategy for prophylactic breast cancer vaccination. *Nat Med* 2010;16:799–803.
- 55 Carr-Brendel V, Markovic D, Ferrer K, Smith M, Taylor-Papadimitriou J, Cohen EP: Immunity to murine breast cancer cells modified to express MUC-1, a human breast cancer antigen, in transgenic mice tolerant to human MUC-1. *Cancer Res* 2000;60:2435–2443.
- 56 Yuan S, Shi C, Liu L, Han W: MUC1-based recombinant *Bacillus Calmette-Guerin* vaccines as candidates for breast cancer immunotherapy. *Expert Opin Biol Ther* 2010;10:1037–1048.
- 57 Yuan S, Shi C, Ling R, Wang T, Wang H, Han W: Immunization with two recombinant *Bacillus Calmette-Guerin* vaccines that combine the expression of multiple tandem repeats of mucin-1 and colony stimulating-factor suppress breast tumor growth in mice. *J Cancer Res Clin Oncol* 2010;136:1359–1367.
- 58 Lee AK, Rosen PP, DeLellis RA, Saigo PE, Gangi MD, Groshen S, Bagin R, Wolfe HJ: Tumor marker expression in breast carcinomas and relationship to prognosis. An immunohistochemical study. *Am J Clin Pathol* 1985;84:687–696.
- 59 Wrba F, Reiner A, Markis-Ritzinger E, Holzner JH, Reiner G, Spona J: Prognostic significance of immunohistochemical parameters in breast carcinomas. *Pathol Res Pract* 1988;183:277–283.
- 60 Cohen C, Sharkey FE, Shulman G, Uthman EO, Budgeon LR: Tumor-associated antigens in breast carcinomas. Prognostic significance. *Cancer* 1987;60:1294–1298.
- 61 Nagamatsu Y, Oka T: Purification and characterization of mouse alpha-lactalbumin and preparation of its antibody. *Biochem J* 1980;185:227–237.
- 62 Ren J, Stuart DI, Acharya KR: Alpha-lactalbumin possesses a distinct zinc binding site. *J Biol Chem* 1993;268:19292–19298.
- 63 Vilotte JL, Soulier S: Isolation and characterization of the mouse alpha-lactalbumin-encoding gene: interspecies comparison, tissue- and stage-specific expression. *Gene* 1992;119:287–292.
- 64 Vilotte JL, Soulier S, Mercier JC: Sequence of the murine alpha-lactalbumin-encoding cDNA: interspecies comparison of the coding frame and deduced pre-protein. *Gene* 1992;112:251–255.
- 65 Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD, Muller WJ: Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. *Proc Natl Acad Sci U S A* 1992;89:10578–10582.
- 66 Guy CT, Cardiff RD, Muller WJ: Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease. *Mol Cell Biol* 1992;12:954–961.
- 67 Tuohy VK, Jaini R: Prophylactic cancer vaccination by targeting functional non-self. *Ann Med* 2011;43:356–365.
- 68 Bailey AJ, Sloane JP, Trickey BS, Ormerod MG: An immunocytochemical study of alpha-lactalbumin in human breast tissue. *J Pathol* 1982;137:13–23.
- 69 Martin RH, Glass MR, Chapman C, Wilson GD, Woods KL: Suppression by bromocriptine of the serum lactalbumin peak associated with human lactogenesis. *Clin Endocrinol (Oxf)* 1981;14:363–366.
- 70 Kwon YJ, James E, Shastri N, Frechet JM: In vivo targeting of dendritic cells for activation of cellular immunity using vaccine carriers based on pH-responsive microparticles. *Proc Natl Acad Sci U S A* 2005;102:18264–18268.
- 71 Tacke PJ, Zeelenberg IS, Cruz LJ, van Hout-Kuijer MA, van de GG, Fokkink RG, Lambeck AJ, Figdor CG: Targeted delivery of TLR ligands to human and mouse dendritic cells strongly enhances adjuvanticity. *Blood* 2011;118:6836–6844.
- 72 Tacke PJ, de V, I, Torensma R, Figdor CG: Dendritic-cell immunotherapy: from ex vivo loading to in vivo targeting. *Nat Rev Immunol* 2007;7:790–802.
- 73 Cuzick J, Decensi A, Arun B, Brown PH, Castiglione M, Dunn B, Forbes JF, Glaus A, Howell A, von MG, Vogel V, Zwierzina H: Preventive therapy for breast cancer: a consensus statement. *Lancet Oncol* 2011;12:496–503.
- 74 Goss PE, Ingle JN, es-Martinez JE, Cheung AM, Chlebowski RT, Wactawski-Wende J, McTiernan A, Robbins J, Johnson KC, Martin LW, Winkquist E, Sarto GE, Garber JE, Fabian CJ, Pujol P, Maunsell E, Farmer P, Gelmon KA, Tu D, Richardson H: Exemestane for breast-cancer prevention in postmenopausal women. *N Engl J Med* 2011;364:2381–2391.
- 75 Watson CJ, Gusterson BA: A prophylactic vaccine for breast cancer? *Breast Cancer Res* 2012;12:310.