Developing a clinical development paradigm for translation of a mammaglobin-A DNA vaccine

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Keywords: breast cancer • clinical trial • DNA vaccine • immunotherapy • mammaglobin-A

Breast cancer is the most common cancer and is second only to lung cancer as the leading cause of cancer death among American women. The latest Cancer Facts & Figures 2015 [1] estimates that 231,840 new cases of invasive breast cancer will be diagnosed in 2015, representing 29% of all new cancer cases among women in the USA. Although the number of deaths from breast cancer has declined in recent years, 40,290 women are predicted to die of breast cancer in 2015 [1]. Clearly, innovative strategies for breast cancer prevention and therapy must be considered a public health priority.

Cancer vaccine therapy

It has long been known that there is a dynamic relationship between the immune system and cancer. This dynamic relationship has been studied in detail, ultimately resulting in the establishment of the cancer immunoediting concept [2]. Evading immune destruction has been recognized as an emerging hallmark of cancer [3]. Recent successes in cancer immunotherapy have highlighted the importance of the immune system in controlling cancer, and cancer immunotherapy was identified by *Science* magazine as the 2013 breakthrough of the year [4]. Both immune checkpoint blockade (anti-CTLA-4 and anti-PD-1) therapy and chimeric antigen receptor therapy have demonstrated spectacular results in clinical trials [5,6].

These dramatic successes in cancer immunotherapy have renewed interest in can-

cer vaccine therapy [7]. Cancer vaccines are typically designed to stimulate the immune system to mount a cell-mediated immune response against one or more tumor-associated antigens. Cancer vaccines have excellent safety profiles and several have now received US FDA approval including Gardasil and Cervarix for prevention of cervical cancer and Provenge for treatment of metastatic prostate cancer. Investigators are now pursuing several different cancer vaccine platforms including autologous cellular vaccines, DNA vaccines and protein or peptide vaccines, often in combination with conventional cancer treatment regimens. We recently completed an open-label Phase I clinical trial (Clinicaltrials.gov identifier: NCT00807781) evaluating the safety and immunogenicity of a DNA vaccine designed to express the human breast cancer-associated mammaglobin-A antigen. Results of the trial demonstrate the safety of the mammaglobin-A DNA vaccine, with preliminary evidence to suggest that the mammaglobin-A vaccine is able to induce mammaglobin-A-specific IFN- γ -secreting CD8 T cells in treated patients. These patients also had improved progression-free survival [8].

Mammaglobin-A is an exceptional target for breast cancer vaccine therapy

Mammaglobin-A is a member of the secretoglobulin superfamily (family 2A member 2). The mammaglobin-A gene (SCGB2A2) was first identified using a differential screen-

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ing approach directed at the isolation of novel human breast cancer-associated genes [9]. Since its original identification, mammaglobin-A has proven to be an important molecular marker for the detection of metastatic breast cancer, and it has been the focus of over 300 publications in the medical literature. The exquisite tissue specificity and near universal overexpression of mammaglobin-A makes it an attractive target for a breast cancer vaccine with potential for use in a breast cancer prevention and/or treatment setting. Evidence of preexisting immunity to mammaglobin-A in breast cancer patients, and strong preclinical data provided additional rationale for clinical translation of a DNA vaccine targeting mammaglobin-A.

Phase I clinical trial of a mammaglobin-A DNA vaccine demonstrates the safety of the vaccine, with strong preliminary evidence of biologic efficacy

A total of 53 patients met all of the eligibility criteria and were enrolled in a Phase I clinical trial between December 2009 and May 2013 [8]. After enrollment, 39 patients were considered screen failures and 14 patients were vaccinated. The mammaglobin-A DNA vaccine was remarkably safe, and no significant adverse events were observed. Eight of the 14 vaccinated patients were HLA-A2⁺ and were studied with in-depth immune monitoring analyses using peripheral blood mononuclear cells collected before, during and after vaccination. Following vaccination there was a significant increase in the frequency of CD8/MAM-A2.1⁺ T cells as measured by flow cytometry ($0.9 \pm 0.5\%$ vs $3.8 \pm 1.2\%$; $p < 0.001$). ELISPOT analysis similarly demonstrated a significant increase in the number of mammaglobin-A specific IFN- γ producing CD8 T cells (41 ± 32 vs 215 ± 67 spm; $p < 0.001$). A comparison between vaccinated patients and patients who were screen failures based on HLA type suggested that mammaglobin-A DNA vaccination was associated with improved progression-free survival [8].

Future perspective

Recent experience has provided significant insights into the challenges associated with clinical translation of cancer vaccines. Initial cancer vaccine studies were typically performed in the context of metastatic cancer, using response criteria that were developed for cytotoxic therapies. In addition, surrogates for vaccine efficacy remain imperfect. We have tried to address these limitations in our ongoing studies of the mammaglobin-A DNA vaccine.

First, cancer vaccines are very different from cytotoxic chemotherapies, and the clinical development paradigm for cancer vaccines is very different. The

clinical development paradigm for cytotoxic agents involves initial testing in patients with metastatic disease to determine the maximum tolerated dose (Phase I), and then to assess biologic efficacy (Phase II). However, it is now commonly believed that generation of an effective antitumor immune response will be difficult in patients with metastatic cancer [10]. Metastatic breast cancer is no exception; metastatic breast cancer is associated with an increase in the prevalence of regulatory T cells [11,12] and immature myeloid cells [13,14] in the peripheral blood and in the tumor microenvironment, and these cells are capable of inhibiting endogenous or elicited antitumor immune responses. Patients with metastatic breast cancer may also have compromised immune systems related to prior cytotoxic therapy. The current clinical development paradigm in the cancer vaccine field emphasizes the early assessment of vaccine efficacy in an appropriate clinical context [15], and this paradigm has been endorsed by the FDA. As detailed below, vaccination in the context of neoadjuvant endocrine therapy is an appropriate clinical context to assess breast cancer vaccine efficacy.

Second, current surrogates for vaccine efficacy are imperfect. Many cancer vaccines have successfully induced immune responses in the peripheral blood, but have failed to induce an antitumor response. It is now known that regulatory networks present in the tumor microenvironment restrain antitumor immune responses. One of the limitations of almost all current cancer vaccine trials is that assessment of biologic efficacy is dependent on imperfect surrogates (immune response in the peripheral blood), and a detailed analysis of antitumor immune responses in the primary tumor is not possible. We believe that there is an excellent opportunity to test the efficacy of breast cancer vaccines in the setting of neoadjuvant endocrine therapy. Breast cancer patients with clinical T2 N0 estrogen receptor-positive breast cancer are often treated with neoadjuvant endocrine therapy (aromatase inhibitors) with the goal of decreasing tumor size and facilitating breast conservation therapy. This provides an excellent window of opportunity to treat patients with an investigational breast cancer vaccine and then rigorously assess antitumor immune responses both in the peripheral blood and in the primary tumor.

Finally, the DNA vaccine platform is a remarkably flexible platform as DNA vaccines are relatively easy to manufacture, with the ability to genetically manipulate the encoded antigens, and/or incorporate other genes to amplify the immune response. Of note, the recent development of electroporation devices that can be used in human clinical trials has increased the potential for clinical translation of DNA vaccines, as electroporation can dramatically increase DNA uptake

by muscle cells, antigen expression and immunogenicity [16–19]. The importance of electroporation to the successful clinical translation of DNA vaccines was recently highlighted and emphasized in a high-profile review [20], and in the introduction to a special issue of *Vaccine* [21].

Building on the success of our Phase I clinical trial, we have recently initiated a Phase Ib clinical trial of the mammaglobin-A DNA vaccine in patients receiving neoadjuvant endocrine therapy. We will use immunohistochemistry, flow cytometry and molecular analyses to study the impact of mammaglobin-A DNA vaccination on the primary tumor. To date, very few cancer vaccine trials have been performed in the neoadjuvant setting, and we are convinced that analysis of the immune response in the primary tumor following vaccination is likely to be significantly more informa-

tive than studies of the immune response in the peripheral blood, providing important insights into how to improve current cancer vaccine platforms.

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