VINCENT K. TUOHY, PhD
Mort and Iris November Distinguished Chair in Innovative Breast Cancer Research, Cleveland Clinic; Staff, Department of Immunology, Lerner Research Institute, Cleveland Clinic; Professor, Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH; Chief Science Officer, Shield Biotech, Inc.

Bench-to-bedside challenges in developing immune protection against breast cancer

ABSTRACT

Despite the success of childhood vaccination against infectious diseases, vaccines are lacking against diseases that occur with age. We are developing a vaccine to prevent breast cancer. This article explains the vaccine strategy, how we think the vaccine will work, and how we plan to move forward through clinical trials.

KEY POINTS

"Retired" tissue-specific self proteins may substitute for unavailable pathogens as vaccine targets for mediating immune prevention of adult-onset cancers.

Vaccination against the retired breast-specific protein alpha-lactalbumin provides safe and effective immune protection against the development of breast tumors in several mouse models.

Alpha-lactalbumin is overexpressed in most human triple-negative breast cancers (TNBC), the most aggressive and lethal form of human breast cancer.

Forthcoming are clinical trials designed to prevent the initiation of TNBC in otherwise healthy cancer-free women, as well as trials designed to prevent recurrence of TNBC in women already diagnosed with this disease.

The author is the inventor of vaccines based on the retired self-protein strategy, and these vaccines have been licensed to Shield Biotech, Inc., a privately owned company. The author is the Chief Science Officer of Shield Biotech and may in the future receive commercialization revenues for this technology. The author acknowledges that there is a potential conflict of interest related to his relationship with Shield Biotech and asserts that to the best of his ability he has taken all measures in this report to avoid any inappropriate bias associated with the commercial goals of the company.

Medical Grand Rounds articles are based on edited transcripts from Division of Medicine Grand Rounds presentations at Cleveland Clinic. They are approved by the author but are not peer-reviewed.

doi:10.3949/ccjm.81gr.14006

The most proven, effective way to control disease is through prophylactic vaccination. The childhood vaccination program is a testament to this disease prevention approach, and in its current form protects us from diseases caused by 16 different pathogens.[1]

Childhood immunization ends in the teen years with recommended vaccination against multiple strains of human papillomavirus that are associated with several cancers, most notably cervical carcinoma.[2] However, even though we have known for over 30 years that the immune system can provide considerable vaccine-induced protection against the development of cancer,[3] we have not produced any vaccines that prevent cancers that commonly occur with age, such as breast and prostate cancer, which afflict 1 of 8 women and 1 of 6 men, respectively.[4,5]

The lack of an adult vaccine program that provides protection against such commonly occurring adult-onset cancers represents a glaring health care deficiency and a challenge for this current generation to protect coming generations.

THE 'RETIRED' PROTEIN HYPOTHESIS

Given that most cancers are not associated with any disease-inducing pathogens, at what targets can we aim our immune system to induce safe and effective protection against these commonly occurring adult-onset cancers?

Perhaps an understanding of the natural aging process may provide us with insights regarding possible vaccine targets. As we age, there is a decline in expression of many tissue-specific proteins, often to the point where they may be considered "retired" and no longer found at detectable or immunogenic lev-

els in normal cells. Examples of this natural aging process include the pigment proteins as our hair whitens, certain lactation proteins when breastfeeding ceases, and some ovarian proteins as menopause begins and production of mature egg follicles ceases. If these retired proteins are expressed in invigorated emerging tumors, then preemptive immunity directed against these retired proteins would attack and destroy the emerging tumors and ignore normal tissues, thereby avoiding any complicating collateral autoimmune damage.

Thus, we propose that retired tissue-specific self-proteins may substitute for unavailable pathogens as targets for mediating safe and effective immune protection against adult-onset cancers such as breast cancer.

SAFE AND EFFECTIVE PREVENTION OF BREAST CANCER IN MICE

To test this retired-protein hypothesis for immunoprevention of breast cancer, we selected alpha-lactalbumin as our vaccine target, for two reasons:

- Alpha-lactalbumin is a protein expressed exclusively in lactating breast tissue and is not expressed at immunogenic levels in either normal nonlactating breast tissues or in any of 78 other normal human tissues examined.[6–8]
- Alpha-lactalbumin is expressed in most human triple-negative breast cancers (TNBC),[9,10] the most aggressive and lethal form of breast cancer, and the predominant form that occurs in women with mutations in the breast cancer 1, early-onset gene (*BRCA1*).[11,12]

We propose that retired tissue-specific self-proteins may substitute for unavailable pathogens as targets for cancer vaccines

We found that alpha-lactalbumin vaccination consistently inhibited the formation and growth of breast tumors in three different mouse models commonly used in breast cancer research.[13] More importantly, the observed immune protection against the development of breast cancer in mice occurred in the absence of any detectable autoimmune inflammatory damage in any normal tissues examined. Thus, we concluded that alpha-lactalbumin vaccination could provide healthy women with safe and effective immune protection against the more malignant forms of breast cancer.

FROM BENCH TO BEDSIDE

How then do we determine whether alpha-lactalbumin vaccination prevents the development of TNBC in otherwise healthy cancer-free women, and whether it prevents recurrence of TNBC in women already diagnosed with TNBC? Our initial approach will involve two phase 1 clinical trials designed to determine the safety of the vaccine as well as the dose and number of vaccinations needed to induce optimum tumor immunity.

The first (phase 1a) trial will involve vaccination of women recently diagnosed with TNBC who have recovered with the current standard of care. These women will be vaccinated in groups receiving various doses of both recombinant human alpha-lactalbumin and an appropriate immune adjuvant that activates the immune system so it responds aggressively to the alpha-lactalbumin and creates the proinflammatory T-cell response needed for effective tumor immunity. This trial will simply provide dosage and safety profiles of the vaccine and will thereby lay the groundwork for subsequent (phase 2 and 3) trials designed to determine whether alpha-lactalbumin vaccination is effective in preventing recurrence of TNBC in women already diagnosed with this disease.

The dosage and number of immunizations shown to provide optimum immunity in the phase 1a trial will be used in a second (phase 1b) trial designed primarily to determine the safety of alpha-lactalbumin vaccination in healthy cancer-free women who have elected to undergo voluntary prophylactic mastectomy to reduce their breast cancer risk. Most of the women who elect to have this surgery have an established family history of breast cancer or a known *BRCA1* mutation associated with high breast cancer risk, or both.[11,12] Consenting women will be vaccinated against alpha-lactalbumin several months before their mastectomy, and their surgically removed breast tissues will be examined extensively for signs of vaccine-induced autoimmune damage. Thus, this trial will determine the safety of alpha-lactalbumin vaccination in healthy cancer-free women and will lay the groundwork for subsequent phase 2 and 3 trials designed to determine whether alpha-lactalbumin vaccination is effective in preventing TNBC in

women at high risk of developing this form of breast cancer.

We estimate that completing our preclinical studies, obtaining permission from the US Food and Drug Administration to test our investigational new drug, and completing both phase 1 clinical trials will require about 5 years. Thereafter, completion of phase 2 and 3 trials designed to prevent both recurrence of TNBC in women already diagnosed with this disease and occurrence of TNBC in otherwise healthy, cancer-free women will likely take at least another 5 years, so that this vaccine will likely not be available to the general public before 2024.

TO SUM UP

Although our immune system is potentially capable of protecting us from some cancers, we currently have no immune protection against cancers we commonly confront as we age. We propose that tissue-specific self proteins that are retired from expression with age in normal tissues but are expressed at immunogenic levels in emerging tumors may substitute for unavailable pathogens as targets for immunoprevention of adult-onset cancers that commonly occur with age. We know that the retired breast-specific protein, alpha-lactalbumin, is overexpressed in TNBC and that vaccination with alpha-lactalbumin provides safe and effective protection from breast cancer in preclinical mouse studies. Clinical trials are planned to ultimately determine whether alpha-lactalbumin vaccination can prevent recurrence of TNBC in women already diagnosed with this disease and prevent the initiation of TNBC in women at high risk of developing this most aggressive and lethal form of breast cancer.

***ACKNOWLEDGMENT**: This work was supported by a grant from Shield Biotech, Inc., Cleveland, OH. In addition, the author wishes to recognize and express his sincere gratitude for the support and encouragement received from numerous organizations that have been instrumental in making this work possible, including November Philanthropy, Brakes for Breasts, the Breast Health and Healing Foundation, the Toni Turchi Foundation, the Coalition of Women Who Care About Breast Cancer, the Sisters for Prevention, the Previvors and Survivors, the Champions of the Pink Vaccine, the Race at Legacy Village, the National Greek Orthodox Ladies Philoptochos Society, the Daughters of Penelope Icarus Chapter 321, Can't Stop Won't Stop, the Babylon Breast Cancer Coalition, and Walk With A Doc.*

REFERENCES

1. **Centers for Disease Control and Prevention**. Immunization schedules. www.cdc.gov/vaccines/schedules/. Accessed September 4, 2014.
2. **Schiller JT, Lowy DR**. Understanding and learning from the success of prophylactic human papillomavirus vaccines. Nat Rev Microbiol 2012; 10:681–692.
3. **Van Pel A, Boon T**. Protection against a nonimmunogenic mouse leukemia by an immunogenic variant obtained by mutagenesis. Proc Natl Acad Sci USA 1982; 79:4718–4722.
4. **Siegel R, Naishadham D, Jemal A**. Cancer statistics, 2013. CA Cancer J Clin 2013; 63:11–30.
5. **National Cancer Institute**. Surveillance, Epidemiology, and End Results (SEER) Program. Previous version: SEER cancer statistics review 1975–2010. http://seer.cancer.gov/csr/1975_2010/. Accessed September 4, 2014.
6. **Uhlen M, Oksvold P, Fagerberg L, et al**. Towards a knowledge-based human protein atlas. Nat Biotechnol 2010; 28:1248–1250.
7. **Pontén F, Gry M, Fagerberg L, et al**. A global view of protein expression in human cells, tissues, and organs. Mol Syst Biol 2009; 5:337.
8. **The Human Protein Atlas**. www.proteinatlas.org. Accessed September 4, 2014.
9. **Rhodes DR, Yu J, Shanker K, et al**. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia 2004; 6:1–6.
10. **ONCOMINEdatabase**. www.oncomine.org/resource/login.html. Accessed September 4, 2014.
11. **Atchley DP, Albarracin CT, Lopez A, et al**. Clinical and pathologic characteristics of patients with *BRCA*-positive and *BRCA*-negative breast cancer. J Clin Oncol 2008; 26:4282–4288.
12. **Comen E, Davids M, Kirchhoff T, Hudis C, Offit K, Robson M**. Relative contributions of *BRCA1* and *BRCA2* mutations to "triple-negative" breast cancer in Ashkenazi women. Breast Cancer Res Treat 2011; 129:185–190.
13. **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.

***ADDRESS**: Vincent K. Tuohy, PhD, Department of Immunology, Lerner Research Institute, NB30, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195; e-mail: tuohyv@ccf.org*