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Magnitude of antigen-specific T-cell immunity the month after completing vaccination series predicts the development of long-term persistence of antitumor immune response

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ABSTRACT

Background For best efficacy, vaccines must provide long-lasting immunity. To measure longevity, memory from B and T cells are surrogate endpoints for vaccine efficacy. When antibodies are insufficient for protection, the immune response must rely on T cells. The magnitude and differentiation of effective, durable immune responses depend on antigen-specific precursor frequencies. However, development of vaccines that induce durable T-cell responses for cancer treatment has remained elusive.

Methods To address long-lasting immunity, patients with HER2+ (human epidermal growth factor receptor 2) advanced stage cancer received HER2/neu targeted vaccines. Interferon-gamma (IFN-γ) enzyme-linked immunosorbent spot measuring HER2/neu IFN-γ T cells were analyzed from 86 patients from three time points: baseline, 1 month after vaccine series, and long-term follow-up at 1 year, following one in vitro stimulation. The baseline and 1-month post-vaccine series responses were correlated with immunity at long-term follow-up by logistic regression. Immunity was modeled by non-linear functions using generalized additive models.

Results Antigen-specific T-cell responses at baseline were associated with a 0.33-log increase in response at long-term follow-up, 95% CI (0.11, 0.54), p=0.003. 63% of patients that had HER2/neu specific T cells at baseline continued to have responses at long-term follow-up. Increased HER2/neu specific T-cell response 1 month after the vaccine series was associated with a 0.47-log increase in T-cell response at long-term follow-up, 95% CI (0.27, 0.67), p=2e-5. 74% of patients that had an increased IFN-γ HER2 response 1 month after vaccines retained immunity long-term. As the 1-month post-vaccination series precursor frequency of HER2+IFN-γ T-cell responses increased, the probability of retaining these responses long-term increased (OR=1.49 for every one natural log increase of precursor frequency, p=0.0002), reaching an OR of 20 for a precursor frequency of 1:3,000

Conclusions Patients not destined to achieve long-term immunity can be identified immediately after completing the vaccine series. Log-fold increases in antigen-specific precursor frequencies after vaccinations correlate with increased odds of retaining long-term HER2 immune responses. Further vaccine boosting or immune checkpoint inhibitors or other immune stimulator therapy should be explored in patients that do not develop antigen-specific T-cell responses to improve overall response rates.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Increased human epidermal growth factor receptor 2 (HER2) -specific T-cell immunity is associated with improved survival and decreased recurrences in HER2+breast cancer. Vaccines for HER2+breast cancer need to augment this immune response to be effective.

WHAT THIS STUDY ADDS

⇒ Patients not destined to achieve long-term immunity can be identified immediately after completing a vaccine series.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Early identification of patients who fail to respond to vaccine may allow the use of boosters or other immune stimulator therapy to improve response rates.

INTRODUCTION

Increased human epidermal growth factor receptor 2 (HER2)-specific Th1 T cells have been associated with improved survival in HER2+metastatic breast cancer and decreased recurrence in local HER2+breast cancer.[1 2] Therefore, the goal of HER2+vaccines is to increase HER2+T-cell immunity. Immunologic memory generated by vaccines has long been the surrogate endpoint for vaccine efficacy for infectious diseases. Adaptive cellular immune responses from B and T cells both contribute to protective immunity.[3] Where antibodies are unable to neutralize free

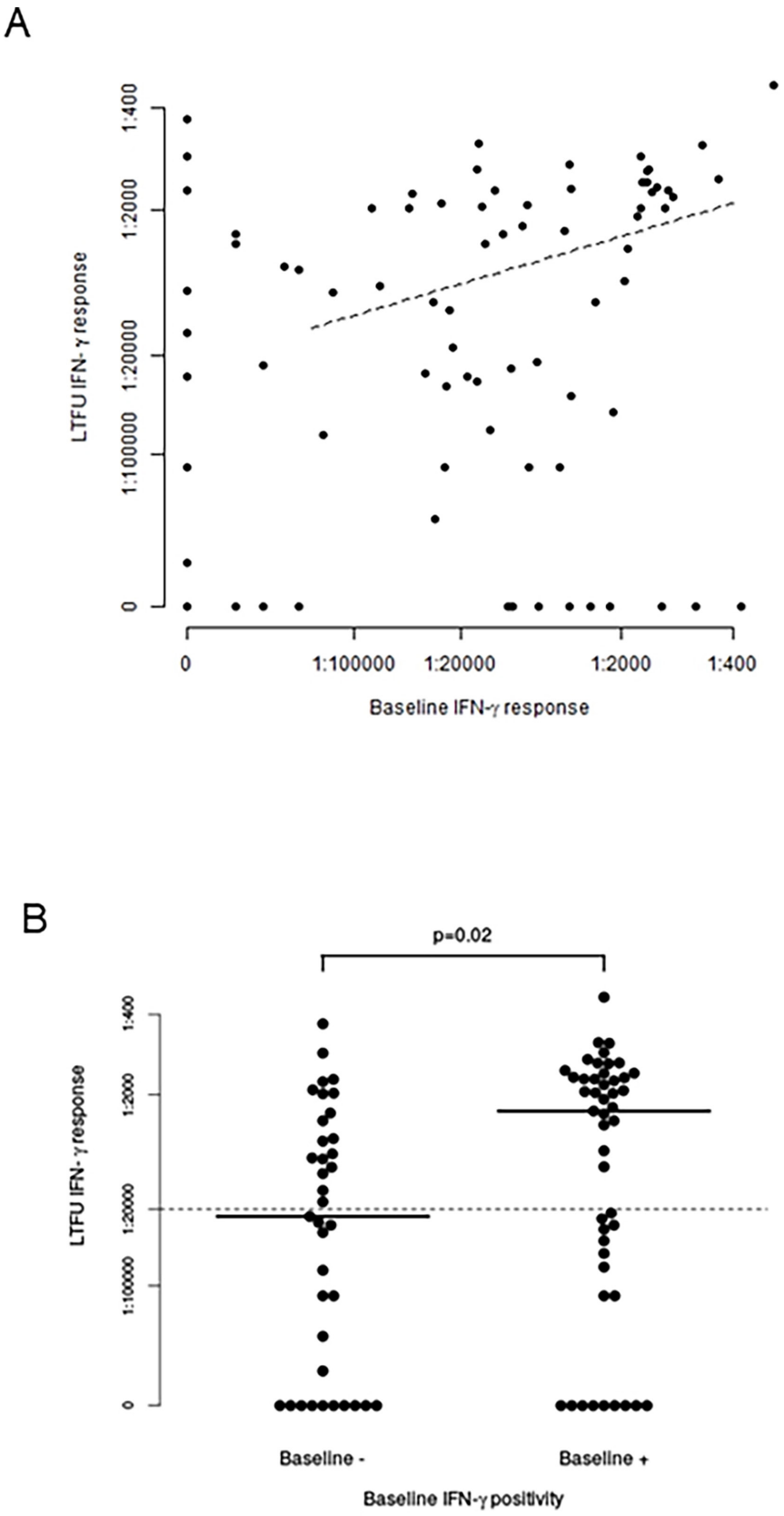

Figure 1 (A) The scatter plot of T-cell responses in long term follow-up (LTFU) against T-cell responses at baseline in the log transformed scale. Y-axis is T-cell precursor frequency as 1:X (representing a calculated 1:frequency of IFN-γ–secreting cells in 10^6 PBMCs) of the IFN-γ response to HER2 antigen in LTFU, X-axis is T-cell precursor frequency as 1:X (representing a calculated 1:frequency of IFN-γ-secreting cells in 10^6 PBMCs) of the IFN-γ response to HER2 antigen at baseline (n=83). Dotted line is the fitted linear line. (B) The distributions of T-cell responses at LTFU by the positivity of T-cell responses at baseline. HER2, human epidermal growth factor receptor 2; IFN-γ, interferon-gamma; PBMC, peripheral blood mononuclear cells.

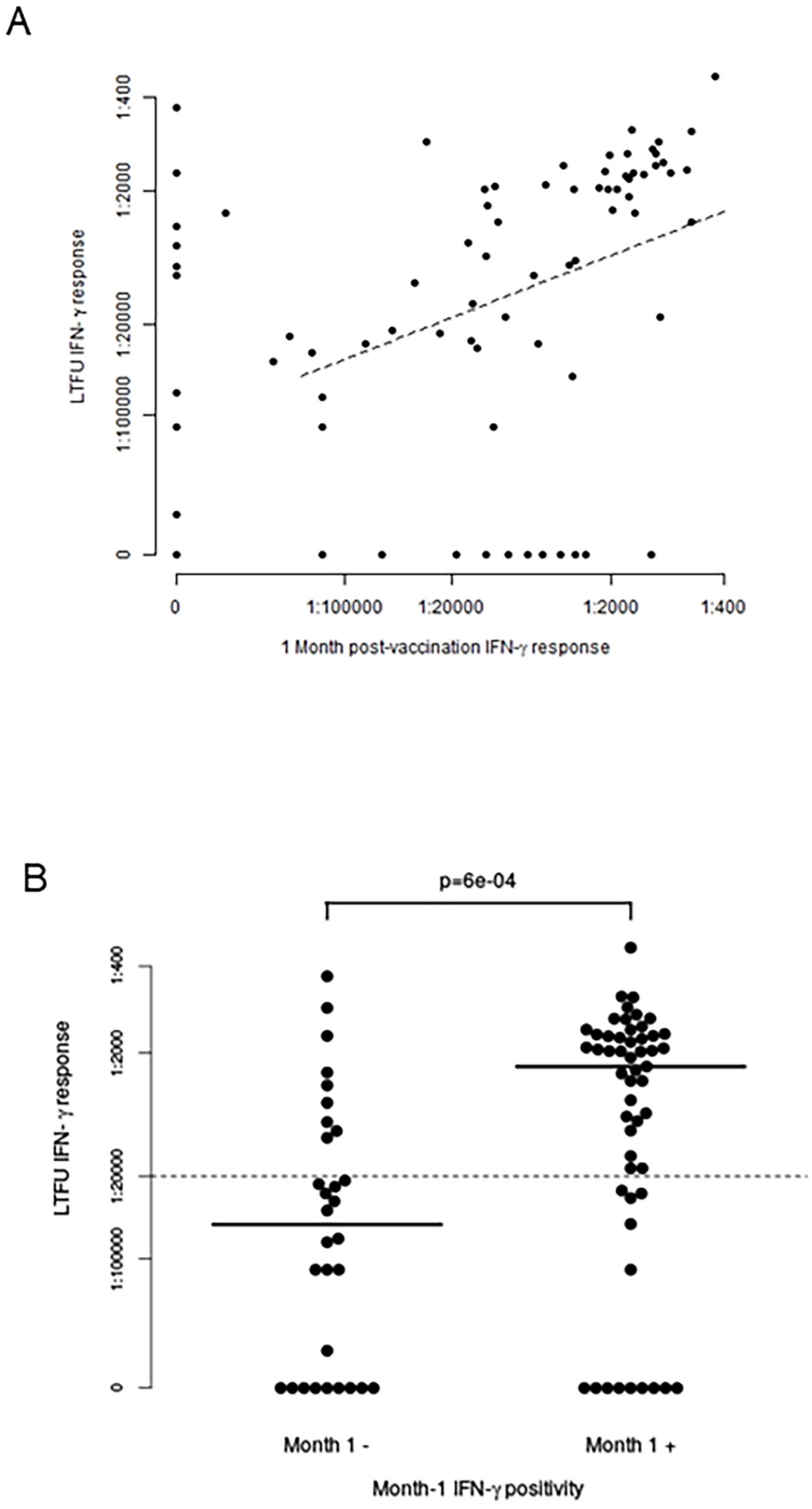

Figure 2 (A) The scatter plot of T-cell responses in long-term follow-up (LTFU) against T-cell responses at month 1 in the log transformed scale. Y-axis is T-cell precursor frequency as 1:X (representing a calculated 1:frequency of IFN-γ-secreting cells in 10^6 PBMCs) of the IFN-γ response to HER2 antigen in LTFU, while X-axis is T-cell precursor frequency as 1:X (representing a calculated 1:frequency of IFN-γ-secreting cells in 10^6 PBMCs) of the IFN-γ response to HER2 antigen at baseline (n=83). Dotted line is the fitted linear line (B) and the distribution of T-cell responses at LTFU by the positivity of T-cell responses 1-month post-vaccination. HER2, human epidermal growth factor receptor 2; IFN-γ, interferon-gamma; PBMC, peripheral blood mononuclear cells.

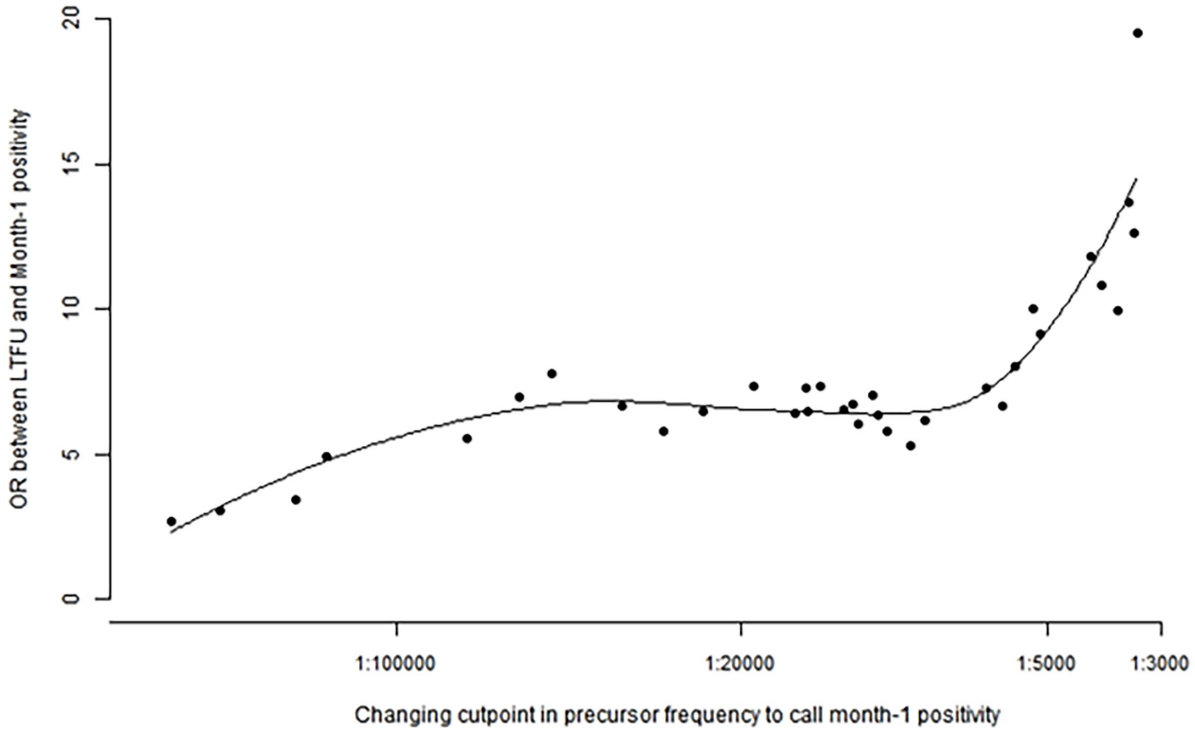

Figure 3 X axis is 1-month post-vaccination precursor frequency used to define the cut-off for positivity (defined as values greater than 1:20,000 interferon-γ-secreting cells per 1×10^6 PBMC (peripheral blood mononuclear cells), and Y axis is the OR of achieving positive immunity at long-term follow-up with the dichotomized 1-month post-vaccination precursor frequency at a defined cut point. The smooth line is the fitted loess curve. LTFU, long-term follow-up; PBMC, peripheral blood mononuclear cells.

pathogens in the extracellular environment, the immune response must rely on T cells. Vaccines eliciting memory T-cell responses are important for killing infected cells and releasing cytokines to inhibit the growth of pathogens.[4] Vaccines target pathogens harbored within cells need T-cell immunity, for example, vaccines for herpes zoster and malaria.[5–8] The magnitude and differentiation of an effective immune response to an antigen also depends on antigen-specific T-cell precursor frequencies.[9 10] Developing vaccines that specifically induce long-lived T-cell responses for cancer treatment continue to present these challenges.[11]

Antigen-specific T cells have been shown to be effective in controlling cancer.[12] Since developing memory T cells specific to cancer antigens are essential in controlling disease, efforts are underway to design cancer vaccines similar to vaccination approaches for infectious disease.[13] Using vaccines in the adjuvant setting where there is minimal or no residual disease or in prophylaxis, where the host is immunocompetent provides the best parallels to vaccines used against microbial and viral pathogens. As the frequency of self-antigen-specific T-cell precursor frequency has been shown to determine the quality of antitumor responses,[14] we evaluated if there is a level of immunity seen prior to administering vaccines that could predict the persistence of immune responses. We also questioned whether there was a level of immunity that could be achieved after the vaccine series that would predict the persistence of immunity up to a year after active immunizations had ended.

PATIENTS AND METHODS

Patient population

Immune-response data were collected from subjects enrolled in three clinical trials evaluating HER-2/neu (HER2) specific vaccination (NCT00436254, NCT00194714, and NCT00343109). The trials were approved by the Fred Hutchinson Cancer Center/University of Washington Cancer Consortium Institutional Review Board. Patients with stage III or IV HER2-positive cancers were eligible.[15 16] All subjects had breast or ovarian cancer with HER2/neu overexpression in their tumor as assessed by immunohistochemistry. A total of 104 subjects were enrolled, among whom 86 patients had immune response data for 12 months after the end of immunizations (defined as long-term follow-up) as well as immune response data for either pre-vaccine and month-1 post-vaccine series. Patients who met these criteria were all patients with breast cancer. Among 86 patients analyzed, 84 had immune response data for pre-vaccination and 83 had immune response data 1-month post-vaccination series. The vaccination series was 3–6 months apart. Vaccination was by intradermal injection using a 27-gage 0.5-inch needle of study vaccine with 100 µg recombinant granulocyte macrophage colony

stimulating factor as an adjuvant in the upper arm. The vaccines were an HER2 polypeptide vaccines containing immunogenic epitopes of HER2 (p369–384, p688–703, and p971–984) (NCT00194714, NCT00343109) or the 1790 base pair region of full-length intracellular domain (ICD) region of HER2 encoded into plasmid DNA (NCT00436254).[15–17] A tetanus vaccine was administered intramuscularly using a 25-gage 1-inch needle in the deltoid region of the arm as a control. If patients had undergone an axillary lymph node dissection vaccines were administered in the contralateral arm and if they had bilateral axillary dissections they received vaccine in the thigh. Vaccines were quantified and vialed by the Biologics Production Facility at the Fred Hutchinson Cancer Research Center (Seattle, Washington, USA) and Multiple Peptide Systems (San Diego, California, USA) under Good Manufacturing Practice (GMP) conditions. Immune monitoring was performed to assess baseline, 1-month post-vaccination series, and 1-year long-term immune response. Baseline clinical characteristics for all subjects are reported in online supplemental table 1. Exclusion criteria did not include restrictions on body mass index or body weight, smoking status, or concomitant drug use. Patients enrolled in other treatment studies, with a history of cardiac disease, with a history of pulmonary disease other than controlled asthma, and with active autoimmune disease were excluded. Subjects with an active immunodeficiency disorder such as HIV were also excluded. Immunocompetence was evaluated by tetanus toxoid (tt) immunization.

ELISpot

Antigen-specific T-cell responses were analyzed with 10-day interferon-gamma (IFN-γ) enzyme-linked immunosorbent spot (ELISpot) assays as previously described. Data were available for all patients for IFN-γ secreting responses for HER2 ICD and tt as specificity control. Data were reported as T-cell precursor frequency, corrected spots per well per 1×10^6 peripheral blood mononuclear cells (PBMC) with subtraction of background wells, calculated 1:frequency of IFN-γ-secreting cells in 10^6 PBMCs per published methods. Cells were plated at a density of 200,000 cells/well in replicates of 6. T-cell immune responses were defined to be positive for values greater than 1:20,000 IFN-γ secreting cells.[18]

STATISTICAL ANALYSIS

When used as a continuous measure, immune response data were transformed by adding 1 and then taking the natural log. Logistic regression was used to assess the correlation between responses at both baseline or 1 month after the vaccine series a positive immune response at long-term follow-up. The OR, the ratio of odds of an event between two levels of a predictor, was used to summarize the strength of association. An interaction between response at baseline or 1 month after the vaccine series with the long-term follow-up outcome was tested by fitting an appropriate term in the logistic regression model. We also examined the association between 1 month after the vaccine series immune values and positive immune response at long-term follow-up by modeling the 1 month value as a continuous variable (rather than a binary positive/negative variable). In addition, we modeled immunity at 1 month after the vaccine series by non-linear functions using generalized additive models.[19] Each of these models used logistic regression with positive response at long-term follow-up as the outcome.

RESULTS

Patients with higher pre-existent HER2 T-cell responses to HER2 were more likely to develop higher HER2 T-cell responses lasting greater than 6 months after immunization than those that did not have pre-existing responses

After log-transformation, patients' HER2 type I T-cell responses at long-term follow-up linearly increased with their baseline T-cell responses (1-log increase of baseline T-cell response was associated with a 0.33-log increase in T-cell response at long-term follow-up, 95% CI (0.11, 0.54), p=0.003, figure 1A). Consistently, long-term follow-up T-cell responses for patients with positive baseline T-cell responses were significantly higher than long-term follow-up T-cell responses for patients with negative baseline responses (p=0.02, figure 1B). Of the 37 patients negative for HER2-specific T-cell immune response at baseline, 18 (49%) were found to be positive at a long-term follow-up time point. Of 47 patients who were positive for HER2-specific Th1 immunity at baseline, 29 (62%) continued to be positive at the long-term follow-up time point. The OR for long-term positive immunity (positive at baseline vs negative at baseline) was 1.7 (95% CI 0.71 to 4.07, p=0.23). Similar analyses were performed for the positive specificity control tt. The median baseline tt-specific responses for patients with positive ICD responses at long-term follow-up was 1:3,289, range of 0 to 1:486 compared with a median of 1:7,246 for patients with negative ICD responses, p=0.27) (online supplemental figure 1A).

The level of HER2-specific immunity achieved 1 month after vaccination series was a stronger predictor of persistent immunity than the presence of pre-existent immunity responses

After log-transformation, HER2 IFN-γ T-cell response at long-term follow-up was more likely as T-cell response 1 month after the end of the vaccination series increased. The magnitude of the increased response at long-term follow-up was larger than that seen in the association of baseline level and response at long-term follow-up. Specifically, a 1-log increase of month-1 T-cell response was associated with 0.47-log increase in T-cell response at long-term follow-up, 95% CI (0.27, 0.67), p=2e-5, figure 2A. Consistently, long-term follow-up T-cell responses for patients with positive month-1 T-cell responses were higher than long-term follow-up T-cell responses for patients with

negative month-1 responses (p=6e-4, figure 2B). One month after the end of the vaccination series, 53 patients were positive for HER2 ICD IFN-γ T-cell responses; 39 (74%) retained positive immunity in long-term follow-up. One month after vaccination, 30 patients were negative. Interestingly, 9 (30%) of patients developed their first immune response in long-term follow-up highlighting the need for prolonged immune monitoring in cancer vaccine trials (figure 2). Taken collectively, patients who had immunity 1 month after completing the vaccination series had higher odds of having persistent HER2-specific immunity than those who did not have HER2-specific immunity 1 month after completing the vaccination series, OR=6.50 (95% CI 2.41 to 17.52, p=0.0002), regardless of whether the patients had pre-existing immunity prior to vaccination. Further, the correlation between baseline and 1 month after the vaccine series T-cell responses was R=0.64. Having baseline HER2 immunity did not significantly improve the prediction of the long-term follow-up positivity by 1 month after the vaccine series (p=0.17), nor did baseline HER2 immunity modify the immunity at 1 month after the vaccine series or the immunity at long-term follow-up positivity (p=0.30).

The median immunity measured as IFN-γ responses at 1-month post-vaccination series against tt among patients with or without positive tetanus responses at long-term follow-up were 1:2,116 (range 0 to 1:302) and 1:3,703 (range 0 to 1:387), respectively (p=0.17). (online supplemental figure 1B).

A higher level of immunity achieved 1 month after the vaccination series is associated with a higher probability of retaining HER2 specific Th1 immunity in long-term follow-up

As the 1 month after the vaccination series T-cell precursor frequency increased, the odds of retaining a robust HER2 specific positive response in long-term follow-up increased (OR=1.49 for every one natural log increase of month-1 precursor frequency, p=0.0002). Adding curvature to the logistic model with a linear term for the log of the 1-month post-vaccine series precursor frequency by the generalized additive model did not further improve the prediction. These results suggest a higher level of immunity achieved 1 month after vaccinations associated with a higher probability of retaining HER2-specific Th1 immunity in long-term follow-up. If positivity at 1-month post-vaccine series—a dichotomous measure by an arbitrary cut point—would be used to predict and classify long-term follow-up positivity, the OR estimates gradually increase with the values of cut points, reaching 20 for precursor frequency 1:3,000 (figure 3).

DISCUSSION

Long-lasting immunity from cancer vaccines can be predicted from immune responses measured 1 month after completion of the vaccination series, and the magnitude of antigen-specific T-cell immunity seen correlates with the likelihood that the response will persist. Existing CD8 T-cell memory has been shown to influence the magnitude of naïve CD8 T-cell responses.[20] The ability of T cells to acquire an effector memory cell phenotype depends on the CD8 T-cell differentiation state, which is modulated by the number of pre-existing memory T cells.[21] Signaling through the cytokine we have measured, IFN-γ, has also been shown to control the magnitude of T-cell responses and memory differentiation.[22]

The frequencies of memory T cells are known to influence the effectiveness of immune response when challenged with antigen or boosted after vaccines. When there is an abundance of antigen-specific T cells, boosting can lead to five to eight divisions, long-term survival of effector-memory T cells, and preservation of their proliferative potential. However, when there is a low frequency of memory T cells, boosting leads to contraction of effectors, senescence and poor protective memory.[10] When this principle was studied in self-antigens using mouse tumor models with adoptive transfer of T cells, the addition of naïve antigen-specific T cells increased vaccine-elicited tumor immunity.[14] Our findings do not necessarily mean that more precursors will always provide an advantage as there may be a threshold beyond which more precursors led to decreased responses due to intraclonal competition, although this may also be overcome with CD4 T-cell help.[14 23] Our findings may also reflect low-avidity T-cell clones due to the high antigen loading doses used, which may represent a limitation of this study.

Knowing the threshold number of antigen-specific T cells secreting IFN-γ measured at 1 month after the vaccination series that will predict long-term immune responses identifies an early, reliable time point and benchmark to estimate the success of vaccination. However, we acknowledge that a limitation of this study is that other post-vaccination time points were not studied, so it is not clear if other longitudinal time points might prove superior. Generation of post-vaccination immunity in the form of antigen-specific T-cell responses has correlated with survival in melanoma, prostate cancer, breast cancer, biliary tract cancer, and ovarian cancer.[16 24–28] This provides an early window to employ booster vaccines in those that fail to respond, vaccinate with a heterologous platform, or target alternate antigens if those are available. Boosters have been used in therapeutically vaccinated patients with breast cancer with waning levels of antigen-specific T cells, but have not necessarily shown an advantage in disease-free survival.[29 30] Although multiple vaccinations and boosters have been associated with clinical responses,[26] applying this strategy universally should be used with caution as data from preclinical models have suggested that repeated boosting may not sustain central memory T cells and may adversely affect overall survival.[31] Patients given booster vaccinations with a peptide-targeted human papillomavirus 16 (HPV16) for treatment of low-grade cervical dysplasia augmented a Th2 response.[32] HPV represents a foreign non-self antigen, and boosters in this setting may lead to T-cell exhaustion driven by chronic exposure to antigen.[4] However, this

may be overcome with immune checkpoint inhibition to expand T cells primed with an HPV-directed vaccine.[33 34] This synergy could also be a strategy exploited to treat other malignancies.[35–40] If patients who are not destined to achieve adequate immunity can be identified after the initial round of vaccinations, boosters may be given to this selectively targeted group. Alternate adjuvants that favor Th1 immunity or vaccine platforms that allow a heterologous boost are strategies that may also bring higher yields in this subgroup of patients.[41 42] But the effectiveness of a specific adjuvant or platform may vary depending on the antigen and a patient's own immune phenotype.[43]

We conclude that patients at risk of failing to achieve long-term immunity can be identified immediately after completion of therapeutic cancer vaccinations. The magnitude of antigen-specific T cells detected after completion of vaccination most strongly correlates with persistent long-term immunity. Boosters, checkpoint inhibitors or alternate vaccine targets should then be explored in this subgroup of patients to improve overall response rates.

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Contributors JBL and MLD were responsible for conceptualization and study design. JBL, JYD, JLR, JL, KMH, and TAG were responsible for data analysis. All authors contributed to writing the manuscript and had final approval. JBL is responsible for the overall content as guarantor.

Competing interests JBL has received grant funding from Merck, AstraZeneca, Precigen, ArsenalBio, Volastra, Nurix, Aminex Therapeutics through his institution and is a consultant for Verismo Therapeutics. JD is employed by Grail. SS has received grant funding from Veana, IMV Inc, and Stanford Burnham Prebys. MLD has grant funding from Precigen, Veanna, Bavarian Nordic, and Aston Sci. MLD also holds shares in Epithany and is an inventor on patents held by the University of Washington. The authors declare there are no other competing interests.

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