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TITLE: Phase II Study of HER-2/Neu Intracellular Domain Peptide-Based Vaccine Administered to Stage IV HER2 Positive Breast Cancer Patients Receiving Trastuzumab

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13. ABSTRACT (Maximum 200 Words) The cytotoxic T lymphocyte (CTL) has been considered the primary immune effector involved in mediating an anti-tumor response. Recent studies have demonstrated that "sensitization" of HER2 overexpressing tumor cells with trastuzumab, in vitro, will enhance the function of CTL specific for HER2. We have completed a Phase I study of a HER2 peptide based vaccine in women with HER2 overexpressing breast cancers without toxicity, particularly autoimmune or cardiac toxicity. This proposal outlines a Phase II clinical trial designed to estimate survival in Stage IV HER2 positive breast cancer patients with no evidence of disease and receiving trastuzumab and a HER2 ICD peptide based vaccine. In addition to survival we will assess the safety of combined immunotherapy, the immunogenicity of the approach, and whether the development of HER2 specific immunity correlates with clinical response. If we find that vaccinating against breast cancer after optimal treatment prevents or slow disease recurrence resulting in improved survival, more universal vaccine approaches can be rapidly developed and tested for the adjuvant treatment of all breast cancers.				
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Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	7
Reportable Outcomes.....	7
Conclusions.....	7

INTRODUCTION

The subject of this grant is to determine whether HER2 intracellular domain (ICD) peptide vaccine, administered in combination with trastuzumab, will enhance the effectiveness of this vaccine for treatment of patients with stage IV HER2-positive breast cancer.

The primary purpose of this grant is to determine the overall survival benefit in Stage IV HER2 positive breast cancer patients vaccinated with a HER2 ICD peptide-based vaccine while receiving maintenance trastuzumab.

The scope of the work includes a Phase II single arm study of a HER2 ICD peptide based vaccine given concurrently with trastuzumab. Patients enrolled will be HER2 over expressing stage IV breast cancer patients who have been treated to a clinical complete remission or have stable bone only disease and are within 6 months of starting maintenance trastuzumab. The primary objective is an estimate of overall survival (OS) compared to a historical control of patients treated with chemotherapy and trastuzumab (55% at 2 years). We hypothesize that the overall survival rate at 2 years with vaccination, if successful, would be 75%. 52 patients will provide 92% power to detect a statistically significant increased survival rate compared to the fixed historical rate of 55% at the one-sided significance level of .05. Secondary objectives are the assessment of the toxicity of the combined approach as well as the immunogenicity of HER2 ICD peptide vaccination. If there is evidence to suggest that the true rate of Grade IV toxicity exceeds 5% or the true rate of Grade III-IV toxicity exceeds 10% then the trial will be stopped for safety concerns. Immunogenicity of the approach will be evaluated as the ability of the vaccine to elicit HER2 ICD specific T cell immunity, to elicit epitope spreading, and to stimulate both a CD4+ and CD8+ immune response. Immune response and epitope spreading will then be modeled as time-dependent covariates in Cox proportional hazards regression models for OS to assess the correlation of each of these outcomes with the hazard of mortality.

BODY

Task 1: To assess the potential clinical impact of the administration of a HER2 ICD peptide-based vaccine to Stage IV breast cancer patients receiving concurrent trastuzumab monotherapy

a. Reconstruct and vial the HER2 ICD peptide vaccine

The work plan has been delayed due to an initial delay in vaccine manufacture by our supplier. The peptides were produced by Multiple Peptide Systems (MPS), California under Good Manufacturing Practice (GMP) conditions. At the time the production was supposed to begin, MPS experienced a backlog of orders due to insufficient manufacturing space. For this reason, our manufacture was delayed 6 months. The peptides were shipped to the University of Washington in December, 2004 rather than June. All three shipments came in December 2004. The first peptide shipment arrived separately of the second and third peptide shipments which arrived together on December 22, 2004. The peptides were delivered directly to our laboratories and then transported to the Biologics Production Facility at the Fred Hutchinson Cancer Research Center (FHCRC) the same day.

The Biologics Production Facility began vialing the ICD peptide-based vaccine the week of February 28, 2005. After vialing was complete and stability of the product was established an additional 2 additional weeks were required to perform sterility testing and all other QA/QC tests. The ICD peptide vaccine was not available to be transported to the Investigational Drug Pharmacy at UW until April 1, 2005.

The vialled product from ICD Vaccine lot 6002 will be evaluated at regular intervals for product stability. A Stability Study Log for lot 6002 is maintained. The study log lists the testing dates, the vials reserved for the study and provides a summary table to record data for each time point tested. All reserved stability vials are stored under the same conditions as the final product, $-20 \pm 2^{\circ}\text{C}$. At each stability time point reserved vials are removed from storage and visually inspected for appearance. MALDI-TOF mass spectrometry and High Performance Liquid Chromatography (HPLC) are used to confirm the stability.

Initial data from MALDI-TOF mass spectrometry and HPLC for the lot 6002 stability study was collected on 03/24/05. Analysis was performed by the FHCRC Proteomics Shared Resource. HPLC was on a MAGIC 2002 (Micro Analytical Gradient Integrated Chromatograph) from Michrom BioResources, Inc (Auburn, CA), with a Michrom Magic Bullet column (5 micron 200 angstrom pore size, C18 packing material, 38 mm column length). MALDI-TOF mass spectrometry was performed on a Voyager-DE PRO Biospectrometry Workstation equipped with delayed extraction and a reflectron from Applied Biosystems (Foster City, CA). The first stability time point, at approximately 90 days, is scheduled for 05/31/05.

Vials from ICD Vaccine lot 6002 have been reserved as an In-house Reference Standard for use in future lot release testing. A total of 24 vials from lot 6002 have been reserved. The storage condition for the In-house Reference Standard is the same as the final product, $-20 \pm 2^{\circ}\text{C}$. An In-house Reference Standard inventory/storage log is maintained.

An amendment to our original IND BB 6524 was submitted on April 12, 2005. The FDA recommended minor changes which were made to the protocol and submitted to our Human Subjects Division (IRB) on May 18, 2005. These modifications are currently in the process of being reviewed by the assigned committee members. Thus, the protocol only began to accrue patients this month.

b. Enroll and treat patients

We have not enrolled any subjects into this study during the annual review period of April 27, 2004 to April 26, 2005. Our accrual to this protocol began in May, 2005. This delay is due to the delay in reconstructing the peptide vaccine, as mentioned above.

As this is a Phase II study, the protocol is very specific in that that all subjects that enroll in this study must be enrolled within 6 months of initiating maintenance trastuzumab. We are consistently identifying potential subjects for this study, but by

the time the vaccine was ready for administration many of them were no longer eligible to participate due to this eligibility inclusion criteria.

Currently we have 6 patients waiting to be scheduled to start the study. We have established collaboration with a large breast cancer practice in Southern California, "Breastlink" which refers patients to us specifically for immune based therapies. In addition, this study is a priority protocol within the breast cancer program at the UW/FHCRC. We plan an aggressive accrual to correct for the manufacturing delay.

c. Interim statistical analysis after 25 patients have been followed for 1 year

Not applicable for this reporting period. We understand that once we have enrolled 25 subjects that have been followed for 1 year we should perform an interim analysis of the data.

d. Final analysis of response

Not applicable during this reporting period.

Task 2: To evaluate the safety of administering a HER2 ICD peptide-based vaccine to Stage IV breast cancer patients receiving trastuzumab monotherapy

a. Evaluate immediate toxicity associated with the vaccine

Not applicable to this reporting period. We will use the NCI Common Toxicity Criteria for Adverse Events Version 3.0 to grade toxicities. We will pay particular attention to local reactions associated with the injection site and systemic reactions to include but not limited to fever, malaise, myalgia, nausea and headache.

b. Determine whether there is any cardiac toxicity associated with the co-administration of the HER2 ICD peptide based vaccine with trastuzumab

Not applicable to this reporting period. We will document any abnormal cardiac events observed by us at clinic visits or reported to us by the subjects or physicians.

c. Evaluate for any potential toxicities due to the generation of an immune response to HER2

Not applicable to this reporting period.

Task 3: To determine the immunogenicity of a HER2 ICD peptide-based vaccine in patients with Stage IV breast cancer receiving concurrent trastuzumab monotherapy

a. Determine the immunogenicity of the approach by assessing the T cell response to HER2 ICD

We have established overlapping peptide pools for the HER2 ICD and have demonstrated in sorted samples that these peptides, as antigens, are equivalent to recombinant protein. The standardization of this assay is significant as the use of peptide pools ensures reproducibility of evaluation over time and removes any potential inherent immunogenicity associated with recombinant protein use. Sample analysis will begin this month.

- b. Determine the incidence of epitope spreading to the HER2 ICD or other peptides in the immunizing mix (intermolecular epitope spreading)*

Not applicable to this reporting period.

- c. Determine the incidence of epitope spreading to other immunogenic proteins associated with breast cancers (extramolecular epitope spreading)*

Not applicable to this reporting period.

- d. Assess the absolute magnitude of the CD4+ and CD8+ HER2 specific immune responses generated after active immunization*

Not applicable to this reporting period.

- e. Evaluate the generation of HER2 specific antibody immunity and antibody avidity*

Not applicable to this reporting period.

- f. Determine whether overall survival is associated with the development of HER2 specific T cell response or epitope spreading after active immunization*

Not applicable to this reporting period.

KEY RESEARCH ACCOMPLISHMENTS

Not applicable to this reporting period.

REPORTABLE OUTCOMES

Not applicable to this reporting period.

CONCLUSIONS

The vaccine has been manufactured, vialled, and tested for quality assurance. The study has been approved by both the FDA and our Human Subjects Division and is currently enrolling patients.

REFERENCES

None

APPENDICES

None