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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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14. ABSTRACT
The primary purpose of this grant is to determine the relapse free survival benefit with locally advanced and stage IV HER2 positive breast cancer patients vaccinated with a HER2 ICD peptide-based vaccine while receiving maintenance trastuzumab. The scope of work includes a Phase II single arm study of a HER2 ICD peptide-based vaccine given concurrently with trastuzumab. Fourteen patients have been enrolled during the last reporting period. All adverse events reported for these fourteen subjects are of low grade. Patients have developed significant T cell immune responses to the vaccine as we as epitope spreading indicating a Type I immune response is being elicited.

15. SUBJECT TERMS
Phase II, Clinical Trial, HER2, Trastuzumab

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INTRODUCTION

The scope of this study includes a Phase II single arm study of a HER2 ICD peptide based vaccine given concurrently with trastuzumab. Enrolled patients include either: (1) patients with locally advanced HER2-positive breast cancer (Stage IIIB and IIIC) who are in complete remission and within 1 year of diagnosis and initiating treatment with chemotherapy and trastuzumab or (2) Stage IV HER2-postive breast cancer patients who are in their first complete remission and defined as NED (no evidence of disease) or have stable bone only disease and are within 6 months of starting maintenance trastuzumab. The primary objective is to estimate relapse free survival compared to a historical control of patients treated with chemotherapy and trastuzumab (44% at 4 years). We hypothesize that the relapse free survival rate at 4 years with vaccination, if successful, would be 65%. Fifty-two patients will provide 92% power to detect a statistically significant increased survival rate compared to the fixed historical rate of 44% at the one-sided significance level of p=0.05.

Secondary objectives include 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 overall survival (OS) to assess the correlation of each of these outcomes with relapse.

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. Construct and vial the HER2 ICD peptide vaccine. This task has been completed. The vaccine product (lot 6002) continues to be monitored at specific intervals for product stability. A Stability Study Log for lot 6002 is maintained. The study log lists the testing dates 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 ± 2°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.

Testing is performed regularly; Table 1 provides a list of test times and outcomes. In the last three tests we have observed dimerization of this vaccine. We have developed an ELISPOT assay to assess the ability of our stored vaccine to stimulate peptide specific T cell immune responses. In the assay, we use four concentrations of ICD vaccine and peptide mixture (0.1, 1, 10 and 20ug/ml) respectively to stimulate T cell responses in donors. According to the data from 10 day ELISPOT assay, the stored ICD peptide based vaccine exhibited similar ability to elicit peptide specific T cell responses *in vitro* as compared to recently constructed and purified peptides. This assay serves as a functional validation of the continuing immunogenicity of the stored vaccine.

Table 1: Product Stability Testing Results

Testing Days	Stability Conditions Met?	Dimerization	Dimerization Outcomes
90	YES	None	Not applicable
180	YES	None	Not applicable
270	YES	None	Not applicable
360	YES	None	Not applicable
540	YES	None	Not applicable
720	YES	9%	Still able to elicit peptide specific T cell responses as compared to recently constructed and purified peptides.

1080	YES	14.8%	Still able to elicit peptide specific T cell responses as compared to recently constructed and purified peptides.
1440	YES	18.8%	Assay is on-going at this time

b. Enroll and treat patients. This study was officially approved by the US Army Medical Research and Materiel Command (USAMRMC) Human Subjects Research Review Board (HSRRB) on June 1, 2006. To date we have enrolled 23 subjects with 13 subjects being enrolled in the last reporting period (April 29, 2008 – April 26, 2009). Table 2 demonstrates the study status of all enrolled subjects through April 26, 2009.

Table 2. Study Enrollment Table

Study Time Point	Number of subjects completed to specified time point	Off Study
Vaccine 1	1	1[a]
Vaccine 2	2	0
Vaccine 3	4	0
Vaccine 4	3	0
Vaccine 5	2	1[b]
Vaccine 6	0	0
FU 1 (Month 7)	3	1[a]
FU 2 (Month 5)	2	0
FU 3 (Month 14)	0	0
FU 4 (Month 18)	6	0
Total 23		**3**

[a] Disease progression

[b] MUGA scan performed by the subject's oncology showed an ejection fraction decrease; subject also developed pneumonia and it was agreed the subject would not return to Seattle for 6th vaccine.

Realizing that our original targeted study population (Stage IV patients who are trastuzumab naïve) was rapidly shrinking due to recent changes in standard treatment as discussed above, we discussed the possibility of including Stage IIIC subjects in addition to the Stage IIIB and IV subjects (already approved). This is based on current literature which is showing that Stage IIIC and Stage IIIB patients are similar in terms of treatment (both groups receive both neoadjuvant and adjuvant chemotherapy in combination with trastuzumab for up to 12 months) and RFS and OS is similar in both groups.

For an amendment, which includes a change to study enrollment to be approved for implementation, we are required to get approval from our Institutional Review Board (IRB), U.S. Army Medical Research and Material Command (USAMRMC) Office of Research Protections (ORP) and the Food and Drug Administration (FDA) prior to implementing the modifications. We were ultimately approved to include stage IIIC breast cancer subjects to this study in addition to stage IIIB and IV breast cancer subjects on August 29, 2008. The approval timeline is summarized below in Table 3.

Table 3: Summary of Required Regulatory Approvals for Addition of Stage IIIC Patients

Regulatory Agency	Approval Date
Fred Hutchinson Cancer Research Center – Cancer Consortium IRB	July 27, 2008
USAMRMC ORP	August 6, 2008
FDA	August 29, 2008

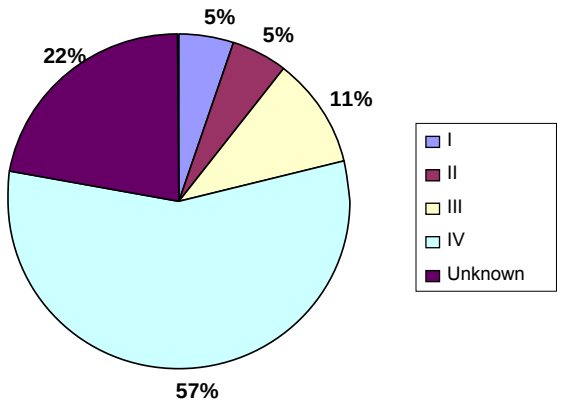
Given the changing population of patients and the fact that we've already enrolled some Stage IV patients, we will continue to enroll Stage IV patients until enrollment of the Stage IIIB/C patients is complete, recognizing that the power to detect a statistically significant difference compared to a historical benchmark will be severely limited due to the relative paucity of Stage IV patients. Nonetheless, an observed outcome that is superior to the historical benchmark will be considered to be encouraging in this population. As a result of the population changes we are tracking the stage of disease of enrolled subjects. Table 4 summarizes the total enrollment by stage.

Table 4: Summary of Stage of Disease of Enrolled Subjects

Stage of Breast Cancer	Total Number of Subjects
Stage IV	19
Stage IIIB	3
Stage IIIC	1

As reported in a quarterly report dated January 2009 we were recently featured on the November 13, 2008 episode of ABC World News with Charles Gibson. This episode was aired nationally and also posted on the ABC World News website with links to the TVG website. As a result, we received numerous phone calls and emails from patients across the country. Overall, there were a total of 102 new inquiries on our vaccine studies over six days. As demonstrated in Figure 1 the largest response was from Stage IV subjects and we believe this may account for a surge of Stage IV subjects ultimately being enrolled.

Figure 1: Potential Candidate Demographics by Stage of Disease



c. Interim statistical analysis after 25 patients have been followed for 1 year. Not applicable for this reporting period. It is understood that once we have enrolled 25 Stage IIIB and IIIC subjects that have been followed for 1 year we should perform an interim analysis of the data.

d. Final analysis of response. Not applicable for 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. We use the NCI Common Toxicity Criteria (CTC) for Adverse Events Version 3.0 to grade toxicities. We 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. Table 5 summarizes the most common adverse events experienced during the last reporting period.

Table 5. Summary of Most Common Adverse Events during Last Reporting Period

Adverse Events (AE)				
	All AE		Possibly, Probably, or Definitely Related	
Most Common Adverse Events	n	% of all AE	n	% of All Related AE
Injection site reaction	17	19	17	24
Myalgias	5	6	5	7
Lymphocytes	5	6	5	7

Leukocytes	4	4	4	6
Hypocalcemia	4	4	3	4
Fatigue	3	3	3	4
Headache	3	3	2	3
Arthralgia	3	3	3	4
Hypoprotenemia	3	3	3	4

	All AE		Possibly, Probably, or Definitely Related	
Adverse Event Grading	**n**	**%**	**n**	**%**
Grade 1	75	84	60	86
Grade 2	14	16	10	14
Grade 3	0	0	0	0
Grade 4	0	0	0	0
Grade 5	0	0	0	0

Please note that Table 5 records the most common adverse events regardless of severity. For example, a subject may have an injection site reaction at each of the three vaccines where another may not. Each one of these injection site reactions is recorded for that one subject. We have recorded a total of 89 individual adverse events for the reporting period: April 29, 2008 to April 26, 2009.

External Monitoring

As part of our Data Safety Monitoring Plan an independent monitor, assigned by the Clinical Trials Support Office at the Fred Hutchinson Cancer Research Center (FHCRC), verifies consent documentation for all newly enrolled subjects in addition to reviewing a select amount of data collected since the previous monitoring visit for randomly selected subjects. All regulatory documentation is reviewed including all IND documentation. We were monitored twice since the last reporting period: August 11-13, 2008 and January 28-29, 2009. There were no major findings. We are due again for monitoring in August 2009.

Medical Monitor Review

According to our Data Safety Monitoring Plan (DSMP) we are scheduled to meet with the Medical Monitor and related clinical research staff members bi-annually (twice a year). Prior to each meeting the Medical Monitor, Dr. Disis and related clinical research staff members are provided with an agenda and a Safety and Performance Report which includes total enrollment, adverse event reporting for and recently approved modifications and amendments for the reporting period. Meeting minutes are reviewed, approved and signed by the Medical Monitor before submitting the information to the Fred Hutchinson Cancer Research Center – Cancer Consortium IRB for review. Since the last Annual Report we have met with our Medical Monitor on June 30, 2008 and December 22, 2008. Our next Data Safety Monitoring Plan meeting is scheduled for June 2009.

b. Determine whether there is any cardiac toxicity associated with the co-administration of the HER2 ICD peptide based vaccine with trastuzumab. When subjects are enrolled we will closely monitor and document any abnormal cardiac events observed by us at clinic visits or reported to us by the subjects or physicians. All subjects have documentation of a MUGA/ECHO scan within 6 months for eligibility assessment and if that MUGA/ECHO scan is greater than 60 days old at time of eligibility we perform a MUGA/ECHO scan at their baseline visit. A follow-up MUGA/ECHO scan is performed again at 4 months post-vaccine. Table 6 compares ejection fractions at baseline and 4 Months Post-Last Vaccination.

Table 6: Baseline and 4 Month Post-Last Vaccine EF Evaluation

Subject # (n=23)	Pre-vaccine EF	4 months post-vaccine EF
12001	68%	60-65% (Echocardiogram)
12002	61%	65%
12003	65%	52%
12004	64%	Have not received follow-up documentation. Emailed physician for copy of report, we did not get a response.
12005	59%	57.5%
12006	64%	61%
12007	60%	Subject's disease progressed prior to follow-up visit.
12008	66%	51-53%
12009	56%	45.8% [a]
12010	51-52%	Subject went off Herceptin before this visit. A MUGA was not performed by her oncologist

[a] Primary oncologist is aware of EF drop. Off study per subject and last communication with her was March 25, 2008.

Two cardiac events have been observed, both reported during vaccination:

1. Grade 1 palpitations – Patient reports one episode of palpitations while watching TV and resolved spontaneously after about 5 minutes. No other related symptoms were reported. No other reports of palpitations have been reported since this one episode. Last episode of palpatations was one year ago while the subject was on chemotherapy.
2. Grade 2 Hypotension – After vaccination 4, which included a large blood draw, patient felt presyncopal and she had to sit down. The medics were called and she had a BP of 90/50. She received IVF in transit, in ER and also when discharged home. Patient did not lose consciousness. Resolved by following study visit.

It should be noted that both of these events were for the same subject (ID#: 12010).

c. Evaluate for any potential toxicities due to the generation of an immune response to HER2. The toxicities we would expect to see for an autoimmune response to HER2 would include: (1) skin reactions such as rashes, (2) gastrointestinal events such as severe diarrhea, (3) pulmonary events, (4) change in kidney function such as a change in creatinine or (5) cardiotoxicity. All of these toxicities are closely monitored, by a credentialed clinician such as a physician and/or physician's assistance, at each clinic visit. These toxicities are recorded and monitored by routine review of systems, clinical laboratory results, and other clinical assessment (i.e. chest x-rays, MUGAs, etc.). To date our toxicity reporting does not indicate any of our 23 subjects have developed an immune response to HER2. All toxicities on study have been of a low grade either grades 1 or 2 (Table 4).

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 evaluated the T cell responses to the three ICD peptides included in this vaccine and overlapping peptide pools for the HER2 intracellular domain (ICDpm) using a standard 10 day IFN-gamma(g) ELISPOT assay in eight patients. In this assay, PBMN before and after vaccinations were stimulated with p776, p927, p1166 and ICDpm respectively on Day 1, and re-stimulated on Day 8. The spots of IFN-g secreted after the stimulations were counted on day 10 using an ELISPOT plate reader. Our results show that p776 specific response (antigen specific cells/10^6 PBMN) increased 5 fold (pre vs. post: 74 ± 42 vs. 374 ± 143; mean ± SE; n=8. p=0.063), the p927 specific response increased 12 fold (pre vs. post: 39 ± 23 vs. 465 ± 184; p=0.037), and p1166 response increased 6 fold (pre vs. post 101 ± 48 vs. 618 ± 201; p=0.025) after the vaccination. The response to ICDpm increased 5 fold (pre vs. post: 126 ± 56 vs. 599 ± 198; p=0.037). Our group has previously established that ICDpm are equivalent to HER2 recombinant protein. Thus, the response to ICDpm may be an indicator of

successful immunization. In contrast, the response to tetanus toxoid (TT) did not increase post vaccination (p=0.512). Figure 2 shows these results. Among the eight patients, seven (88%) developed immunity to p776 and p1166, six (75%) developed immunity to p927 and the ICDpm (Figure 3).

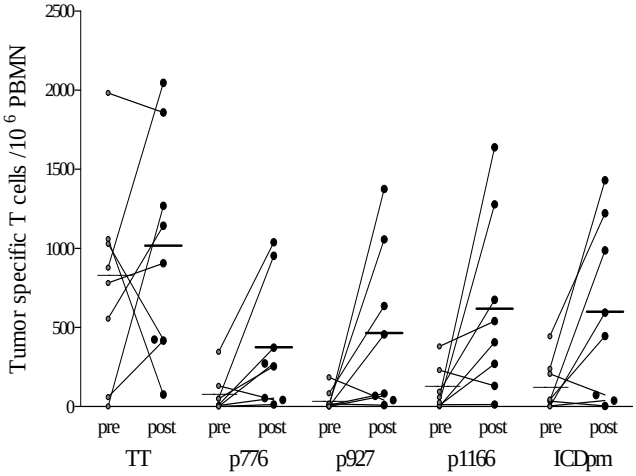


Figure 2. HER2 ICD peptide and protein T cell immunity elicited after the active vaccination. The bars indicate the mean.

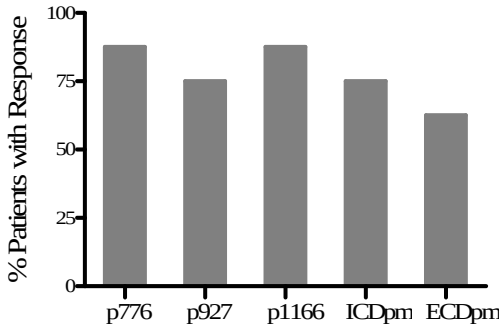


Figure 3. HER2 ICD peptide vaccine stimulated HER2 specific immunity in the majority of the patients.

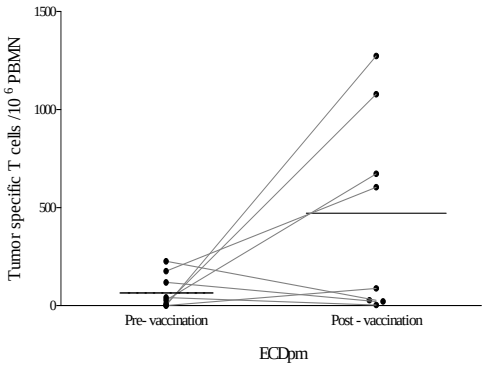


Figure 4 HER2 ECD immunity elicited after ICD peptide vaccination. The bars indicate the mean.

<u>b. Determine the incidence of epitope spreading to the HER2 ICD or other peptides in the immunizing mix (intermolecular epitope spreading).</u> We have evaluated the T cell responses to overlapping peptide pools for the HER2 extracellular domain (ECD pm), which is not included in the vaccine. We found that patients developed significant responses to ECDpm (pre vs. post: 75 ± 31 vs. 471 ± 181; n=8; p=0.049) (Figure 3). Among the eight patients, five (63%) developed epitope spreading. Our group has recently demonstrated that the patient's survival was significantly associated with the development of epitope spreading following vaccination.

In addition, we evaluated the serum levels of TGF-beta (b) in patients before and after vaccination using a human TGFb1 ELISA kit (eBioscience, San Diego, CA). TGF-b is an immunosuppressive cytokine secreted by tumor and Treg cells. We found that the levels of serum TGFb decreased in 7 of the 9 patients evaluated after vaccination. The mean level of hTGFb was 2,269 (± 857) pg/ml before the vaccination, and decreased to 1276 (± 381) pg/ml after 3rd vaccine and maintained at 1293 (± 609) pg/ml after 6th vaccine (mean ± SE, n=9; Figure 4). Thus, the mean level of serum TGFb decreased more than 40% after vaccination, although it did not reach significant differences. Our group has demonstrated that serum levels of TGFb is correlated with levels of $CD4^+CD25^+$ Treg cells. The decreased levels of serum TGFb may be an indicator that the Treg were not increased during the CD4+ targeted immunization, and may predict a better prognosis as elevated levels of serum TGFb are associated with an increased risk of relapse in breast cancer patients (Bates GJ et al J Clin Oncol 2006).

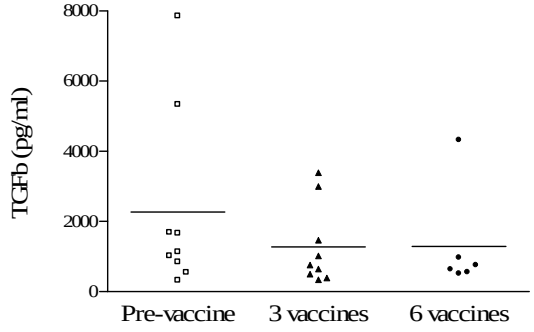


Figure 5. Levels of TGFb decreased in the serum of patients after vaccination with HER2 ICD peptides. The bars indicate the mean.

We further analyzed the correlation between the change of serum levels of TGFb post vaccination and HER2 ICD vaccine –induced T cell response at the same time point. We found that the greater the magnitude of HER2 specific T cell response, as demonstrated by IFNg secretion, the greater of decrease in serum TGFb. The increased T cell response to immunizing peptides significantly correlated with decreased levels of TGFb (p=0.0045, r=0.742, Fig 6A). The correlation between increased epitope spreading T cell response and decreased levels of TGFb was more significant

(p=0.0003, r=0.825, Fig 6B). In contrast, there was no correlation when evaluating the magnitude of tt response with change in TGF-b levels (p=0.839; r=0.041). Thus, the increasing numbers of tumor antigen specific T cells elicited via HER ICD peptide vaccination was associated with a decrease in serum TGF-b levels.

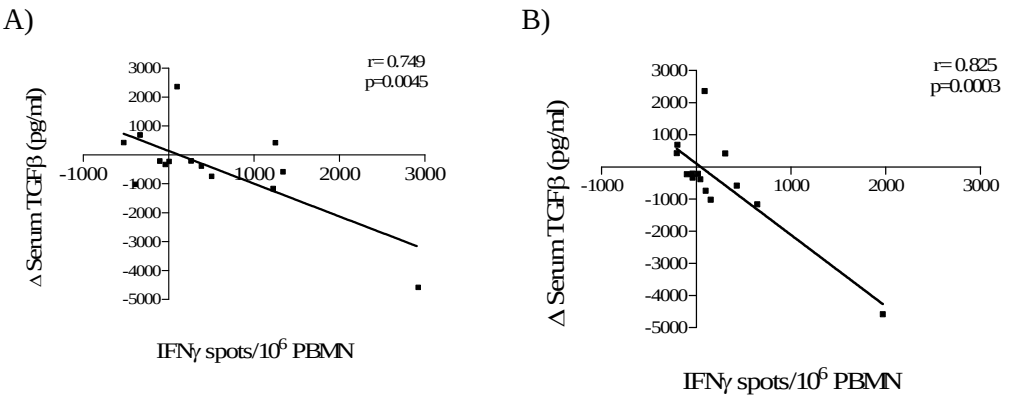


Figure 6. HER2 ICD peptide vaccine induced ICD peptide response (A) and epitope spreading (B) after immunization were associated with a decrease in serum TGF-beta levels. X axis: IFNg secretion (post-pre vaccine); Y axis: serum levels of TGFb (Post-pre vaccine).

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

- Substantially increased enrollment
- Demonstrated significant augmentation of immunity in vaccinated patients
- Demonstrated epitope spreading in the majority of patients evaluated to date

REPORTABLE OUTCOMES

CONCLUSIONS

We began study enrollment on December 29, 2006. We have since enrolled 23 subjects who are at varying phases of vaccination and follow-up. To date we have observed only low grade adverse events (Grades 1 & 2) most of which were expected. We will continue to enroll Stage IIIB/IIIC subjects to reach 25 subjects for the interim statistical analysis after 25 patients have been followed for 1 year.

Although we are performing our immunologic analysis as patients have completed early studies suggest we are both significantly augmenting a vaccinated immune response as well as generating epitope spreading in the majority of patients.

In order to successfully accomplish the scope of work for this project, the grant officer has granted us continued funding through May 2010.