AD_____

Award Number: **W81XWH-11-1-0704**

TITLE: **Chemotherapy Necessitates Increased Immune Control of HHVs: A Cause of Persistent Inflammation Enabling Protracted Fatigue in Breast Cancer Survivors**

PRINCIPAL INVESTIGATOR: Jessica E Thaxton, PhD, MsCR

CONTRACTING ORGANIZATION: Medical University of South Carolina
Charleston SC 29425

REPORT DATE: October 2014

TYPE OF REPORT: Annual Summary Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2014	Annual Summary	15 Sept 2013 -14 Sept 2014

4. TITLE AND SUBTITLE
Chemotherapy Necessitates Increased Immune Control of HHVs: A Cause of Persistent Inflammation Enabling Protracted Fatigue in Breast Cancer Survivors

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0704

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Jessica Thaxton, PhD, MsCR

email: Thaxton@musc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of South Carolina
Charleston, SC 29425

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT

12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited

14. ABSTRACT
This work hypothesizes that chemotherapy can permanently alter the balance between the immune system and chronic herpes virus infections. We predicted that herpes virus-driven inflammatory cytokines exacerbate cancer treatment related fatigue (CTRF). Here we report the significant finding that high C-reactive protein (CRP) levels, indicative of underlying inflammatory milieu, are predictive of fatigue in a retrospective cohort of n=108 breast cancer survivors. Survivor HHV burden, as assessed by number of viruses carried by a subject was not predictive of fatigue or CRP levels. Further, type of HHVs carried by subjects was not predictive of fatigue or CRP levels. We further sought to assess CRP levels, as a predictive biomarker of breast cancer survivor fatigue, in women actively undergoing chemotherapy treatment for breast cancer. In a cohort of n=20 subjects we found a significant increase in CRP levels between EBV+ and EBV/CMV double positive subjects. Moreover, EBV/CMV double positive individuals showed increased serum IFN-γ. High IFN-γ levels were significantly associated with enhanced serum hs-CRP. Taken together, fatigued breast cancer survivors had higher levels of hs-CRP. Similarly, CMV/EBV double positive breast cancer patients undergoing chemotherapy showed higher hs-CRP levels than CMV-/EBV+ subjects. CRP and viral status in subjects actively undergoing breast cancer therapy predict the patient population at risk for CTRF.

15. SUBJECT TERMS
breast cancer, chemotherapy, immunology, human herpes viruses, survivor fatigue

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	14	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

INTRODUCTION:

This work hypothesizes that chemotherapy can permanently alter the balance between the immune system and chronic herpes virus infections. We predicted that herpes virus-driven inflammatory cytokines exacerbate cancer treatment related fatigue (CTRF). Here we report the significant finding that high C-reactive protein (CRP) levels, indicative of underlying inflammatory milieu, are predictive of fatigue in a retrospective cohort of n=108 breast cancer survivors. Survivor HHV burden, as assessed by number of viruses carried by a subject was not predictive of fatigue or CRP levels. Further, type of HHVs carried by subjects was not predictive of fatigue or CRP levels. We further sought to assess CRP levels, as a predictive biomarker of breast cancer survivor fatigue, in women actively undergoing chemotherapy treatment for breast cancer. In a cohort of n=20 subjects we found a significant increase in CRP levels between EBV+ and EBV/CMV double positive subjects. Moreover, EBV/CMV double positive individuals showed increased serum IFN-γ. High IFN-γ levels were significantly associated with enhanced serum hs-CRP. Taken together, fatigued breast cancer survivors had higher levels of hs-CRP. Similarly, CMV/EBV double positive breast cancer patients undergoing chemotherapy showed higher hs-CRP levels than CMV-/EBV+ subjects. CRP and viral status in subjects actively undergoing breast cancer therapy predict the patient population at risk for CTRF.

Specific Aim 1: To determine whether the number of HHV infections and/or the type of HHV infection carried by an individual contributes to protracted fatigue in BC survivors.

Specific Aim 2: To monitor fatigue levels, HHV infections, and HHV-specific immunity in BC patients during chemotherapy to assess the impact of therapy on immune control of HHVs and CTRF outcomes.

BODY:

In the past year we have completed our collection and analysis phase of specific aim 1 and we have adopted a significantly modified experimental approach in order to achieve the aims initially proposed in specific aim 2. In this summary we report our analysis of n=108 breast cancer survivor samples for HHV status of EBV, CMV, HSV-1, and VZV. We present our analysis of HHV sero-status and subject reported fatigue scores. Further, we report the finding that, of cytokines assayed in sera, we found a significant association between C-reactive protein and subject reported fatigue score. Secondly, we report obtainment of n=20 patient samples from breast cancer patients actively undergoing chemotherapy treatment. We show analysis of CMV and EBV sero-status and demonstrate that, in agreement with our data from breast cancer survivors, we found that CRP levels are greater in CMV/EBV subjects and is correlated with IFN-γ levels in these subjects. Thus, in accordance with our initial hypothesis, CMV/EBV double positive sero-status may predispose women to experience greater CTRF and CRP levels are a biomarker in this patient population. The conclusion of this report will discuss future collection of n=20 subject samples from breast cancer patients undergoing chemotherapy. We will use these samples to assess associations among CRP levels, IFN-γ levels, CMV/EBV sero-status, and virus-specific T cell responses.

Specific Aim 1:

We first aimed to test if an association existed between the number and/or type of HHVs a breast cancer survivor carried and CTRF experienced by a subject. In collaboration with Dr. Kerri Winters-Stone at Oregon Health & Science University, we obtained n=120 breast cancer survivor serum samples and matched fatigue data. Survivors were cancer free for two to ten years and fatigue scores were a result of the PROMIS fatigue survey (NIH) where 5 was the least level of fatigue and 40 was the greatest level of fatigue a patient could report.

We analyzed n=120 subject samples for IFN-γ, TNF-α, IL-10, neopterin, and CRP via ELISA and recorded serum cytokine levels as continuous variables. For each subject we obtained baseline fatigue scores and categorized these as continuous outcomes. Further, each subject serum samples was assayed for n=4 human herpes viruses, CMV, EBV, VZV, and HSV-1 and viral status was categorized as a dichotomous variable (yes or no). 109 subjects had complete data sets with no missing variables. Of 109 subjects n=55, n=75, n=99, n=101 were HSV-1, CMV, EBV, and VZV+, respectively. 5 subjects carried only one virus, 24 subjects carried 2 viruses, 42 subjects carried three viruses, and 38 subjects carried all four viruses.

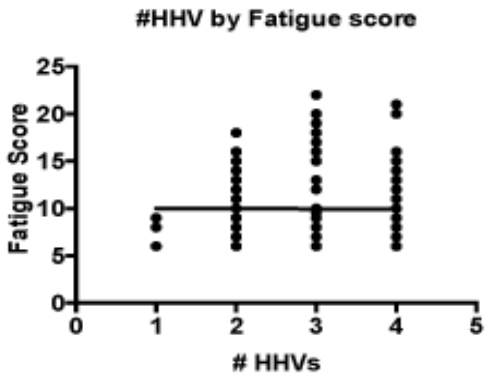


Figure 1. Number of HHVs carried is not predictive of CTRF in breast cancer survivors.

We did not find any significant associations between the number of viruses carried by a subject and basal fatigue scores as assessed by linear regression analysis. Figure 1 shows the lack of slope in the linear regression generated to predict a relationship between fatigue score and number of viruses carried by and individual. Similarly, we did not detect a significant

relationship as assessed by linear regression analysis, between the type of herpes virus carried by a subject and reported fatigue score (Figure 2). For these data we tested whether an association existed among subjects that were all carriers of EBV, CMV, HSV-1, or VZV. As Figure 1B shows the mean fatigue score for all subjects was 9.75 (n=180) and the mean for each herpes virus fell within a range between 9.25 and 11.25. Importantly, EBV was the only herpes virus where carriers in our survivor cohort (indicated by 1) showed a mean fatigue score (9.8) greater than non-carriers (indicated by 0, mean is 9.2). All non-HSV-1 carriers (0) averaged a 9.84 fatigue score and carriers (1) averaged 9.74. All non-CMV carriers (0) averaged a 9.9 fatigue score and carriers (1) averaged 9.6. All non-VZV carriers (0) averaged 11.2 fatigue score and carriers (1) averaged 9.7.

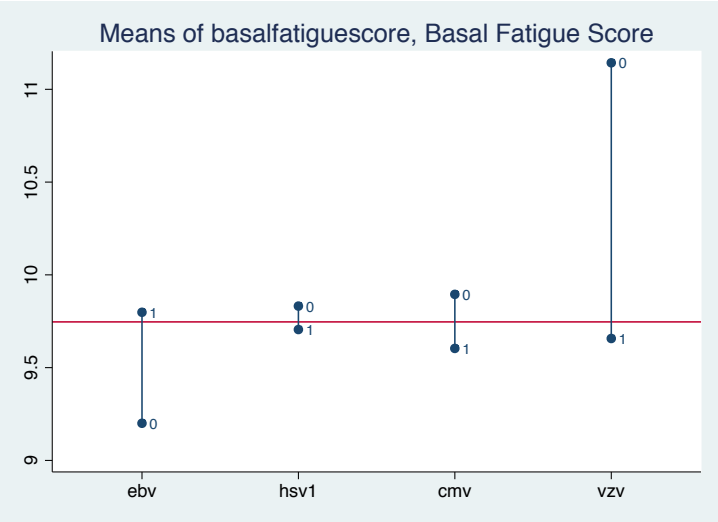


Figure 2. Type of HHV carried by breast cancer survivors is not predictive of CTRF.

We next analyzed n=120 subject samples for IFN-γ, TNF-α, IL-10, neopterin, and CRP via ELISA and recorded serum cytokine levels. We firs tested a subset of serum samples to determine specific dilutions at which to test the entire cohort of serum samples. We undertook IFN-γ analysis at 1:4 serum dilutions. TNF-α, and IL-10 assays were performed at 1:1 dilutions of serum per samples and neopterin ELISAs were performed at 1:2 dilutions. Finally CRP analysis was performed at 1:4000 dilution factor. Due to the fact that we did not find a significant association among type of viruses carried or number of viruses carried and fatigue score, we first tested whether an association existed between type or number of viruses carried and individual cytokine levels. We found that type or number of viruses carried showed no detectable relationship with specific cytokine levels among subjects (ex: CRP by fatigue score Figure 3).

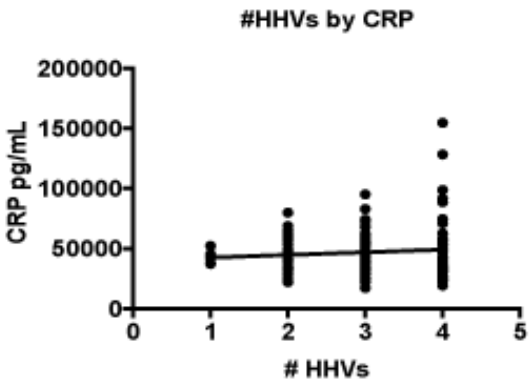
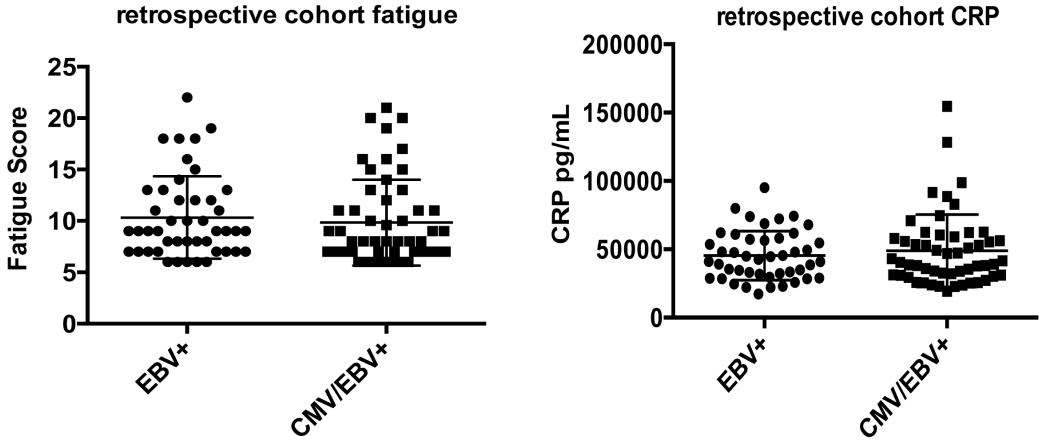


Figure 3. Graphic example of cytokine by fatigue score analysis.

Due to our findings presented under the Specific Aim 2 section of this report we assessed associations between fatigue score and EBV+ or CMV/EBV double positive viral status. We reasoned that the majority of subjects in our Specific Aim 1 cohort were VZV positive, and thus we would not be able to separate groups into positive and non-positive for VZV. Further, HSV-1 positive subjects cannot be assessed for virus-specific T cells due low levels of virus specific T cells allotted by the host to control viral foci as

well as the lack of available laboratory tools available to HSV-1-specific T cell responses. Our assessment of 97 breast cancer survivors, for EBV alone or EBV/CMV double positive viral status indicated that 45 subjects were positive for EBV alone and 53 were positive for both viruses. We next sought to determine if a relationship existed between fatigue score and EBV+ or EBV/CMV double positive status (Figure 4A). For our survivor subjects we were unable to detect a meaningful relationship for the aforementioned variables. We sought to determine if CRP levels correlated with EBV+ or EBV/CMV double positive viral status, based on our finding in significant aim 2. We did not detect a meaningful relationship for the aforementioned outcomes (Figure 4B).

Figure 4. A. EBV+ or EBV/CMV+ status is not significantly correlated with CTRF in breast cancer survivors. B. EBV+ or EBV/CMV+ status is not significantly correlated with CRP levels in breast cancer survivors.



We reasoned that a detectable association might not exist in survivor populations between CRP and EBV/CMV viral status. Viral reaction and immune-shaping most likely occurs over the course of chemotherapy and upon immune-reconstitution post treatment. CRP levels during cancer treatment are associated with viral status (Specific Aim 2), but there may not be a detectable association in survivor populations as CRP may be high due to other inflammatory parameters in the body at the time CTRF is measured in survivors. Thus, we aimed to determine if a relationship existed directly between subject reported fatigue score and CRP levels in survivor populations. As Figure 5 demonstrates, linear regression analysis showed a significant relationship between high fatigue score and high serum levels of CRP ($p=0.0006$). Taken together these data indicate that CRP in survivor populations is predictive of high levels of fatigue. These data agree with recently published results that indicate that CRP levels are predictive of highly fatigued survivor populations [1,2]. Our results in specific aim 2 lend insight to the potential mechanism that may lead to, or are predictive of, CTRF prior to and during the course of chemotherapy in breast cancer patients.

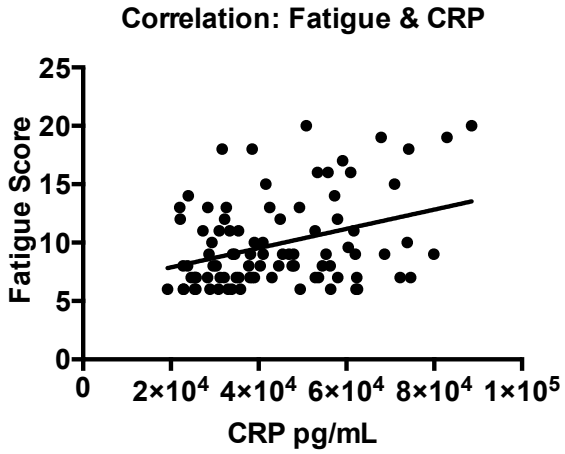


Figure 5. CRP levels predict CTRF in breast cancer survivors.

Specific Aim 2: Our initial experimental plan called for identification, recruitment, enrollment, fatigue survey administration and sample collection of n=70 breast cancer patients. We aimed to correlate subject fatigue score during chemotherapy to subject EBV+ or CMV/EBV double positive status and to associate these data with subject-specific peripheral blood T cell virus-specific responses. Our study suffered a setback when our collaborating medical oncologists, Rita Kramer MD and Neil Christensen MD, unexpectedly resigned from Medical University of South Carolina. These two MDs were the only breast cancer medical oncologists involved in research at MUSC. The clinical trials office with whom we have worked with to trouble-shoot subject enrollment/recruitment did not have any feasible solutions for our study over the past eight months. Thus, active breast cancer subject recruitment and enrollment was not possible from February 2014 to present.

Table 1. Breast cancer subject samples by histological type and stage.

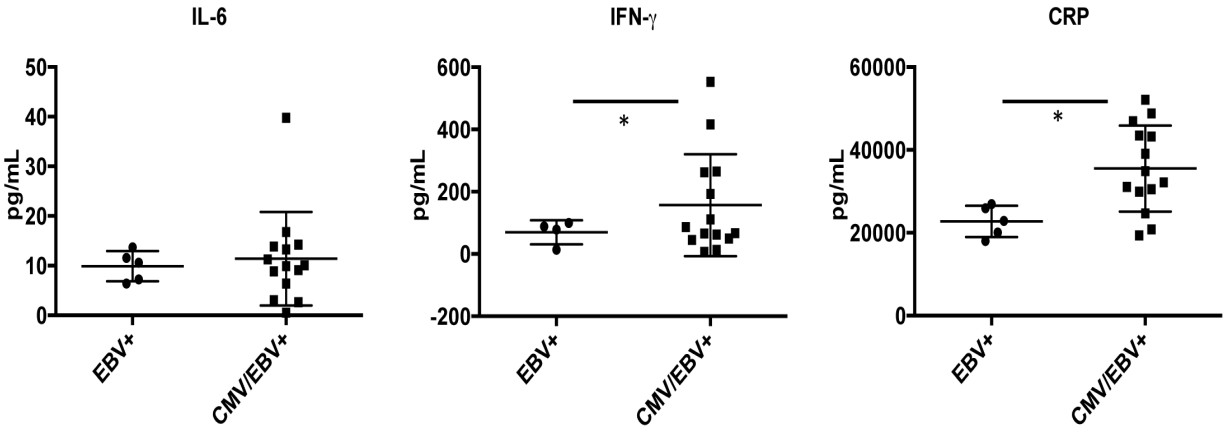
Histology	Stage
invasive ductal carcinoma	T1cNO(ITC)Mx
lobular carcinoma	T1bN1Mx
lobular carcinoma	T2NxMx
invasive ductal carcinoma	T1aN1a
invasive ductal carcinoma	T1bN1a
invasive ductal carcinoma	T1cN1
invasive ductal carcinoma	T2Nx
invasive ductal carcinoma	T1bNO
invasive ductal carcinoma	
invasive ductal carcinoma	T3N2a
invasive ductal carcinoma	T1cNO
invasive ductal carcinoma	T1cN1a
invasive ductal carcinoma	T1bNx
invasive ductal carcinoma	T1bNO
ductal carcinoma	T1cN1a
invasive ductal carcinoma	T1bN1a
focal residual ductal carcinoma	
invasive lobular carcinoma	T1cNOMx
invasive ductal carcinoma	T1aNO
invasive ductal carcinoma	T1aNOMx

We adapted our experimental design and were able to produce significant results from our adaptation. Further, due to our findings presented below in n=20 breast cancer subjects, we have a feasible experimental approach that will answer our initial scientific questions by the close of our study in April 2015. We obtained n=20 banked breast cancer patient samples, serum and plasma, from the Bio-repository at MUSC. Table 1 depicts the de-identified patient samples that were tested. Histological and stage data demonstrate that subjects ranged in breast cancer type, stage, and metastatic activity. We first tested subject serum for CMV and EBV sero-positivity. Since IL-6 cytokine production has previously been reported to indicate subject fatigue level, we undertook IL-6 ELISAs on subject serum as a measure of subject fatigue. [3] IFN-γ and CRP are direct products of virus-specific T cell activation, thus we undertook IFN- γ and CRP ELISAs on subject serum as an indirect measure of virus-specific T cell activity. [4,5].

Finally, we reasoned that frozen plasma from subjects would contain a buffy coat layer of peripheral blood mononuclear cells. Thus, we undertook a protocol to isolate PBMC from patient plasma samples (1 mL of plasma was available to us) to assess whether we could detect virus-specific T cell responses in these samples. Upon obtainment of PBMC from subjects (2.56x10^3 cells were recovered on average) we plated PBMC with two EBV or two CMV immune-dominant virus-specific peptide pools, EBNA-1, BZLF-1, IE1, or pp65, respectively. We assessed IFN-γ responses from live-gated CD3/CD8+ T cell populations.

Serum Cytokines: Our assessment of IL-6 showed EBV+ subjects had a mean value of 9.893 ± 1.363 pg/mL and CMV/EBV double positive subjects had a mean value of 11.40 ± 2.519 pg/mL. Two tailed t-test between the two virus groups and IL-6 levels showed no significant correlation. Our assessment of CRP showed EBV+ subjects had a mean value of 22767 ± 1687 pg/mL and CMV/EBV double positive subjects had a mean value of 35511 ± 2775, pg/mL. Two tailed t-test between the two virus groups and CRP was significant, p=0.0011. We next assessed IFN- γ between viral groups and found mean values of 69.82 ± 19.27 pg/mL for the EBV+ group and 156.9 ± 43.72 pg/mL for the double positive virus group. Two tailed t-test analysis with Welch's correlation of these means proved significant, p=0.0359 (Figure 6). Taken together, these data show that CMV/EBV double positive viral status is significantly associated with enhanced CRP and IFN-γ levels in serum of breast cancer patients actively undergoing therapy.

Figure 6. Breast cancer patient viral status predicts IFN-γ and CRP levels.



We next aimed to determine whether CRP and IFN-γ levels were associated with one another on a per patient basis in the EBV+ group or in the CMV/EBV double positive group. We performed linear regression analysis on the four samples from our cohort of breast cancer patients that were EBV+ only and found no correlation, through linear regression analysis, between CRP and IFN-γ ($p=0.2549$). Strikingly, in the double positive virus group, a linear regression of CRP and IFN- γ levels of each subject within this groups showed a significant association where $p=0.0432$ (Figure 7). These data demonstrate that virus double positive breast cancer patients, who are actively undergoing therapy, have elevated IFN- γ and CRP levels.

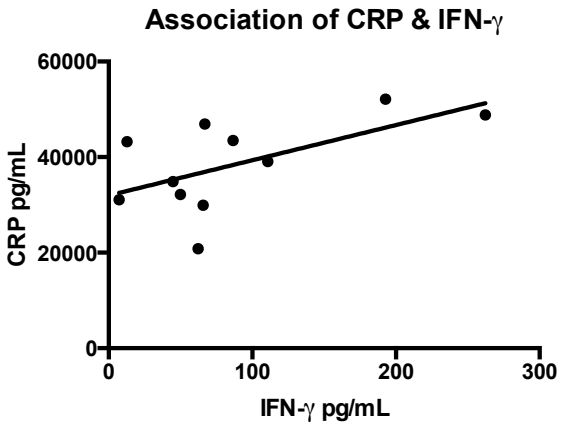


Figure 7. CRP and IFN-γ levels are significantly association in CMV/EBV double positive breast cancer patients only.

In our final arm of analysis we aimed to determine if an association exists between virus-specific T cells and subject fatigue. These data are important to confirm the abovementioned data and to demonstrate an underlying cause of subject fatigue that is measurable during the lympho-depletive window of chemotherapy. Our attempt to obtain virus-specific T cell cytokine levels from plasma samples collected from Table 1 subjects was hindered by low cell numbers and low cell viability. We were only able to process 1 mL of plasma. Cell numbers recovered were too low to accurately analyze flow cytometric data. EBV specific responses averaged 3.4 ± 3.3 (EBNA-1) 0.85 ± 1.7 (BZLF-1) and CMV specific response 1.9 ± 2.4 (IE1). These responses were real in four of 16 samples analyzed as determined by isotype control and un-stimulated cell group negative gating. When averaged as a whole these data were null due to the high level of standard deviation in the majority of samples.

Due to our significant findings presented here it is important to test the virus-specific T cell response from PBMC isolated from patients. Frozen plasma is a less than ideal product from which to take PBMC. Currently, we have n=12 frozen PBMC samples prepared from this study and we aim to collect between n=10 and n=20 more subject samples. We wish to perform the acquisition of data from peptide re-stimulation and subsequent flow cytometric analysis at one time due to inter-experimental variations. We have enlisted the help of Dr. Carolyn Britten and Clinical Trial Office Director Terri Matson for the collection of our remaining samples. Thus our remaining analysis will be to determine CMV and EBV sero-status on n=20 (approx.) subject samples. We will measure serum for CRP and IFN-γ levels and we will measure virus-specific T cell responses from PBMC of subjects.

KEY RESEARCH ACCOMPLISHMENTS

- Performed CMV, EBV, HSV, VZV analysis on n=160 retrospective BC subject samples
- Performed IL-10, TNF-α, IFN-γ analysis on 67 retrospective BC subject samples
- Obtainment of n=160 basal fatigue scores from retrospective BC subject samples
- Statistical analysis of HHV status, cytokine measures, and basal fatigue score among n=160 retrospective subject samples
- Performed hs-CRP analysis on n=160 retrospective BC subject samples
- Statistical analysis of hs-CRP, basal fatigue score, and EBV/CMV sero-status data from n=160 subject samples retrospective BC subject samples
- Renewal of prospective protocol by MUSC IRB
- Obtainment of n=20 breast cancer subject samples, plasma & serum, from MUSC biorepository
- Performed IL-6, CRP, IFN-γ, CMV and EBV analysis on n=20 subject samples
- Performed peptide re-stimulation on n=20 plasma subject samples & flow cytometric analysis for virus-specific T cell IFN-γ production
- Statistical analysis of IL-6, CRP, IFN-γ, CMV, and EBV data from n=20 subject samples
- Establishment of breast cancer mentorship research team

Dr Stephen Ethier	Chair in Breast Cancer Diagnosis, Treatment, and Research
Dr Elizabeth Garret-Meyer	Director of Biostatistics, HCC
Dr Chanita Hughes-Halbert	Chair in Cancer Equity. Cancer Disparities
Dr Rita Munn Kramer	Breast Cancer Oncologist
Dr Zihai Li	Chair Microbiology & Immunology

REPORTABLE OUTCOMES:

- Identification of CRP as a biomarker of patient reported fatigue in breast cancer survivors
- Identification of CRP and IFN-γ as significantly associated with CMV/EBV double positive status in subjects actively undergoing chemotherapy for breast cancer
- Manuscript in preparation:
  C-reactive protein and CMV/EBV viral status identify an at risk populations for cancer treatment related fatigue among breast cancer patients
  Submission: February 2015, Brain Behavior and Immunity
- Thesis defense of Masters in Clinical Research, July 2014
- Receipt of Masters in Clinical Research, July 2014 cum: GPA 4.0

CONCLUSION:

We are in line with our SOW for SAI and we have made significant progress on SAII. We have completed our sample collection and analysis from SAI and these data have allowed us to focus the goals of SAII to achieve a significant publication. This work can be concluded by the completion of this study in April 2015 with the collection of 10-20 more patient samples and subsequent analysis listed above. The finding the CRP levels correlate with CTRF in breast cancer survivors coupled to our finding that CMV/EBV+ breast cancer patients show higher CRP and IFN-γ levels will produce a significant contribution to this our field.

Statement of Work:

September 2012-October 2012

1. Submit IRB to MUSC for expedited review to use samples from OHSU	Accomplished
2. Meet with MUSC research team to plan prospective patient sample collection	Accomplished
3. Prepare and submit full review IRB to MUSC for human subjects use approval	Accomplished

November 2012-February 2012

4. Receive retrospective cohort samples from OHSU	Accomplished
5. Perform HHV analysis on patient samples (VZV, EBV, CMV, HSV-1) to determine seropositive/negative status	Accomplished
6. IP-10 flow based assay, neopterin assay sample analysis	Accomplished

February 2013-July 2013

7. Coordinate coded fatigue data with serology and inflammatory protein results	Accomplished
8. Statistical consultation/analysis for fatigue score with HHV type or #	Ongoing
9. If necessary include total 285 subject data for enhanced significance	Accomplished
10. Preparation of data and production of manuscript 1	No significant findings
11. Patient recruitment and sample collection for SA2 begins	Accomplished

August 2013-January 2014

12. Assess patient recruitment rates and sample collection efficacy	Accomplished
13. Meet with study team for SA2 to revise and/or insure study maintenance	Accomplished
14. Patient recruitment and sample collection for SA2 continues	Accomplished
15. HHV analysis of baseline samples for SA2 to determine sero-status	Accomplished
16. PCR of CMV+/EVB+ for viral DNA in sero+ samples from 4th cycle 3-6 month follow up to determine if viral DNA is detectable	Not feasible
17. IP-10 flow cytometry based assay	Not feasible

February 2014-May 2014

18. Assess patient recruitment rates and sample collection efficacy	Accomplished
19. Meet with study team for SA2 to revise and/or insure study maintenance	Accomplished
20. Patient recruitment for SA2 is completed	Not feasible
Alternative n=20 subject samples collected from biorepository	Accomplished

21. HHV analysis of baseline samples for SA2 to determine sero-status Accomplished
22. PCR of CMV+/EVB+ for viral DNA in sero+ samples from 4th cycle Not feasible
23. Coordinate patient fatigue data with HHV sero-status and inflammatory data Accomplished
24. Statistical analysis/consultation for significance between SA1 parameters and SA2 (long-term vs short-term fatigue associations) Accomplished
25. Preparation and submission of manuscript 2 from data (24) Ongoing

June 2014-June2015 (revised beow)

26. Sample collection and 1 year patient follow-ups complete Not feasible
27. Finalize serum analysis of HHVs for all time points obtained (changes from baseline if detectable)
28. Finalize viral DNA PCR for EBV/CMV for all time points obtained (changes from baseline or 4th cycle to long-term follow-up time points if detectable)
29. Finalize IP-10, neopterin cytokine detection for all time-points obtained
30. Flow cytometry assays performed for immune cell changes between patient time-points collected for sero+ individuals: EVB/CMV peptide restimulation
31. Coordinate fatigue data with sero-status, viral-DNA outcomes, inflammatory cytokine status (IP-10, neopterin) and PBMC-virus specific immune activity data
32. Analysis/consultation for statistical significance for measured parameters (30)
33. Prepare, submit 2 manuscripts (manuscripts 3 and 4 from these data)

October 2014-April 2015 (Revised)

Collection of n=10 to n=20 subject samples, serum & PBMC by January 2015
CMV, EBV, CRP, IFN-g Serum analysis February 2015
CMV, EBV virus-specific T cell stimulation and intracellular cytokine measurement February 2015
Complete Manuscript March 2015
Manuscript submission April 2015

References:

1 Crosswell, A. D., Lockwood, K. G., Ganz, P. A. & Bower, J. E. Low heart rate variability and cancer-related fatigue in breast cancer survivors. *Psychoneuroendocrinology* **45**, 58-66, doi:10.1016/j.psyneuen.2014.03.011 (2014).

2 Orre, I. J. *et al.* Higher levels of fatigue are associated with higher CRP levels in disease-free breast cancer survivors. *Journal of psychosomatic research* **71**, 136-141, doi:10.1016/j.jpsychores.2011.04.003 (2011).

3 Zick, S. M. *et al.* Preliminary differences in peripheral immune markers and brain metabolites between fatigued and non-fatigued breast cancer survivors: a pilot study. *Brain imaging and behavior*, doi:10.1007/s11682-013-9270-z (2013).

4 Bennett, J. M. *et al.* Inflammation and reactivation of latent herpesviruses in older adults. *Brain, behavior, and immunity* **26**, 739-746, doi:10.1016/j.bbi.2011.11.007 (2012).

5 Li Pira, G. *et al.* A registry of HLA-typed donors for production of virus-specific CD4 and CD8 T lymphocytes for adoptive reconstitution of immune-compromised patients. *Transfusion*, doi:10.1111/trf.12754 (2014).