**UNIVERSITY OF WASHINGTON**
**DEPARTMENT OF MEDICINE**
**DIVISION OF ONCOLOGY**

**Protocol 135**

# Phase I/II Randomized Study of Combination Immunotherapy With or Without Polysaccharide Krestin (PSK®) Concurrently with a HER2 ICD Peptide-Based Vaccine in Patients with Stage IV Breast Cancer Receiving HER2-Targeted Monoclonal Antibody Therapy

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**Table of Contents** **Page**

## SYNOPSIS

**Title**

Phase I/II Randomized Study of Combination Immunotherapy With or Without Polysaccharide Krestin (PSK®) Concurrently with a HER2 ICD Peptide-Based Vaccine in Patients with Stage IV Breast Cancer Receiving HER2-Targeted Monoclonal Antibody Therapy

**Study Population**

Patients with Stage IV HER2+ breast cancer who have been treated with definitive therapy and are receiving maintenance HER2-targeted monoclonal antibody therapy (defined as trastuzumab monotherapy or combination trastuzumab and pertuzumab therapy); and are currently without evidence of disease (NED) or have stable-bone only disease.

**Rationale**

Hot-water extracts of mushrooms have been used in traditional medicine in China, Japan, and other Asian countries for centuries. PSK was isolated in Japan from the *Trametes versicolor* mushroom in the 1960s and has been studied in more than 48 clinical trials for various types of cancers. Randomized clinical trials have shown low toxicity profile and improved survival in breast cancer patients treated with PSK. Pre-clinically, *in vitro* studies have shown PSK to be a potent TLR-2 agonist that activates dendritic cell maturation, activates human NK cells, induces pro-inflammatory cytokine/chemokine secretion and potentiates trastuzumab-mediated ADCC against breast cancer cells. PSK has also been shown to potentiate antitumor effects of anti-HER2 mAb therapy in neu-transgenic mice. We hypothesize that the use of PSK will augment the innate anti-tumor immune responses specifically NK-cell activity. Additionally, PSK could potentially augment HER vaccine induced Th1 immunity and collectively the combination therapy could result in enhanced eradication of micrometastatic disease which could prevent relapse in patients with optimally treated HER2+ Stage IV Breast Cancer.

Oral PSK at 3000 mg/day has been shown to be safe with and without chemotherapy in breast cancer. Moreover, combination immunotherapy with HER2-directed immunotherapy (HER2 ICD vaccination and HER2-targeted monoclonal antibody therapy) has been shown to be safe and immunogenic. However, the safety of PSK when added to HER2-directed immunotherapy is less well known and warrants further investigation. This study will allow us to better define the safety and immunomodulatory effects of this combined immunotherapy regimen.

**Objectives**

*Primary*

1. To evaluate the safety of PSK when given with HER2-directed immunotherapy.

*Secondary*

1. To evaluate the effect of PSK on NK cell functional activity when given with HER2-directed immunotherapy.

*Tertiary*

1. To investigate the effect of PSK when given with HER2-directed immunotherapy on:
    a. Serum levels of pro-inflammatory cytokine and/or chemokines.
    b. Intermolecular epitope spreading.
    c. Serum TGF-β levels.
    d. Progression free survival (PFS) and overall survival (OS).

**Study Design**

A phase I/ II double-blind randomized two-arm clinical trial designed to examine the safety and immune effects of PSK when given as combination HER2-directed immunotherapy (a HER2 ICD peptide vaccine and HER2-targeted monoclonal antibody therapy) in patients with Stage IV HER2+ breast cancer. Eligible patients will be enrolled and randomized in equal numbers to one of 2 arms (15 patients per arm).

**Arm 1**:

***HER2 ICD vaccine + HER2-targeted monoclonal antibody therapy* + Placebo***: Patients will receive HER2 ICD peptide vaccine administered concomitantly with HER2-targeted monoclonal antibody therapy and placebo product.

**Arm 2**:

***HER2 ICD vaccine + HER2-targeted monoclonal antibody therapy* + PSK***: Patients will receive HER2 ICD peptide vaccine administered concomitantly with HER2-targeted monoclonal antibody therapy and PSK.

*****Trastuzumab (or trastuzumab and pertuzumab) will be administered per standard of care by patient's primary oncologist.

**Number of Patients** A total of 30 patients will be enrolled and randomized in equal numbers to one of 2 arms with PSK or placebo.

**Outcome Measures**

*Primary Endpoint:*

1. *Evaluation of safety of PSK when administered with HER2-directed immunotherapy.* Evaluation of safety and systemic toxicity will include physical examination and clinical labs performed at various timepoints. Toxicity grading will be evaluated per CTEP CTCAE 4.0 and monitoring of adverse events will be done per FDA and NCI guidelines. Additionally, stopping rules for accrual to study will become operational if there is sufficient evidence of excessive toxicity.

*Secondary Endpoint:*

1. *Evaluation of the effect of PSK on NK cell functional activity.* PBMC will be obtained and cryopreserved at baseline (prior to start of PSK) and then at 4, 8, and 16 weeks. PBMC will subsequently be thawed and used to determine the effect of PSK on NK cell functional activity specifically, induction of IFN-gamma production and CD107a expression in NK cells, via flow cytometry. Augmentation of NK cell activity is defined by a 2-fold increase in NK cell IFN-gamma production and CD107a expression from baseline after 4 weeks of oral administration of PSK.

*Tertiary Endpoints:*

1. *Assessment of the effect of PSK on pro-inflammatory serum cytokine and/or chemokines when administered with a HER2-directed immunotherapy.* Serial blood draws will be done before initial dose of PSK (time 0) and 30 min, 4 hours, and 24 hours post-initial PSK dose to assess serum cytokines/chemokines (i.e., IL-6, IFN-γ, and TNF-α) via Luminex analysis. Serum cytokine/chemokine levels at different timepoints post-PSK will be compared to baseline levels. Serum will be cryopreserved and subsequently be thawed at time of analysis.
2. *Assessment of the effect of PSK on intermolecular epitope spreading when administered with HER2-directed immunotherapy*. IFN-γ ELISPOT assay will be used to evaluate T cell precursor frequency to specific breast tumor antigens (HER2, IGFBP-2, TOPO-IIα, p53, and PRAME) at various timepoints. A positive immune response will be defined as a post-vaccination T cell precursor frequency >1:20,000 antigen-specific PBMCs. In patients with a baseline precursor frequency >1:20,000, a positive post-vaccination immune response will be defined as a 2-fold increase in antigen-specific PBMC. PBMC will be cryopreserved and subsequently be thawed at time of analysis.
3. *Assessment of the effect of PSK on serum TGF-β levels when administered with HER2-directed immunotherapy.* Serum levels of TGF-β will be assessed with ELISA at various timepoints to determine incidence and magnitude of

serum TGF-β reduction pre- and post-combination immunotherapy. Serum will be cryopreserved and subsequently be thawed at time of analysis.

4. *Evaluation of PFS and OS*. Evaluation of PFS and OS will be followed and compared between the treatment arms. Though not statistically powered to this endpoint, large differences if observed between the treatment groups will be noted and described.

## Appendices

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| Appendix A | Zubrod Performance Status Scale |
| Appendix B | Schedule of Events |
| Appendix C | Data Safety Monitoring Plan |

**Abbreviations:**

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| ADCC | antibody-dependent cell-mediated cytotoxicity |
| ALND | axillary node dissection |
| APC | antigen presenting cells |
| BUN | blood urea nitrogen |
| CBC | complete blood count |
| CFC | cytokine flow cytometry |
| CMP | Complete metabolic panel |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CTEP | Cancer Therapy Evaluation Program |
| CTL | cytotoxic T lymphocytes |
| DC | dendritic cell |
| DTH | delayed type hypersensitivity |
| DSMP | Data Safety Monitoring Plan |
| ECD | extracellular domain of HER2 |
| FISH | fluorescence *in situ* hybridization |
| rhuGM-CSF | rhu granulocyte macrophage colony stimulating factor |
| GMP | good manufacturing practice |
| HER2 | HER-2/neu |
| HPLC | high pressure liquid chromatography |
| ICD | intracellular domain of HER2 |
| id. | intradermal |
| IFNγ | interferon-gamma |
| IHC | immunohistochemistry |
| LVEF | left ventricular ejection fraction |
| MBC | Metastatic breast cancer |
| MHC | major histocompatability complex |
| MUGA | multigated acquisition |
| NED | no evidence of disease |
| NK | Natural Killer Cell |
| OS | overall survival |
| PBMC | peripheral blood mononuclear cells |
| PSK | Polysaccharide Krestin |
| PFS | progression free survival |
| Th | T helper cells |
| TLR | Toll-like receptor agonist |
| TNF | Tumor necrosis factor |

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| UPN | unique patient number |

## 1. Introduction

Patients with advanced breast cancer have been shown to have defects in anti-tumor immunity including impairment of natural killer (NK) cells.[1-3] NK cells are key components of innate immunity which play a fundamental role in tumor immune surveillance. Recent findings in breast cancer patients show peripheral blood and tumor-infiltrating NK cells to have tumor-promoting alterations in their activating and inhibitory receptors which correlate with decreased cytotoxic NK cell function.[3] Additionally, inhibitory factors in the tumor microenvironment including TGF-beta (TGF-β) have been shown to be directly involved in the impairment of NK cells' phenotype and functions. It is postulated that invasive breast tumors escape NK cell antitumor immunity via a strong inhibitory microenvironment resulting in tumor progression and recurrence. Thus, novel approaches to improve NK cell function and overcome tumor cell-mediated NK suppression are needed.

Toll-like receptor agonists (TLRs) are a group of pathogen recognition receptors that can recognize the pathogen associated molecular patterns (PAMPs) from virus, bacteria, and fungus resulting in initiation of innate immunity. Several TLR agonists have been shown to activate NK cells, either directly or indirectly via dendritic cells (DC).[4-6] Activation of NK cells can result in direct NK cell mediated cytotoxicity of tumor. Activated NK cells also secrete proinflammatory cytokines and chemokines which regulate and direct immune responses.[7] Specifically, activated NK cells secrete IFN-γ which can promote maturation and differentiation of DCs toward a Th1 profile and drive the activation of adaptive immunity. Our group has been studying the immune modulatory and anti-tumor potential of Polysaccharide Krestin (PSK), a hot water extract from *Tremetes Versicolor* mushroom as a means to enhance NK cell function via stimulation of TLR2. Our pre-clinical studies have shown that PSK: (1) is a selective TLR2 agonist with potent antitumor effects which are dependent on enhanced PSK-induced NK and CD8 T cells activity[8], (2) can activate human NK cell function and enhance trastuzumab-mediated antibody-dependent cell-mediated cytotoxicity (ADCC)[9], (3) can induce NK cell and gamma-delta T cell secretion of multiple inflammatory cytokines including IFN-γ resulting in DC maturation and activation[8, 9] and (5) may also work as a vaccine adjuvant to significantly augment antigen-specific T cell immunity. Collectively, these findings suggest the potential of PSK to augment antitumor immunity via stimulation of DCs and NK cells and also to enhance the therapeutic effect of trastuzumab and HER2 vaccination in breast cancer patients.

We hypothesize that the use of PSK will enhance the innate anti-tumor immune responses specifically NK-cell activity and result in prolonged upregulation of IFN-γ production; this could also result in stimulation of adaptive Th1 immunity. Thus, we have designed a phase I/II randomized study of combination immunotherapy with oral PSK administered concurrently with trastuzumab and HER2-targeted peptide vaccination. Our primary and secondary objectives are to assess the safety of PSK as a component of combination immunotherapy and to evaluate effect of PSK on NK cell functional activity. Additionally, we will investigate if PSK stimulates circulating proinflammatory cytokine/chemokines, enhances HER2 vaccine-induced T cell immunity (epitope spreading) and decreases serum TGF-β.

## 2. Background

**A. Natural killer (NK) cell deficiency has been found in patients with breast cancer.** NK cells are part of the innate immunity that plays a fundamental role in tumor immune surveillance. As surveillance cells in the tumor microenvironment, DCs and NK cells have been shown to infiltrate tumors and monitor the presence of different antigen derived from tumors.[10] Thus, NK cells limit not only the emergence of viral infections but also of cancers by surveillance of "missing-self" and "induced-self" ligands, and by direct recognition of pathogen-associated molecules. Unfortunately, NK cell maturation and cytotoxic function is frequently impaired in breast cancer patients.[1-3] Moreover, NK cell activity of IFN-γ production has been shown to be significantly impaired in invasive breast tumors, especially in advanced stage disease.[3]

The activation of NK cells is regulated by a balance of signals transduced by activating and inhibitory receptors expressed on the surface of NK cells. More recently, it has been shown that breast tumor cells can alter NK cell function through modulation of their surface receptors.[3] Specifically, expression of activating NK cell receptors (i.e., NKp30, NKG2D) were significantly decreased while expression of inhibitory receptors (i.e., NGK2A) were increased in both peripheral blood NK cells (p-NK) and breast tumor infiltrating NK cells (Ti-NK); and this alteration was correlated with decreased NK cell function, especially cytotoxicity.[3] Moreover, impairment of Ti-NK cytotoxicity was more pronounced when compared to that of peripheral NK cells. Additionally, in situ levels of inhibitory factors such as TGF-β and

regulatory T cell infiltrates correlated with the NK cell impaired phenotype and functions. These data support the notion that breast tumors are able to model their environment to evade NK cell antitumor immunity which likely contributes to tumorigenesis. Thus, further investigation of immunomodulators such as PSK to restore NK cell maturation and activity within an immunosuppressive tumor microenvironment and prevent breast cancer progression and/or recurrence is warranted.

**B. Mushroom extract PSK is a TLR2 agonist that activates human NK cells.** Hot-water extracts of mushrooms have been used in traditional medicine in China, Japan, and other Asian countries for centuries. Among other properties, the anticancer and immunomodulatory effects of certain mushroom extracts have been described and studied in depth in the lab and in clinical trials, mainly in Asia. PSK was isolated in Japan from the *Trametes Versicolor* mushroom in the 1960s and has been studied in more than 48 clinical trials, mainly for colorectal, gastric, esophageal, lung, and breast cancer. Based on the findings of randomized PSK clinical trials which showed a low toxicity profile and improvements in patient survival, PSK is commonly used as adjuvant or neoadjuvant treatment with or without chemotherapy for patients with colorectal, breast, and gastric cancer in Japan.[11-14]

TLRs play a crucial role in the innate immune response and the subsequent induction of adaptive immune responses against microbial infection or tissue injury.[15] Specifically, TLRs are a group of pathogen recognition receptors that can recognize the pathogen associated molecular patterns (PAMPs) such as dsRNA or ssRNA, unmethylated DNA, or lipopeptide from virus, bacteria, and fungus. TLRs can induce strong anti-tumor activity by regulating the functions of the immune cells that infiltrate the tumor microenvironment. The potential of TLR agonists, such as polyI:C (TLR3), resiquimod (TLR7/8), and CpG (TLR9), to activate NK cells, either directly or indirectly via dendritic cells (DC), have been shown in both pre-clinical and clinical studies.[4-6] Our group has been studying the immune modulatory and anti-tumor potential of PSK. Our research studies have demonstrated that PSK is a potent TLR2 agonist (Fig. 1). Pre-clinically, *in vitro* studies have shown that PSK can activate human NK cells to produce IFN-γ and lyse tumor cells; and induce secretion of pro-inflammatory cytokines and chemokines (e.g., IL-12, TNF-α, MIP-1α, and MIP-1β).

NK cells represent a highly specialized lymphoid population with potent cytolytic activity against tumor or virus infected cells. There are two subpopulations of NK cells, the $CD56^{bright}$ NK cells which are mainly responsible for cytokine production and the $CD56^{dim}$ NK cells that are mainly responsible for the cytotoxic effect of NK cells.[16] Our preclinical studies have shown that PSK can activate both subpopulations of NK cells and stimulates both the IFN-γ production and cytotoxicity of NK cells (Fig. 2).[9]

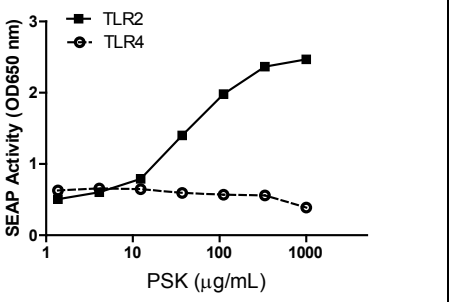



**Figure 1.** PSK selectively activates TLR2. HEK cells transfected TLR2 or 4 were stimulated with serial dilutions of PSK (0.5-1000 μg/mL, 24 hr). PSK also had no effect on TLR3, 5, 7, 8, 9 (data not shown).

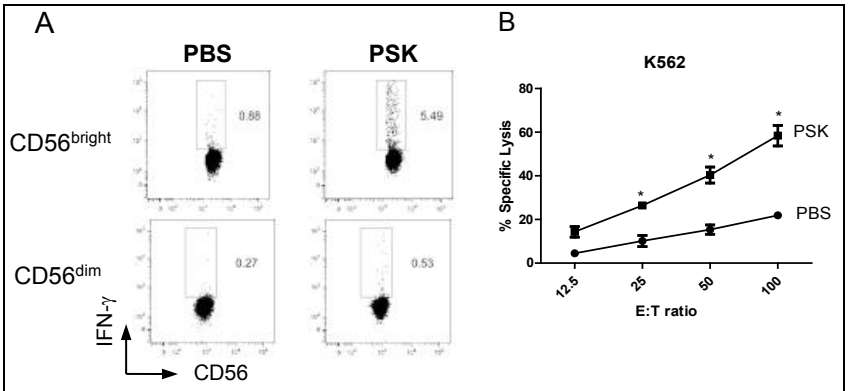



**Figure 2.** PSK stimulates IFN-γ production and augments the cytotoxicity of NK cells. (A) Intracellular staining showing that PSK induces IFNγ production by NK cells. (B) PSK-stimulated PBMC have enhanced lysis against K562. Data shown is representative of results from 3 donors.

In a recent Phase 1 study of Trametes versicolor (Tv) in women with breast cancer conducted by Standish et al, NK cell activity increased to approximately 2-fold of baseline after 4 weeks of oral administration of Tv.[17] Thus, the immune measure of PSK-induced NK cell activity in this study will be assessed with functional NK flow cytometry assays used in our previously published papers and at similar time points as described in Section 7.C.2. (Outcome Measures).[18]

**C. PSK enhances trastuzumab mediated ADCC and may improve its clinical efficacy.** Trastuzumab is a humanized anti-HER2 monoclonal antibody (mAb), and is the first HER2-targeted therapy approved by FDA. Trastuzumab has significantly advanced the clinical management of patients with HER2+ breast cancer by prolonging disease-free survival in patients with locally advanced breast cancer, and progression-free and overall survival in patients with metastatic breast cancer.[19, 20] Trastuzumab inhibits tumor cell growth through multiple mechanisms including signaling blockade and downregulating the HER2/neu receptor. One of the major mechanisms is believed to be ADCC, in which the tumor cells are coated with trastuzumab and then lysed by immune cells via binding of Fc gamma receptor (FcγR) to the Fc portion of the mAb.[21] Increase in tumor infiltrating NK cells after trastuzumab therapy has been found in human breast cancer biopsy samples[22, 23] and FcγR gene polymorphism can impact the clinical response to trastuzumab.[24] NK cells constitutively express FcγRIIIA (CD16) and are the major effectors of ADCC.[25] Therefore the function of NK cells may impact the efficacy of ADCC and clinical response to trastuzumab.[26]

In our pre-clinical studies, we have found that PSK significantly enhanced trastuzumab-mediated ADCC against two breast cancer cell lines, SKBR3 and MDA-MB-231. Furthermore, we showed that combination of oral PSK and a HER2-targeted mAb therapy in mice resulted in enhanced anti-tumor effect.[18] Patients enrolled to this study will have metastatic breast cancer which has been optimally treated to a state of NED (no evidence of disease) and therefore off cytotoxic chemotherapy. However, our patient population will continue use of maintenance trastuzumab monotherapy or combination trastuzumab and pertuzumab therapy as dictated per standard of care. Although not a study outcome, combination of PSK with trastuzumab could potentially enhance NK-cell-mediated ADCC of residual microscopic disease (i.e., HER2+ tumor cells) in our study patients.

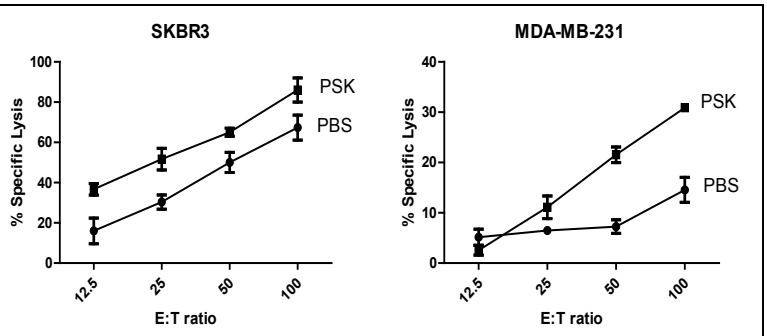
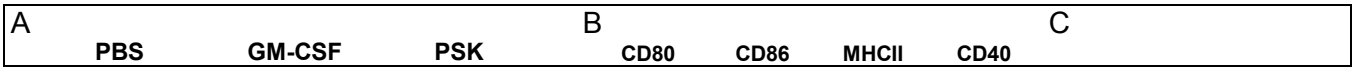



**Figure 3**. PSK enhances trastuzumab-mediated ADCC in lysing of SKBR3 and MDA-MB-231 breast cancer cells. PSK or PBS-stimulated PBMCs were incubated with 3000 target cells at the indicated E:T ratio. Shown are specific lysis at each E:T ratio (mean±sem of triplicate wells).

**D. PSK can activate DC and augment antigen-specific T cells when used as a vaccine adjuvant.** TLR2 is highly expressed on DC, and treatment with PSK can induce DC maturation and secretion of multiple inflammatory cytokines, including TNF-γ, IL-12p40, and IL-12p70.[8, 9] Furthermore, PSK-induced IFN-γ produced by NK cells and gamma-delta T cells can reciprocally activate DC. Therefore, we hypothesize that PSK may work as vaccine adjuvant to augment antigen-specific T cells. Our pre-clinical studies in a mouse model of vaccination with ova peptide antigen p323 has shown that PSK as a vaccine adjuvant significantly enhanced ova-specific T cell immunity. Specifically, BALB/c mice were given a vaccine of OVA p323-339 peptide at the dose 0.5 mcg per mouse concurrently with an intradermal (ID) adjuvant of either PSK (1000 mcg), GM-CSF (5 mcg), or PBS. For two days following initial injection, adjuvant was given alone once per day and animals were sacrificed on Day 4. Draining lymph nodes (dLN) were harvested for evaluation of DC activation and antigen-specific T cells. dLNs from mice that received GM-CSF or PSK were enlarged compared to dLNs from mice in the PBS control group (Fig. 4A); and the percentage and number of $CD11c^+$ DC were significantly increased in GM-CSF and PSK treated mice per FACS analysis. The expression of activation markers, CD80, CD86, MHCII, and CD40 were also up-regulated in DC isolated from dLNs from PSK treated animals (Fig. 4B). The number of ova antigen-specific T cells, as evaluated in an IFN-γ ELISPOT assay was also significantly increased in mice that received PSK as adjuvant (Fig. 4C). Although PSK was given ID in these experiments, we hypothesize that oral PSK may also enhance the effect of vaccination. Indeed, a recent publication by Tanaka et al has shown that combining peptide vaccine with oral ingestion of *Lentinula endodes* mycelia extract enhances anti-tumor activity in B16 melanoma-bearing mice.[27]



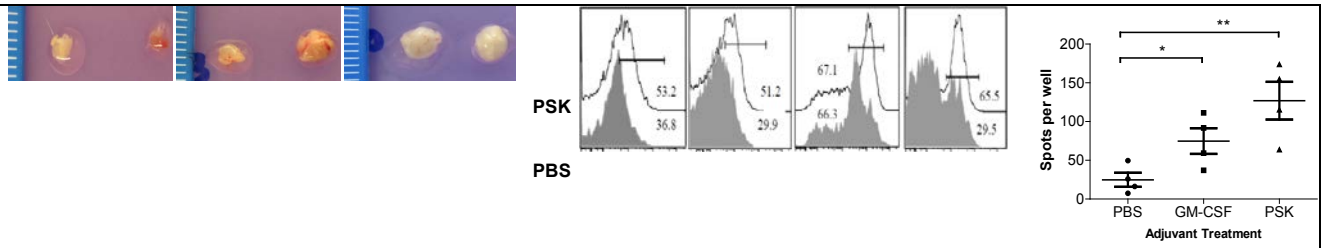



**Figure 4.** PSK as vaccine adjuvant stimulates dendritic cell (DC) activation *in vivo* and enhances antigen-specific T cells. (A) Representative pictures of draining lymph nodes (dLN) from mice vaccinated with ova peptide with PBS, GM-CSF or PSK as adjuvant. (B) The expression of DC activation markers, CD80, CD86, MHCII, and CD40 on DC in dLN from mice received ova plus PSK (empty histogram) or PBS control (shaded histogram). (C) The number of spots per well in IFN-γ ELISPOT assay evaluating ova-specific T cells using dLN cells from mice received ova plus PBS, GM-CSF or PSK.

**E. PSK may augment vaccine-induced HER2 Th1 immunity in optimally treated metastatic breast cancer patients**. Disease relapse in optimally treated HER2+ breast cancer patients who are in complete remission suggest the presence of persistent residual microscopic disease not eradicated by systemic therapy. One approach to the eradication of residual subclinical disease in this high-risk population is the development of tumor vaccines that target HER2, an immunogenic biologically relevant protein. Generation of vaccine-induced HER2-specific Type I inflammatory CD4+ T helper immunity (Th1) could result in immunologic eradication of residual HER2+ tumor cells and potentially prevent disease relapse or tumor spread. Recent evidence indicates that Type I immunity which is associated with the production of high levels of IFN-γ enhances cross priming, the primary method by which immunity is generated against cancer, at the site of cancer. Moreover, vaccine induced Th1 immunity has the potential to generate epitope spreading, a broadening of the immune response to multiple potential antigens in the tumor which may result in more efficient kill of residual tumor cells by T cells. This is particularly important as impaired IFN signaling with associated T cell activation defects have been identified in breast cancer and may be a contributing factor to decreased Th1 immune responses to breast cancer vaccines.

As previously described, our pre-clinical studies have shown that PSK can induce DC maturation and secretion of multiple inflammatory cytokines that result in antigen-specific T cell immunity. Additionally, growing evidence indicates that NK cells have mechanisms to efficiently promote maturation and differentiation of DCs toward a Th1 profile. Specifically, activated DCs in the tumor microenvironment are able to take up tumor antigens and migrate to lymph nodes where they present their antigens to CD8+ T cells and CD4+ T cells which then undergo clonal expansion and are able to infiltrate and destroy tumor in an antigen specific manner. Thus, NK cell activation and resultant IFN-γ production indirectly promote adaptive antitumor T cell responses via activated DCs.[10] Similarly, activated DCs induce potent NK cell activation. Together, NK cell and DC activation are key in the priming process of CD8+ and Th1 immunity and development of adaptive immunity.[10] Recognizing the importance of NK cells and their role in stimulating adaptive immunity, recent immunotherapeutic strategies such DC vaccination have focused on overcoming impaired Th1 immunity via NK cell activation. Specifically, Baek et al, showed that DC vaccines when given with IL-12 were able to induce antigen specific immunity; and NK cell activation with reduction of inhibitory immunity.[28, 29] In a follow-up study, Qi et al, demonstrated the ability of DC vaccination to promote secretion of Th1 cytokines and significantly increase the number of peripheral blood NK cells resulting in tumor specific CD8+ T cell expansion and further differentiation into IFN-γ producing effector cells.[29]

We have previously demonstrated our ability to induce tumor-specific immunity and also epitope spreading in patients with HER2+ breast cancer.[30, 31] Moreover, epitope spreading was associated with potential survival benefit in vaccinated patients.[32] Key elements to inducing HER2-specific Th1 immunity and epitope spreading in the majority of HER2+ breast cancer patients were the use of vaccines which elicit Th1 immunity given in combination with GM-CSF as adjuvant and our targeted patient population (optimally treated patients to state of NED). The addition of immunomodulatory agents such as PSK as adjuvants to vaccines may help enhance, sustain, and skew the immunogenicity of antigens toward a Th1 phenotype via robust NK cell activation, allowing for development of robust and durable vaccine-induced Th1 immunity; and potentially prevention of cancer recurrence. Thus, we propose to investigate if PSK when given orally with HER2 peptide based vaccination and trastuzumab can enhance both development and magnitude of HER2 vaccine-induced Th1 T cell immunity specifically, epitope spreading.

**F. PSK may enhance NK cell activation by inhibiting TGF-β production in the tumor microenvironment.** TGF-β is a hormonally active polypeptide that inhibits the proliferation of normal epithelial cells, and enhances matrix formation and angiogenesis.[33] Most epithelial cancer cells lose responsiveness to TGF-β induced growth inhibition.[34, 35] TGF-β is secreted by both breast cancer cells and the tumor stroma resulting in increased proliferative capacity, enhanced epithelial-mesenchymal transition (EMT); and subsequent increased metastatic potential of tumors.[36, 37] TGF-β, a potent immunosuppressive cytokine acts in the tumor microenvironment to blunt immune-surveillance via multiple mechanisms, including suppression of NK cells. Additionally, TGF-β is secreted by T regulatory cells which function to inhibit the development of adaptive immunity to "self-antigens" such as tumor antigens.[38] Moreover, elevated levels of serum TGF-β have been found to be related with disease progression and worse prognosis in breast cancer patients.[39] Recent studies have shown that inhibitory molecules in the tumor microenvironment such as TGF-β directly contribute to impairment of NK cell phenotype and function. As described above, Mamessier et al showed that expression of activating NK cell receptors were significantly decreased while expression of inhibitory receptors were increased in both peripheral blood and breast tumor infiltrating NK cells; and stroma-derived inhibitory factors particularly TGF-β were involved in driving the impaired NK cell activity. Additionally, increased in situ levels of TGF-β and regulatory T cell infiltrates correlated with worse NK cell impairment. Thus, inhibition of the TGF-β via immunomodulation of the tumor microenvironment may restore NK cell activity and result in development of robust tumor antigen specific Th1 immunity. Previous studies have suggested that PSK may modulate the biological activity of TGF-β and thus restore robust NK cell activity. Additionally, enhanced NK cell activity with subsequent increased IFN-γ production may result in decreased TGF-β production in the tumor microenvironment. In a more recent study, PSK was shown to inhibit the Smad pathway, the major regulator of TGF-β signaling.[40] Treatment with PSK inhibited the expression of several TGF-β pathway target genes and prevented TGF-β1-induced EMT.

Studies by our group have demonstrated that circulating levels of TGF-β are significantly elevated in sera from breast cancer patients compared to controls; and that vaccine-induced HER2 specific IFN-γ T cell responses specifically epitope spreading, were associated with reductions in serum TGF-β levels. Additionally, the magnitude of the epitope spreading T cell response elicited with active vaccination was inversely correlated with TGF-β levels assessed in serum throughout immunization. As described in Section 13 (Statistical Considerations) our previous study data suggest a modest decrease in serum TGF-β levels among patients who received HER2 ICD vaccination and trastuzumab alone. Therefore we aim to investigate if administration of PSK with HER2 vaccination and trastuzumab results in enhanced downregulation of TGF-β expression in the tumor and augmented NK cell induced IFN-γ production; and subsequent decrease in detectable circulating serum levels of the cytokine.

**G. Concomitant administration of pertuzamab and trastuzumab has been shown to be safe and effective in treating HER2+ metastatic breast cancer (MBC).** Pertuzumab is a recombinant humanized monoclonal antibody that binds to the extracellular dimerization domain II of HER2, and inhibits heterodimerization of HER2 with other HER family members, including EGFR, HER3, and HER4. Blockade of HER2-HER3 likely represents the most relevant action of.[41] In pre-clinical models, pertuzumab when given in combination with trastuzumab, is associated with a more complete HER2 blockade. Additionally, the treatment combination is more effective in HER2-positive tumor xenografts than when either antibody is given alone.[41] Moreover, enhanced efficacy of the combined use of trastuzumab and pertuzumab when given with docetaxel has now been demonstrated in several clinical studies in HER2+ MBC.[42]

Over the past 2 years, the first-line treatment of HER2+ MBC has been changing and now also includes other HER2 targeted agents specifically pertuzumab (perjeta). In 06/2012, the FDA approved the combination of docetaxel and pertuzumab/trastuzumab for the treatment of HER2+ MBC. This was based on data from the CLEOPATRA study, a large Phase III trial enrolling 808 patients which showed the combination of docetaxel + pertuzumab/trastuzumab improved progression free survival (PFS) by 6 months compared to docetaxel + placebo/trastuzumab.[43] Given the PFS benefit, oncologists started using the triple regimen more readily. As a result, HER2+ MBC patients started receiving indefinite concomitant pertuzamab and trastuzumab as maintenance therapy once docetaxel was stopped. More recently, final results from CLEOPATRA (reported 02/2014) showed that first-line treatment with docetaxel + pertuzumab/trastuzumab in patients with HER2+ MBC significantly improved overall survival (OS) when

compared to docetaxel + placebo/trastuzumab. Median OS was 56.5 months (pertuzumab arm) vs 40.8 months (placebo arm) with difference of 15.7 months.

Extensive cardiac surveillance was conducted in CLEOPATRA and treatment with docetaxel + pertuzumab/trastuzumab did not increase the rate of left-ventricular systolic dysfunction (LVSD) when compared to docetaxel + placebo/trastuzumab. Specifically, the incidence of LVSD (all grades) was 8.6% in placebo arm vs 5.4% in pertuzumab arm. Additionally, the incidence of symptomatic LVSD was 1.8% in placebo arm vs 1.2% in pertuzumab arm. Lastly, incidence of LVEF decline to <50% and by ≥10% points from baseline was 7.4% in placebo arm vs 4.6% in pertuzumab arm; and LVEF recovery to ≥50% was 89.3% in placebo arm vs 88.9% in the pertuzumab arm. Altogether, no increased cardiotoxicity was observed in patients receiving concomitant pertuzumab and trastuzumab with docetaxel when compared to concomitant placebo and trastuzumab with docetaxel. Given the significant OS benefit, and the acceptable toxicity data, it has been recommended that the new standard of care for first-line treatment of HER2+ MBC include docetaxel + pertuzumab/trastuzumab.

## 3. Study Rationale

The proposed oral dose of PSK (3000 mg/day) in this study has been demonstrated to be safe with and without chemotherapy in large randomized breast cancer studies.[44, 45] In a review of PSK use and adverse events (AEs), 114 (1%) AEs were reported from a total of 11,300 individuals who used PSK alone or with other medical treatments (Table 1). Principal AEs were diarrhea (0.25%), nausea (0.24%), vomiting (0.15%), and anorexia (0.15%). These data are taken from the Japanese Ministry of Health and Welfare and are in the PSK Package Insert. Severe PSK-related AEs have not been reported since clinical trials began in Japan in the 1970s thus, PSK appears to be extremely well tolerated.

| **Table 1. Adverse Event Summary for PSK Dose (3g/day) Based on 11,300 Individuals** | |
|---|---|
| Symptomatic | Diarrhea (0.25%) |
| | Nausea (0.24%) |
| | Vomiting (0.15%) |
| | Anorexia (0.15%) |
| | Hives (0.1%) |
| | Stomach discomfort (0.1%) |
| Hematologic | None |

Combination immunotherapy with HER2-directed immunotherapy has also been shown to be both safe and immunogenic in our previous studies. However, the safety of PSK when added to HER2-directed immunotherapy is less well known and warrants further investigation. This study will allow us to better define the safety and immunomodulatory effects of this regimen on the tumor microenvironment and systemic immunity.

## 4. Objectives

### A. Primary objectives:

1. To evaluate the safety of PSK when given with HER2-directed immunotherapy.

### B. Secondary objectives:

1. To evaluate the effect of PSK on NK cell functional activity when given with HER2-directed immunotherapy.

### C. Tertiary objectives:

1. To investigate the effect of PSK when given with HER2-directed immunotherapy on:
   a. Serum of pro-inflammatory cytokine and/or chemokines.
   b. Intermolecular epitope spreading.
   c. Serum TGF-β levels.
   d. Progression free survival (PFS) and overall survival (OS).

## 5. Experimental Agent Preparation

### A. HER2 ICD peptide-based vaccine

The synthetic HER2 peptides to be used in the vaccine (Table 2) are 15 amino acids in length with an amino acid sequence identical to the normal HER2 sequence. This HER2 vaccine has been evaluated in a previous study (see Background) and is covered by BB IND 6524 (Sponsor: Mary L Disis, M.D.).

**Table 2. HER2 ICD peptides**

| HER2 ICD Vaccine | Sequence |
|---|---|
| p776-790 | GVGSPYVSRLLGICL |
| p927-941 | PAREIPDLLEKGERL |
| p1166-1180 | TLERPKTLSPGKNGV |

1. Peptide synthesis and characterization:

   The peptides are manufactured by Multiple Peptide Systems, San Diego, CA. using standard solid phase synthesis techniques employing Boc chemistry. Amino acids are analyzed by TLC and polarimetry prior to use. The peptides are cleaved from the solid support using anhydrous hydrogen fluoride and conventional techniques. Each peptide is purified by HPLC using a Waters Delta-prep system with a gradient of acetonitrile (containing 0.1% trifluoroacetic acid) in water (also containing 0.1% trifluoroacetic acid). The peptides are lyophilized to dryness before being passed over an anion exchange column to exchange the trifluoroacetate for acetate. GMP procedures are followed for the manufacture of these sequences. After formulation is complete, the peptides are characterized by mass spectrometry to confirm their identity, by HPLC for purity using two different elution buffers, and by amino acid analysis for identity and peptide content. The water content of each peptide was determined by the Karl Fischer procedure and the data obtained used to verify the mass balance of the peptides.

   These peptides are vialed as the HER2 ICD by the Fred Hutchinson Cancer Research Center, Biologics Product Facility (a GMP facility).

2. Peptide formulation and stability:

   Prior to formulation, the solubility of each peptide in 10 mM sodium acetate buffer (pH 4.0) is determined. Three peptides were made up in a 10 mM sodium acetate buffer (pH 4.0). The stability of each individual peptide in the acetate buffer, as well as the peptide mixtures, is monitored by HPLC and mass spectrometry. The dissolved peptides are stored at -20 ± 5˚C prior to use. Microbial and sterility testing are conducted to ensure safety. The peptide formulation is labeled to accurately reflect the product identity, concentration, lot number and fill date. These labels are attached to vials immediately post-fill.

**B. GM-CSF**

Recombinant human GM-CSF (rhuGM-CSF; Sargramostatin, LEUKINE) will be used as adjuvant admixed with HER2 vaccine, obtained by commercial suppliers and stored at their specifications. GM-CSF is a growth factor that supports the survival, clonal expansion, and differentiation of hematopoietic progenitor cells including dendritic APCs. In general, the use of GM-CSF is associated with little toxicity; and rhuGM-CSF, when given IV or subcutaneously is well tolerated at doses ranging from 50-500 mcg/$m^2$/day. Severe toxicity is extremely rare in patients treated with rhuGM-CSF. For the current study, rhuGM-CSF will be used at a total injection dose of 100 mcg.

Administration of rhuGM-CSF may aggravate fluid retention in patients with pre-existent edema, capillary leak syndrome, or pleural or pericardial effusions. In some patients with pre-existing renal or hepatic dysfunction, elevation of the serum creatinine or bilirubin and hepatic enzymes has occurred during rhuGM-CSF administration. Dose reduction or interruption of rhuGM-CSF administration has resulted in a decrease to pretreatment values. Occasional transient and reversible supraventricular arrhythmia has been reported in uncontrolled studies, particularly in patients with a previous history of cardiac arrhythmia. Stimulation of marrow precursors with rhuGM-CSF may result in a rapid rise in white blood cell count. If rhuGM-CSF is being used for the purpose of hematopoietic reconstitution, dosing should be stopped if the ANC exceeds 20,000/$cm^3$. The dose being used in this study should not affect ANC levels. RhuGM-CSF may stimulate the growth of myeloid malignancies; therefore caution must be exercised in its use in these malignancies or myelodysplastic syndromes. RhuGM-CSF is a sterile, white, preservative-free lyophilized powder in 250 mcg vials. Reconstituted rhuGM-CSF will be admixed and administered with the HER2 vaccine.

**C. Vaccine Dose Compounding and Dispensing**

The HER2 ICD Vaccine is supplied in single-use vials as a sterile, frozen solution. Each single-use 1.1 ml vial contains equal volumes of ICD peptides, p776-790, p927-941, p1166-1180 (625 mcg of each peptide per ml) for a final concentration of 1.9 mg/ml total ICD peptide mix. The ICD vaccine will be supplied as frozen vials and each vial will carry a label bearing the drug identification and conditions for storage. The University of Washington Investigational Drug Pharmacy (UW IDS) will prepare and dispense the drug for administration per their standard operating procedure as follows:

**Table 4. Description and Composition of Placebo**

| Component | Source | Amount | Function |
|---|---|---|---|
| Mannogem®™ 2080, Granular Mannitol, USP | SPI Pharma (Wilmington, DE) | 1.2 g | Bulking Agent |
| Caramel Color 643[a] | D. D. Williamson & Co., Inc. (Louisville, KY) | 0.3 g | Colorant |
| [a]Class III caramel color used in sauces, gravy, beer, baking, other foods. | | | |

1. Reconstitute rhuGMCSF 250mcg with 0.5ml sterile preservative free water and add rhuGMCSF 125mcg/0.25ml to a sterile empty 2ml vial.
2. Thaw HER2 ICD Vaccine 1.9mg/ml and withdraw 1 ml and add to vial containing the rhuGMCSF. Total volume in vial is 1.25ml. Rotate vial gently to mix.
3. Total dose is HER2 ICD 1500mcg and GMCSF 100mcg in 0.99ml. Divide dose into 3 syringes, containing 0.33ml each for intradermal injection.

**D. PSK Product and Placebo**

This PSK and Placebo characterization and storage are covered by BB IND 114546 (Sponsor: Leanna Standish, PhD, ND). The PSK® and Placebo are to be stored at room temperature (59ºF - 86ºF).

1. Description and Composition of PSK® Clinical Drug Product

   The PSK® clinical drug product is a granular powder, light tan to dark brown in color, which is packaged in unit dose aluminum pouches (Table 3) by First Priority in Ferndale, WA. There are no excipients, other botanical substances, synthetic or highly purified drugs, biotechnology-derived drugs, or other naturally derived drugs in the drug product. Once reconstituted with water, a clear to slightly turbid brownish solution is obtained for oral administration.

**Table 3. Quantitative Composition of the PSK® Clinical Drug Product in Each Unit Dose Pouch**

| Component | Source | Amount | Function |
|---|---|---|---|
| PSK® Bulk Drug Product | Kureha | 1.5 g | Active Ingredient |

2. Description and Composition of Placebo:

   The placebo is a granular powder, light tan to dark brown but mottled with white in color, which is packaged in unit dose aluminum pouches by First Priority in Ferndale, WA. There are no botanical substances, synthetic or highly purified drugs, biotechnology-derived drugs, or other naturally derived drugs in the drug product (Table 4). Once reconstituted with water, a clear to slightly turbid brownish solution is obtained for oral administration.

3. Description and Composition of Container Closure System:

   The primary container closure system for PSK® clinical drug product and placebo is a laminated aluminum pouch. The aluminum pouch is composed of components that are commonly used in the pharmaceutical industry for solid, oral dosage form products. The container closure system is selected based on comparable specifications to that used for the marketed drug product.

4. Description and Composition of Dosage Preparation Kit:

   A shaker cup/lid is provided to facilitate preparation of the oral dosage solution. Briefly, the PSK® clinical drug product or placebo is emptied into the shaker cup, which has already been filled with approximately 4-8 oz. of warm water. The lid is then applied to seal the cup and shaken vigorously to dissolve the powder. The liquid preparation is to be consumed within 15 minutes. After the initial

preparation is consumed, additional warm water is added, shaken and consumed to rinse the cup and ensure the entire dose has been taken.

**E. Experimental Agent Accountability**

The HER2 vaccine, GM-CSF and PSK will be managed by the UW IDS. UW IDS will be responsible for study drug disposition (drug receipt, dispensing, transfer or return). Once IDS receives notification of the subject visit they will begin thawing the vaccine and prepare the appropriate number of syringes ready for injection as described in Section 5.C. UW IDS will deliver the HER2 vaccine, PSK and/or the placebo to the Clinical Research Center, where patients will be seen. The disposal of clinical trial materials that are in UW IDS will be incinerated under a state contract through an EPA licensed facility. Unused PSK pouches will be destroyed.

As part of our Data Safety Monitoring Plan (DSMP) (see Appendix C) an independent monitor will be assigned by the Fred Hutchinson Cancer Research Center Clinical Trials Support Office to monitor our study twice per year, which includes a review of the UW IDS dispensing logs for the vaccine and PSK.

## 6. Subject Selection

### A. Inclusion criteria

1. Patients with stage IV HER2+ breast cancer treated to:
   a. No evidence of disease (NED), or
   b. Stable bone only disease after definitive therapy.
2. HER2 overexpression by IHC of 2+ or 3+ in the primary tumor or metastasis; or documented gene amplification by FISH analysis. IHC ≤ 2+ must have HER2 gene amplification documented by FISH.
3. Patients must continue HER2-targeted monoclonal antibody therapy dosing per standard of care through the entire study period (one year).
   a. HER2-targeted monoclonal antibody therapy is defined as either trastuzumab monotherapy, or trastuzumab and pertuzumab combination therapy administered per standard of care.
4. Patients must be at least 21 days post cytotoxic chemotherapy prior to enrollment.
5. Patients must be at least 28 days post immunosuppressants prior to enrollment.
6. Patients must be at least 28 days from use of any mushroom supplements (examples: turkey tail, reishi, maitake, shiitake,) and agree to withhold them for the entire study period (one year).
7. Patients on bisphosphonates and/or endocrine therapy are eligible.
8. Patients who are having sex that could lead to pregnancy must agree to contraceptive use during the entire study period.
9. Patients must have Zubrod Performance Status Score of ≤ 2 (Appendix A).
10. Patients must have recovered from major infections and/or surgical procedures, and in the opinion of the investigator, not have significant active concurrent medical illnesses precluding study treatment.
11. Laboratory values must be as follows and performed within 30 days of enrollment:
    a. WBC ≥ 3000/$mm^3$
    b. Hgb ≥ 10 g/dl
    c. Serum creatinine ≤ 2.0 mg/dl or creatinine clearance > 60 ml/min
    d. Total bilirubin ≤ 1.5 mg/dl
    e. SGOT ≤ 2.5 times the upper limit of normal
12. Patients must be at least 18 years of age
13. Patients must have adequate cardiac function as demonstrated by normal left ventricular ejection fraction (LVEF) ≥ the lower limit of normal for the facility on MUGA scan or echocardiogram (ECHO) within 3 months of enrollment.

### B. Exclusion criteria

1. Patients with any of the following cardiac conditions:
   a. Restrictive cardiomyopathy

b. Unstable angina within 6 months prior to enrollment
c. New York Heart Association functional class III-IV heart failure
d. Symptomatic pericardial effusion.

2. Patients with any contraindication to receiving rhuGM-CSF based products.
3. Patients with any clinically significant autoimmune disease requiring active treatment.
4. Patients receiving any concurrent immunosuppressants.
5. Patients who are pregnant or breast-feeding.
6. Patients who are simultaneously enrolled in other treatment studies.
7. Patients who have received a previous HER2 breast cancer vaccine.
8. Known hypersensitivity reaction to mushroom products.

Subjects must meet all of the listed criteria in order to be eligible for study.

## 7. Experimental Design

### A. Study design

This will be a phase I/II double-blind randomized two-arm clinical trial designed to examine the safety and immune effects of PSK when given as combination immunotherapy with a HER2 ICD peptide vaccine in patients with Stage IV HER2+ breast cancer receiving HER2-targeted monoclonal antibody therapy. Eligible patients will be enrolled and randomly assigned in equal numbers to one of two treatment arms (15 patients per arm) shown below. However, stagger enrollment of 2 weeks will occur for the first 2 patients in each arm.

**Arm 1**:

***HER2 ICD vaccine + HER2-targeted monoclonal antibody therapy* + Placebo***: Patients will receive HER2 ICD peptide vaccine administered concomitantly with HER2-targeted monoclonal antibody therapy and placebo product.

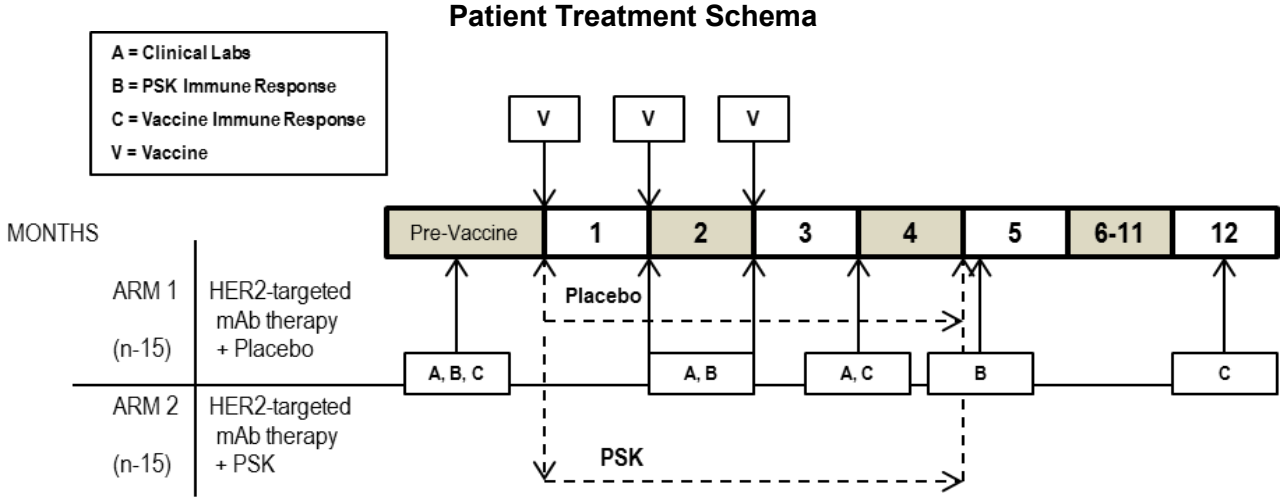
**Arm 2**:

***HER2 ICD vaccine + HER2-targeted monoclonal antibody therapy* + PSK***: Patients will receive HER2 ICD peptide vaccine administered concomitantly with HER2-targeted monoclonal antibody therapy and PSK.

**Trastuzumab (or trastuzumab and pertuzumab) administered per standard of care by patient'sprimary oncologist*.

All study patients will receive the HER2 ICD vaccine administered monthly for a total of 3 vaccines in addition to HER2-targeted monoclonal antibody therapy (administered per standard dosing guidelines). Additionally, patients randomized to Arm 1 will receive continuous placebo product given orally and patients randomized to Arm 2 will receive continuous PSK given orally at 3000 mg/day (1500 mg twice daily before meals). Both the placebo product in Arm 1 and PSK in Arm 2 will be started on the day of the first HER2 vaccine and continued for a total of 4 months. All patients will be followed for immune response at designated timepoints as shown in the patient treatment schema below.

**Patient Treatment Schema**



**B. Sample size**

The study will accrue a maximum of 30 subjects: 15 subjects per arm as described in Section 13 (Statistical Considerations).

**C. Outcome measures**

1. Primary Endpoints:
   a. *Evaluation of safety of PSK when administered with HER2-directed immunotherapy.* Evaluation of safety and systemic toxicity includes physical examination and clinical labs performed at baseline, before each vaccination, and 1 month after the 3rd vaccine. Toxicity grading will be evaluated per CTEP CTCAE 4.0. Type and grade of toxicities noted during treatment will be summarized. Additionally, stopping rules for accrual to the study will become operational if there is sufficient evidence of excessive toxicity as described in Section 12.

2. Secondary Endpoints:
   a. *Evaluation of the effect of PSK on NK cell functional activity.* PBMC will be obtained and cryopreserved at baseline (prior to start of PSK) and then at 4, 8, and 16 weeks. PBMC will subsequently be thawed and used to determine the effect of PSK on NK cell functional activity specifically, induction of IFN-γ production and CD107a expression in NK cells, via flow cytometry. Augmentation of NK cell activity is defined by a 2-fold increase in NK cell IFN-γ production and CD107a expression from baseline and after 4 weeks of oral administration of PSK.

3. Tertiary Endpoints:
   a. *Assessment of the effect of PSK on pro-inflammatory serum cytokine and/or chemokines when given with HER2-directed immunotherapy.* Serial blood draws will be done at baseline before the initial dose of PSK (time 0) and 30 min, 4 hours, and 24 hours after the initial dose of PSK to assess serum cytokines/chemokines (i.e., IL-6, IFN-γ, TNF-α) via Luminex analysis. Serum cytokine/chemokine levels at different timepoints post-PSK will be compared to base-line. Serum will be cryopreserved and subsequently be thawed at time of analysis.

   b. *Assessment of the effect of PSK on intermolecular epitope spreading when given with HER2-directed immunotherapy.* PBMC will be obtained and cryopreserved prior to the 1st vaccine and at 1 and 9 months after the 3rd vaccine (months 4 and 12) by IFN-γ ELISPOT. PBMC will subsequently be thawed and used to determine T cell precursor frequency specific for tumor antigens, HER2, IGFBP-2, TOPO-IIα, p53, and PRAME. A positive immune response will be defined as a T cell precursor frequency >1:20,000 antigen-specific PBMCs. Development of a positive immune response after treatment begins will constitute augmentation in patients without pre-existent immune responses. In patients with pre-existent immune response to the tumor antigens, augmentation is defined as a final response to tumor antigen >2 times the baseline level. Epitope spreading will be defined as the generation of significant immunity to the HER2 ECD as the patients will receive an ICD vaccine.

   c. *Assessment of the effect of PSK on serum TGF-β levels when given with HER2-directed immunotherapy.* Serum TGF-β levels will be assessed via ELISA prior to the 1st vaccine (baseline), and 1, and 9 months after the 3rd vaccine (months 4 and 12) to determine the incidence and magnitude of reduction of serum TGF-β levels pre- and post-combination immunotherapy. A decrease in serum TGF-β levels will be defined as a reduction of ≥ 25% from baseline value to post-vaccination values. Serum will be cryopreserved and subsequently be thawed at time of analysis.

   d. *Evaluation of PFS and OS.* PFS and OS will be followed and compared between study arms. This study will not enroll sufficient numbers of subjects to give statistical power to this endpoint however, large differences if observed between the treatment groups (PSK + HER2-directed immunotherapy *versus* Placebo + HER2-directed immunotherapy) will be noted and described. PFS and OS will

be defined as time from first vaccine to time of first event. While on study, patients will be followed for disease progression by their primary oncologist per conventional practice standards. Information on patient disease status will be obtained biannually from their primary oncologist for up to 3 years after enrollment.

## 8. Plan of Treatment - Arms 1 and 2

The following section outlines the patient evaluation and treatment schedule prior to and during study.

### A. Pre Screen

Potential subjects will be screened for eligibility by a study team member. Standard-of-care procedures, done prior to signing consent, can be used for eligibility assessment as long as they are conducted within required windows (Section 6). During this evaluation a pathology report will be obtained to ensure a patient's tumor is HER2 positive as described in Section 6.

### B. Initial evaluation (may be performed up to 2 weeks prior to initial vaccine visit)

1. Sign consent form before initiating any study procedures and eligibility is confirmed.
2. The clinical trial procedures will occur at the UW CRC. Patients, who meet the inclusion/exclusion criteria after consent, will be assigned a unique study numbers and randomized to either PSK or placebo at time of study visit #1 – Day 1. Following an algorithm developed by the study statistician, a randomization table will be generated and provided to an un-blinded IDS pharmacist. Patients, clinicians, and study staff will be blinded to treatment assignment.
   a. Arm 1: Patients will be assigned to the placebo agent
   b. Arm 2: Patients will be assigned to PSK
3. Medical history and complete physical examination which includes weight, vital signs, symptom assessment, Zubrod scoring, medication/supplement changes (Appendix A).
4. Urine pregnancy testing will be performed on female patients of childbearing potential. If a patient is found to be pregnant, she will not be able to participate in the study.
5. A tetanus-diphtheria vaccine (Td) will be administered as a positive control if patient has not received Td immunization within the last six months. Patients who have experienced any of the following described under a. and b. below will not receive the Td vaccine but will continue all other study related treatment and procedures.
   - Patients that have ever had a life-threatening allergic reaction after a dose of any tetanus or diphtheria containing vaccine, OR have had a severe allergy to any part of a tetanus or diphtheria vaccine.
   - Patients with epilepsy or another nervous system problem, severe pain or swelling after any vaccine containing diphtheria or tetanus, or ever had Guillain Barré Syndrome (GBS).
6. Clinical labs (baseline):
   a. Complete blood count which includes a differential and platelet count
   b. Comprehensive metabolic panel (CMP) which includes electrolytes, creatinine, blood urea nitrogen (BUN), AST, ALT, alkaline phosphatase, and total bilirubin.
7. Research blood includes:
   a. 200 mls for immunologic monitoring, including, cytokine kinetics and an assessment of single polymorphisms and T cell function that may contribute to PSK responsiveness.
8. The research staff will instruct each patient on the mixing and administration of study drug using "dummy" pouches. The study staff will ensure that they do not witness the preparation of the tea in order to preserve the study blind.
9. Immediately after the 1st vaccine, patients will take a total dose of PSK 3000 mg/placebo while in the UW CRC.
   a. 30 minutes (+15 min) after the placebo/PSK dose, 10 mls of blood will be drawn for cytokine kinetics
   b. 4 hours (+/-15 min), after their first dose of placebo/PSK dose, 10 mls blood will be drawn for cytokine kinetics

10. Day 2
    a. 24 hours (+/-30 min) after finishing their first dose of placebo/PSK, patients will return to the UW CRC for an additional 10 mls blood draw for cytokine kinetics
    b. After this blood draw, patients will start taking the placebo/PSK at 1500 mg orally BID (total daily dose = 3000 mg)
    c. Patients will be given a sufficient amount of placebo/PSK which will equal a 30 day supply + 7 additional days as needed, in order to provide continuous dosing until the subsequent study visit
    d. Patients will be given a monthly tea diary

**C. Monthly immunizations**

1. The following procedures and tests will be done prior to each vaccination:
    a. Physical examination which includes weight, vital signs, symptom/toxicity assessment and medication/supplement changes.
    b. Urine pregnancy test in patients capable of child bearing (patients found to be pregnant will not receive further study treatment).
    c. Clinical labs: CBC and CMP.
    d. Research blood: 40 mls for immunologic monitoring
2. Monthly vaccines will be administered intradermally (i.d.) on the arm or leg for 3 months.
    a. Patients with axillary lymph node dissection (ALND) will have vaccine administered to the contralateral arm. Patients with bilateral ALND will have vaccine administered in the thigh. As much as possible each vaccine dose will be given within the same draining lymph node site.
    b. Vaccines may be scheduled 28 + 7 days apart.
3. Post-vaccination monitoring: Patients will be observed on the UW CRC for minimum of 60 minutes and vital signs will be documented.
4. Arm 1: Patients will be given a 1-month supply + 7 additional days of placebo agent and a monthly tea diary.
5. Arm 2: Patients will be given a 1-month supply + 7 additional days of PSK (1500 mg twice daily) and a monthly tea diary.
6. Collect any unused PSK pouches for destruction.

Should patients show evidence of disease progression, they will be removed from the study if their treatment conflicts with the eligibility criteria (e.g., concurrent administration of chemotherapy, immune modulators, steroids, etc.).

**D. Evaluation after the final vaccine**

1. One month after the final vaccination (approximately month 4) patients will undergo:
    a. A physical examination which includes weight, vital signs, symptom/toxicity assessment and medication/supplement changes.
    b. Clinical labs: CBC and CMP
    c. Research blood draw of 200 mls for immunologic monitoring
    d. Arm 1: Patients will be given a 1-month supply of placebo agent and a monthly tea diary.
    e. Arm 2: Patients will be given a 1-month supply of PSK (1500 mg twice daily) and a monthly tea diary.
2. Two months after last vaccine (approximately month 5) patients will undergo research blood draw of 40 mls for immunologic monitoring.
3. Three months after last vaccine (approximately month 6) toxicity information will be collected from the patient's primary oncologist which will include recent notes and labs. Recent imaging reports may be collected if available.
4. Nine months after the last vaccine (approximately month 12) patients will undergo research blood draw of 200 mls for immunologic monitoring.

5. Information on patient's disease status will be obtained biannually from their primary oncologist for up to 3 years from enrollment.

### E. Management of Potential Study Risks

Although patients have a small chance of experiencing an allergic-type reaction to the vaccine, if a reaction were to happen, it would usually occur within one hour of the vaccination. For this reason, all patients will undergo post-vaccination monitoring in the UW CRC which will include observation and monitoring of vital signs for a minimum of 60 minutes.

Patients must have a baseline LVEF measured by MUGA or ECHO equal to or greater than the lower limit of normal for the facility within three months of enrollment. Subsequent LVEF monitoring while on HER2-targeted monoclonal antibody therapy will be done per standard of care by patient's oncologist or as clinically indicated.

## 9. Patient Withdrawal from Study

The Principal Investigator/Study Physician may deem it appropriate to remove a patient from study participation for toxicity (as described in Section 12), recurrent disease, or other reasons.

Patients who are no longer being vaccinated or provided PSK/Placebo may continue to be followed for disease status and/or research blood may be requested to follow immunity. Whether it was the decision of the patient or the Principal Investigator/Study Physician to withdraw participation from the study, it will be reported in the annual renewal report to all appropriate agencies. We will report the reason for the withdrawal and whether it was related to the study.

Patients who have persistent grade 3 or 4 toxicity at time of withdrawal from study treatment will continue to be followed until toxicity resolves or returns to baseline for that patient.

Patients may decide to withdraw from the study at any time and for any reason, at which time all research-related treatment procedures (vaccine and/or PSK/Placebo) will then cease.

## 10. Evaluation and Management of Toxicity

Toxicity grading will be evaluated according to the CTEP CTCAE v. 4.0 common terminology and the DSMP (Appendix C). DLT will be defined as Grade 2 autoimmune reactions and any incidence of Grade 3 or 4 adverse events (AEs) other than those listed in Table 5.

For grade 2 treatment-related reactions, patients will be treated with acetaminophen and/or diphenhydramine or as clinically indicated per standard of care; and will not receive further vaccinations or PSK.

**Table 5: Expected and Allowable Grade 3 Toxicities (CTCAE v4.0) that are not DLT**

| Category | Toxicity/Adverse Event | Allowable Duration |
|---|---|---|
| General disorders and administration | Flu-like symptoms | 1 week |
| Musculoskeletal and connective tissue disorders | Arthralgia | 1 week |
| | Myalgia | 1 week |
| Investigations | Lymphocyte count decreased | 2 week |
| | Hemoglobin decrease | 2 week |
| | White blood cells decreased | 2 week |

Rationale for allowing the above Grade 3 toxicity is based on the following. Our proposed target population (patients with Stage 4 breast cancer who are NED or have stable bone disease) most likely have received ≥2 regimens of chemotherapy which can result in long-lasting grade 1 and 2 hematologic abnormalities specifically decreased WBC, lymphocyte counts and hemoglobin which are clinically insignificant (e.g., do not place patient at significant increased risk and/or require medical intervention). We have extensive experience in vaccinating this patient population and it is expected that despite their Grade 1 and 2 hematologic abnormalities, these patients will have fairly normal and functional bone marrow reserve and not be at increased risk of harm due to transient perturbations in the immune system. Additionally, we have now conducted 3 HER2 vaccine studies in over 100 optimally treated patients with stage 4 NED and/or stable bone disease on trastuzumab monotherapy and have observed only transient Grade 3 hematologic toxicity that did not require medical intervention. While the incidence of transient

Grade 3 hematologic toxicity in our previous vaccine studies is low (<2%) it can occur due to patients having low blood counts at baseline. The combination of trastuzumab and pertuzumab as maintenance therapy has also been extensively studied and found to have an acceptable toxicity profile with no increased cardiotoxicity.[43] Additionally, all patients in our proposed target population will be on either trastuzumab or combination trastuzumab/pertuzumab; and the majority will be on endocrine and/or bisphosphonate therapy. All of these therapies independent of vaccination and PSK can incur flu-like symptoms, myalgias and arthralgias and enhance similar and expected symptoms related to vaccination. Lastly, PSK has not been associated with grade 3 flu-like symptoms, arthralgias, myalgias or hematologic toxicity (lymphopenia, anemia, or leukopenia).

We will monitor patients closely for the above Grade 3 toxicities and should they occur will only be allowed for a limited duration of 1-2 weeks. If any of these Grade 3 toxicities exceed the 1-2 week period, they will constitute DLT and patient would not receive further treatment.

If there is a Serious Adverse Event (Appendix C; Section C), that in the opinion of the Independent Medical Monitor, may constitute increased risk to other study participants (i.e., risk section of consent may need to be amended), un-blinding of treatment assignment for an individual subject may be requested by the medical monitor. The study statistician would provide the code if requested directly by the medical monitor. The study team would remain blinded and would follow the recommendations of the Independent Medical Monitor to amend the necessary study documents.

## 11. Dose Modifications

There will not be any modifications to the vaccine, PSK or placebo doses. There may be allowances for the timing of the administration of vaccine to accommodate patient schedules or special circumstances. Subsequent vaccinations will be scheduled 28 + 7 days from when the previous vaccine was actually administered, not when it should have been administered.

## 12. Accrual and Criteria for Premature Study Termination

Combination immunotherapy with HER2-directed immunotherapy has been shown to be both safe and immunogenic. Additionally, the proposed oral dose of PSK (3000 mg/day) has been demonstrated to be safe with and without chemotherapy in large randomized breast cancer studies with the most common and expected AEs being diarrhea, nausea, vomiting, anorexia, hives and stomach discomfort (incidence of all listed AEs was <1%).[44, 45] Although we expect minimal toxicity from HER2 vaccination administered concomitantly with PSK, criteria for stopping the trial for excessive treatment-related toxicity will be instituted as follows:

The study will be terminated for safety reasons if there is sufficient evidence to suggest that the true incidence of Grade 3 or higher toxicity exceeds 40%, the true incidence of Grade 4 toxicity exceeds 20%, or both. The toxicity rates (adverse events) will be evaluated after every 5th enrolled patient has become evaluable, and sufficient evidence will be defined as observed rates of toxicity that correspond to 1-sided 80% confidence intervals with lower limits greater than 40% (Grade 3 or higher) or 20% (Grade 4). Operationally, these limits would be achieved if 4/5, 6/10, or 9/15 patients experienced Grade 3 or higher toxicity, or if 3/5, 4/10, or 5/15 patients experienced Grade 4 toxicity. Monitoring and reporting of AEs and toxicity rates will be done per the DSMP (Appendix C).

## 13. Statistical Considerations

### A. Study statistics

1. Determination of Sample Size:
   This sample size is chosen to be sufficient for gathering preliminary data on both the safety of PSK and its efficacy in enhancing NK cell activity when combined with HER2 vaccination and HER2 monoclonal antibody therapyin this patient population. Thirty patients will be enrolled and randomized in equal numbers to one of 2 arms (15 patients/arm) unless there is excessive toxicity as defined in Section 12. Up to 5 additional patients may be enrolled per arm to replace subjects who fail to receive study treatment to meet the target accrual of 30 subjects.
2. Randomization:
   Eligible subjects will be enrolled and randomly assigned in equal proportions to one of two treatment arms, Arms 1 (Placebo) and 2 (PSK) via a permuted block design. Following an algorithm developed by the study statistician, a randomization table will be generated and provided to IDS pharmacy.

Patients, clinicians and study staff will be blinded to the treatment assignment. The dispensing UW IDS pharmacy staff will be un-blinded.

a. Randomization will take place following completion of the screening evaluations and eligibility assessment.
b. Patient information will be entered into the study database and patients will be assigned a unique study identification number.
c. The study staff will notify the dispensing pharmacy to request the patient's randomization.
d. Patients will be randomly assigned per the randomization table described above.

3. Statistical Analysis of Study Endpoints:
This is a randomized phase I/II double-blind study designed to examine the safety and immune effects of PSK when given as combination HER2-directed immunotherapy in patients with advanced HER2+ breast cancer.

a. *Evaluation of safety and systemic toxicity among the different treatment arms.* Clinical and/or chemical parameters for all study patients will be evaluated for potential toxicity at baseline, before each vaccine, and 1 month after the third vaccine. Toxicity grading will be evaluated per CTEP CTCAE 4.0 and monitoring of AEs will be done per FDA and NCI guidelines. The type and grade of toxicities noted during treatment will be summarized. AEs will be tabulated per CTCAE 4.0 system organ class and descriptive statistics will be used to summarize toxicity profiles for the PSK and placebo groups. Stopping rules based on toxicity are described in Section 12.

b. *Evaluation of the effect of PSK on NK cell functional activity*. Our previous in vitro studies on PSK have shown that PSK can enhance NK cell function. It significantly induced IFN-γ production and CD107a expression in NK cells. After overnight stimulation with 100ug/mL PSK, the percentage of NK cells that express CD107a is 1.8+/-1.3% in control group and 6.1+/-3.8% in PSK group (n=7, p=0.01). Therefore we chose CD107a expression as the primary marker of NK cell activation. However we do not have direct information on the ability of PSK to activate NK cells after in vivo administration. Based on a recent Phase 1 clinical trial of Trametes versicolor (PSK) in women with breast cancer conducted by Standish et al, NK cell activity increased to approximately 2-fold of baseline after 4 weeks of oral administration of PSK. We hypothesize that oral administration of PSK will have a similar effect and augment NK cell activity by 2-fold while NK cell activity in the placebo group will be unchanged compared to the baseline (pre-treatment) level. Under these assumptions and the assumption that the standard deviation of change in NK cell activity is 1.6, a sample size of 15 will provide 91% power (at the 2-sided significance level of .05) to test the null hypothesis that the change (post-treatment vs. pre-treatment) in CD107a expression is the same in the PSK group as it is in the control group. These assumptions imply a true effect size of 2/1.6 = 1.25.

c. *Assessment of the association between changes in pro-inflammatory serum cytokine and/or chemokines and treatment with PSK*. Serial blood draws will be done at baseline before the initial dose of PSK (time 0) and 30 min (+15 min), 4 hours (+/-15 min), and 24 hours (+/-30 min) after the initial dose of PSK to assess serum cytokines/chemokines (i.e., IL-6, IFN-γ, and TNF-α) via Luminex analysis. Serum cytokine/chemokine levels at different timepoints post-PSK will be compared to baseline levels using linear regression or linear mixed models. A global treatment group comparison will be made for all post-baseline measurements, for the rate of change in cytokine/chemokine levels, and for the 24 hour timepoint.

d. *Assessment of the association between development of intermolecular epitope spreading and treatment with PSK.* Preliminary data show that HER2 Th1 vaccines given with trastuzumab elicit or augment tumor-specific immunity including epitope spreading. We have identified a panel of breast cancer associated antigens that could provide coverage to approximately 61% of all breast cancer patients as an immunogenic profile (HER2, IGFBP-2, TOPO-IIα, p53, and PRAME). We will evaluate PBMC from patients pre- and post-treatment for the development of T cell immunity to these antigens by ELISPOT (indicating epitope spreading). We hypothesize that PSK will boost circulating IFN-γ secreting tumor-specific Th1 CD4+ in treated patients. The development of a positive antigen-specific T cell immune response will be defined as a precursor frequency more robust than 1:20,000 antigen-specific PBMCs. In patients without a pre-existent immune

response, the acquisition of a positive immune response after treatment begins will constitute augmentation. In patients with pre-existent immune response to the tumor antigens, augmentation will have occurred if the final response to tumor antigen is >2 times the baseline level. Significant differences in the levels of immunity to HER2 will be compared between groups using a two-tailed T test. Intramolecular epitope spreading will be defined as generation of significant immunity to the HER2 ECD as the patients will be receiving an ICD vaccine.

The degree of intermolecular epitope spreading in each arm may be summarized as the proportion of the 5 antigens that exhibit T cell augmentation as defined above. If, on average, 2/5 antigens are expected to demonstrate epitope spreading in Arm 1 (vaccine and HER2-targeted monoclonal antibody therapy alone) and the expected rate is 4/5 in Arm 2 (PSK arm), a generalized linear model of binomial proportions with 15 patients per group (two-sided test with $\alpha$=0.05) will have 99% power to detect a difference in proportion of antigens that exhibit epitope spreading, and 77% power to detect a rate of 4/5 compared to 3/5. We will also assess the correlation of reduction in TGF-β level with the extent of epitope spreading, where each is quantified as a continuous linear variable. Linear regression will be used to assess this correlation, and all patients (both PSK and non-PSK patients) will be used for this analysis.

e. *Assessment of the association between changes in TGF-β levels and treatment with PSK.* As previously described, PSK may directly inhibit TGF-β signaling resulting in decreased TGF-β production and enhanced NK cell activity and increased IFN-γ secretion. Additionally, our previous study data demonstrated vaccine-induced HER2 specific IFN-γ T cell responses to be associated with modest reductions in serum TGF-β levels. We hypothesize that addition of PSK to HER2 vaccination and HER2-targeted monoclonal antibody therapy will result in increased NK cell-induced IFN-γ production; and a greater decrease from baseline to post-vaccination serum TGF-β levels compared to HER2 vaccination and HER2-targeted monoclonal antibody therapy alone.

   In our previous vaccine trial, decrease in serum TGF-β levels among patients who receive HER2 ICD vaccination and trastuzumab alone was modest. Specifically, the mean decrease in TGF-β (pre-vaccine vs. post-vaccine) was 294 pg/ml (10% reduction) with a standard deviation of roughly 1000 pg/ml in 23 vaccinated patients. The average pre-vaccine value among these patients was just over 2900 pg/ml. If we assume that the true decrease in TGF-β level among patients who receive PSK is 1450 pg/ml (50%), a sample size of 30 provides 89% power to conclude that change in TGF-β is different for groups with and without PSK, using two-sided tests with $\alpha$=0.05. Actual power should be greater, since the statistical analysis will use the pre-test TGF-β as a covariate in a linear regression model with transformations performed as needed to satisfy regression assumptions.[46] Combined analysis of multiple post-baseline immune measurements (using generalized estimating equations) may also increase precision.

f. *Evaluation of PFS and OS.* PFS and OS (time of first vaccine to time of first event) will be compared between treatment arm cohorts. This study was not designed to be sufficiently powered for any particular hypothesis testing, since time-to-event analyses generally require larger sample sizes than simple two-way comparisons. However, large differences, if observed between the different vaccine treatment groups will be noted and described.

**B. Projected gender and ethnic distribution**

The ethnic and gender distribution chart below (Table 6) reflects estimates of race and gender of the population to be included in this study. These estimates are based on the following general statistics of the population in Washington State based on recent 2010 US Census data. The population pool from which patients will be drawn is 83.8% Caucasian, 7.0% Asian American, 3.9% African American, 1.8% American Indian or Alaska Native and 0.5% Native Hawaiian or Other Pacific Islander. Although men with breast cancer are not excluded from this study, less than 1% of all breast cancers occur in men.

**Table 6. Targeted/Planned Enrollment: Number of Subjects = 30**

| Ethnic Category | Sex/Gender | | |
|---|---|---|---|
| | Females | Males | Total |
| Hispanic or Latino | 3 | 0 | 3 |

| | | | |
|---|---|---|---|
| Not Hispanic or Latino | **26** | **1** | **27** |
| **Ethnic Category: Total of All Subjects *** | **29** | **1** | **30** |
| **Racial Categories** | | | |
| American Indian/Alaska Native | **1** | **0** | **1** |
| Asian | **2** | **0** | **2** |
| Native Hawaiian or Other Pacific Islander | **0** | **0** | **0** |
| Black or African American | **1** | **0** | **1** |
| White | **25** | **1** | **26** |
| **Racial Categories: Total of All Subjects *** | **29** | **1** | **30** |

## 14. Administrative considerations

### A. Institutional review board

In accordance with federal regulations, an Institutional Review Board that complies with the regulations in 21 CFR 56 must review and approve this protocol and the informed consent form prior to initiation of the study.

### B. Consent

The P.I., study physician or their designated physician extender must explain verbally and in writing the nature and duration of the study and possible consequences of the treatment. Patients should also be informed that they may withdraw from the study at any time and for any reason without jeopardizing their future treatment. At the initiation of eligibility screening and prior to enrollment, the investigators or their associates will obtain permission from the patient to access medical records for research purposes. Additionally, patients will remain blinded until publication of study manuscript.

### C. Reporting adverse events

Adverse events will be reported using the NCI's CTEP CTCAE v 4.0. A copy of the CTCAE v 4.0 can be downloaded from the CTEP homepage (http://ctep.info.nih.gov). Guidelines for adverse event reporting to the FDA and the NCI are described in the DSMP, Appendix C.

All serious adverse events are communicated to the Principal Investigator, IRB, FDA, NCCAM, CRC, and Independent Medical Monitor. A status report including accrual, adverse events and death information will be reviewed by the Principal Investigator and the FHCRC – Cancer Consortium Protocol Data Monitoring Committee (PDMC) annually. In addition, the study will be monitored by the CTSO according to the FHCRC – Cancer Consortium monitoring plan.

### D. Confidentiality of patient records

All eligible patients will be assigned a unique study ID number that will not contain any personally identifying information, such as name, initials, medical record number, social security number, etc. The study ID number will be used on all specimens, research study records and associated documents collected in the research record. To maintain confidentiality, we protect the link between the patients' personal identifying information and study ID number by limiting who have access to the patients' chart documentation. Only specified clinical research staff has access to the data, which remains locked at all times when not in use.

All hard copy research records collected on potential and enrolled patients are stored in a locked filing cabinet that is accessed only by approved clinical research staff. This staff which includes the Principal Investigator, study physician(s), and designated clinical research staff are the only ones with access to the link between patients' personal identifying information and their assigned UPN codes.

Protections and security of electronic clinical data it is being performed by the UW Medicine Information Technology (IT) Services. The UW Medicine IT security policy is to protect UW Medicine information and information systems; and ensure compliance with UW policy, state and federal regulations.

### E. Study Team Roles and Responsibilities

Dr. Gwin, the Principal Investigator will be responsible for the oversight of the research. Dr. Gwin is responsible for ensuring that all information and documentation related to the conduct and safety of the study is disseminated to the proper agencies in the proper timeframe. Dr. Gwin will delegate tasks to qualified research staff for this study as needed.

Research physicians/physician extenders will be responsible for conducting consent conferences and eligibility determination at the initial visit. They will perform monthly assessments prior to vaccination. In addition, they will be available to answer any patient questions.

Research Nurses/Coordinators will be responsible for initial screening and scheduling patient visits, that data is entered into the database in a timely manner, so as to have real time data to review and report any safety concerns trends. They will also be responsible for maintaining regulatory documentation to the various agencies involved with this research and ensure that all research team members are following the protocol and all regulations.

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# Appendix A

## Zubrod Performance Scale

**Performance status will be assessed according to the current ECOG performance scale***

| Grade | Description |
|---|---|
| 0 | Fully active, able to carry on all pre-disease performance without restriction |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work |
| 2 | Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours |
| 3 | Capable of only limited self-care, confined to bed or chair more than 50% of waking hours |
| 4 | Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair |
| 5 | Dead |
| | *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.*<br>*The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair. |

# Appendix B

## Schedule of Events

| Visit Time Point | Procedures |
|---|---|
| **Vaccine #1: Day 1 (First Visit)** | • ⊙Consent<br>• ⊙Medical history, physical examination, symptom assessment and medication/supplement changes<br>• ⊙◆Clinical Labs: CBC and CMP<br>• Vital signs and weight<br>• Urine pregnancy test if applicable<br>• Research Labs: 200 mls for immunologic monitoring<br>• Tetanus-diphtheria (Td) immunization<br>• HER2 Vaccination<br>• Administration of total daily dose of PSK or placebo then 10 ml blood draw 30 minutes and 4 hours post-administration for cytokine kinetics |
| **Vaccine #1: Day 2** | • Research Labs: 10 mls for cytokine kinetics at CRC then take Day 2 dose of PSK or placebo as instructed<br>• Take home 30 day supply plus 7 additional days of PSK or placebo |
| **Vaccine #1: Day 2 until Vaccine #2 visit** | • *PSK or placebo daily as instructed and complete daily tea diary* |
| **Vaccine #2: Day 1** | • Interim medical history, physical examination, vital signs and weight<br>• Symptom/toxicity assessment and medication/supplement changes<br>• Review tea diary<br>• ◆Clinical Labs: CBC and CMP<br>• Research Labs: 40 mls for immunologic monitoring<br>• Urine pregnancy test if applicable<br>• HER2 Vaccination<br>• Take home 30 day supply plus 7 additional days of PSK or placebo |
| **Vaccine #2: Day 1 until Vaccine #3 visit** | • *PSK or placebo daily as instructed and complete daily tea diary* |
| **Vaccine #3: Day 1** | • Interim medical history, physical examination, vital signs and weight<br>• Symptom/toxicity assessment and medication/supplement changes<br>• Review tea diary<br>• ◆Clinical Labs: CBC and CMP<br>• Research Labs: 40 mls for immunologic monitoring<br>• Urine pregnancy test if applicable<br>• HER2 Vaccination<br>• Take home of 30 day supply plus 7 additional days of PSK or placebo |
| **Vaccine #3: Day 1 until Month 4 visit** | • *PSK or placebo daily as instructed and complete daily tea diary* |
| **Month 4 (1 month after the last vaccine)** | • Interim medical history, physical examination, vital signs and weight<br>• Symptom/toxicity assessment and medication/supplement changes<br>• Review tea diary<br>• ◆Clinical Labs: CBC and CMP<br>• Research blood: 200 mls<br>• Take home of 30 day supply of PSK or placebo |
| **Month 4: Day 1 until Month 5 visit** | • *PSK or placebo daily as instructed and complete daily tea diary* |
| **Month 5 (2 months after the last vaccine)** | • Research Labs: 40 mls for immunologic monitoring<br>• Send tea diary to study nurse |
| **Month 6 (3 months after the last vaccine)** | • Collection of recent oncologist notes and lab results<br>• Collection of recent imaging reports if available |
| **Month 12 (9 months after last vaccine)** | • Research blood: 200 mls<br>• Collection of physician notes including most recent lab results and imaging reports |

⊙May be done up to 2 weeks prior to initial vaccine visit
◆Clinical Labs: Complete blood count (CBC) includes differential/platelets; and Comprehensive Metabolic Panel (CMP) includes serum electrolytes, creatinine, BUN, AST, ALT, alkaline phosphatase, total bilirubin

# Appendix C

## Data Safety Monitoring Plan

### A. Purpose

To ensure that the Tumor Vaccine Group (TVG) follows NIH/NCI/CTEP/FDA guidelines with respect to: (1) accurate assessment and timely reporting of adverse drug reactions associated with investigational drugs, (2) adherence to protocol, and (3) accurate reporting of data. The DSMP will be approved by Fred Hutchinson Cancer Research Center – Cancer Consortium IRB (FHCRC – CC IRB) in Seattle, Washington.

The Principal Investigator (P.I.), for this project, is responsible for every aspect of the design, delegating responsibility/authority, study conduct and final analysis of the protocol. Regulations defining the responsibilities for assessment and reporting of adverse events (AE), serious AE (SAE), and unexpected AEs are defined by the Code of Federal Regulations: 21 CFR 312.32 and Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 published by the Cancer Therapy Evaluation Program (CTEP), a division of the NCI/NIH. A matrix of reporting requirements and schedules is at the CTEP web-site at http://ctep.infi.nih/gov.

This clinical study will rely upon the monitoring of the trial by the P.I. in conjunction with a Research Nurse, a Statistician, an Independent Medical Monitor, and an Independent Study Monitor assigned by the Clinical Trials Support Office (CTSO) of the University of Washington (UW)/Fred Hutchinson Cancer Research Center (FHCRC) Cancer Consortium.

The Medical Monitor, Robert Montgomery, M.D. will meet openly with the P.I. and/or their designee, Statistician, Research Nurse, Research MD and/or Research Coordinator(s) prior to patients being recruited in order to orient committee participants to the study. The Medical Monitor will then discuss the study every 6 months throughout the study treatment period with the P.I., Research MD(s), Research Nurse(s), Research Coordinator(s), and/or other related clinical research staff. The Medical Monitor will review patient recruitment and retention, adherence to protocol, follow-up, data quality, and participant risk versus benefit.

### B. Objectives

1. To ensure that the Principal Investigator and clinical research staff follow federal and institutional regulatory guidelines with respect to timely reporting of adverse reactions associated with investigational drugs.
2. To define classification of adverse drug reactions as expected or unexpected.
3. To define classification of adverse drug reactions as serious or non-serious.
4. To ensure compliance and accuracy of documentation of adverse drug reactions reportable to: (1) FHCRC – CC IRB, (2) FDA, (3) UW Clinical Research Center, (4) Independent Medical Monitor and (5) NIH - NCCAM.

### C. Adverse event reporting policy and procedures

1. Evaluation of adverse events:

   AEs are graded on a scale of 1-5 and attribution is assigned, using the CTCAE v4 common terminology. Patients are monitored at: (1) eligibility, (2) at each vaccination visit, and (3) at Month 4, for the development of end organ damage by assessing AEs with serum chemistries, liver function studies, complete blood counts and physical exams. Information pertaining to toxicity is recorded into a database AE Log.

2. Definitions of adverse events:

   *Adverse Event* - any unfavorable and unintended sign (including abnormal laboratory finding*), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure.

a. An adverse event **may** include:

- an exacerbation of a pre-existing illness
- an increase in frequency or intensity of a pre-existing episodic event or condition
- a condition detected or diagnosed after study drug administration
- continuously persistent disease or symptoms that were present at baseline and worsen following the start of the study
- *An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:
  - Results in discontinuation from the study;
  - Is associated with clinical signs or symptoms;
  - Requires treatment or any other therapeutic intervention;
  - Is associated with death or another serious adverse event, including hospitalization; or
  - Is judged by the Investigator to be of significant clinical impact.

  If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

b. An adverse event **does not** include:

- medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, or transfusion); however, the condition that leads to the procedure may be an adverse event
- pre-existing diseases, conditions, or laboratory abnormalities present or detected at the start of the study that do not worsen
- hospitalizations or procedures that are done for elective purposes not related to an untoward medical occurrence (e.g., hospitalizations for cosmetic or elective surgery or social/convenience admissions)
- the disease being studied or signs/symptoms associated with the disease unless more severe than expected for the patient's condition
- overdose of study drug without any clinical signs or symptoms

c. *Expected Adverse Event* - an event that may be reasonably anticipated to occur as a result of the study procedure and is described in the Investigators Brochure and/or consent form.

d. *Unexpected Adverse Event* - an AE that is not described in the investigator brochure and/or consent form and is unanticipated. An event that might have been anticipated but is more serious than expected or occurs more frequently than expected, would be considered an unexpected AE.

e. *Life-threatening Adverse Event* - the patient was at substantial risk of dying at the time of the AE or it is suspected that the use or continued use of the product would have resulted in the patient's death.

f. *Serious Adverse Event* - grade 4 or 5 toxicity or any AE occurring at any dose that results in any of the following outcomes: death, a life threatening AE, inpatient hospitalization or prolongation of existing hospitalization due to the AE, a persistent or significant disability/incapacity or a congenital anomaly/birth defect that is unexpected and unrelated.

g. Hospitalizations that do not meet these criteria are:

- reasons described in the protocol, e.g., drug administration, protocol-required testing
- social reason in the absence of an AE
- surgery or procedure planned prior to entry into the trial

Note: Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations; for example, important medical events may not be immediately life-

threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Any AE is considered a serious AE if it is associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

3. Adverse event score
   a. Grade 1 = mild adverse event
   b. Grade 2 = moderate adverse event
   c. Grade 3 = severe adverse event
   d. Grade 4 = life-threatening or disabling adverse event
   e. Grade 5 = death related to adverse event.

4. Attribution of adverse event
   a. 5 (definite): the adverse event is clearly related to the investigational agent
   b. 4 (probable): the adverse event is likely related to the investigational agent
   c. 3 (possible): the adverse event may be related to the investigational agent
   d. 2 (unlikely): the adverse event is doubtfully related to the investigational agent
   e. 1 (unrelated): the adverse event is clearly NOT related to the investigational agent

5. Adverse event reporting

This procedure is outlined below by regulatory agency (Table 1).

**Fred Hutchinson Cancer Research Center: Cancer Consortium IRB** - We will follow the current AE reporting policy of the FHCRC – CC IRB.

**FDA (for trials using an Investigational New Drug (IND)** – Adverse events will be reported per 21 CFR 312.32 by telephone or facsimile transmission (using MedWatch Form 3500A) as soon as possible but no later than 7 calendar days after initial receipt of the information concerning the event. All unexpected, serious adverse events are reported in writing (using MedWatch Form 3500A) to the FDA (Center for Biologics) within 15 calendar days after initial receipt of the information concerning the event. Yearly written progress reports to the FDA will summarize expected or non-serious unexpected AEs.

**NCCAM –** Serious adverse events will be reported to NCCAM Program Officer within 24 hours of FDA notification. A copy of the documents submitted to the FDA will also be submitted to NCCAM within 7-15 calendar days. Submit written summaries of all reviews conducted by the monitoring group to the responsible NCCAM Program Officer within 30 days of reviews or meetings. If reviews are frequent, quarterly reports are sufficient.

**University of Washington Clinical Research Center (UW CRC)** – The CRC will receive copies of the documents submitted to the FHCRC – CC IRB within 7-10 calendar days.

**Independent Medical Monitor -** At a minimum, the medical monitor should comment on the outcomes of serious adverse event(s) and relationship of the AE to the vaccine or prepare an unbiased written report of the event. They should also indicate whether they agree with the details of the report provided by the study investigator.

**Table 1. Adverse event reporting**

| | Expected | Expected | Unexpected | Unexpected |
|---|---|---|---|---|
| | Non-Serious | Serious | Non-Serious | Serious (including grade 4 & 5 toxicity) |
| | Attribution* (Possible, Probable, Definite) | Attribution * (Possible, Probable, Definite) | Attribution * (Possible, Probable, Definite) | Attribution * (Possible, Probable, Definite) |
| FHCRC – IRB | Continuation Review Report | Continuation Review Report | Continuation Review Report | FHCRC Adverse Event Expedited |

| | Expected Non-Serious | Expected Serious | Unexpected Non-Serious | Unexpected Serious (including grade 4 & 5 toxicity) |
|---|---|---|---|---|
| | Attribution* (Possible, Probable, Definite) | Attribution * (Possible, Probable, Definite) | Attribution * (Possible, Probable, Definite) | Attribution * (Possible, Probable, Definite) |
| | | | | Reporting Form – ASAP but within 7 days |
| FDA | Annual Progress Report | Annual Progress Report | Annual Progress Report | FDA Form 3500A – ASAP but within 7 days |
| NCCAM | Annual Review | Annual Review | Annual Review | Notify Program Officer within 24 hours of FDA notification<br><br>FDA Form 3500A – ASAP but within 7-15 days |
| UW Clinical Research Center (CRC) | Copy of Continuation Review Report | Copy of Continuation Review Report | Copy of Continuation Review Report | Copy of FHCRC Adverse Event Expedited Reporting Form |
| Medical Monitor | Bi-annual Meeting (twice a year) | Bi-annual Meeting (twice a year) | Bi-annual Meeting (twice a year) | Copy of FHCRC Adverse Event Expedited Reporting Form |

6. Procedure for reporting serious adverse events:
   a. Identify the classification/attribution of the AE as defined above using CTCAE v4.
   b. After appropriate medical intervention has been instituted, the P.I. or his/her designee will be notified within 24 hours.
   c. File appropriate reports immediately by phone/fax with appropriate agencies, as described in Table 1.
   d. Notify the patient's primary physician or referring physician within a medically appropriate timeframe, depending on the classification of the adverse event.
   e. Submit written reports to appropriate agencies.
   f. Document the AE in the patient's research file, using a progress note to describe the event and treatment, if appropriate.
   g. File copies of all forms/correspondence relating to the AE in the patient's research file.

**D. Clinical trials monitoring operational procedures** (Table 2)

1. Clinical data documentation

   a. *Internal study monitoring*:

   All patients actively enrolled in the study are seen monthly during the treatment period and one month following the final vaccine administration. Clinical labs are evaluated at baseline, before each vaccination and 1 month after the 3rd vaccine for the development of toxicity (adverse events) related to the vaccine. Any clinically significant abnormal lab values may be faxed to the patient's physician. The Research Physician, physician extender or P.I. sees each study participant at each visit and the following evaluations are completed: toxicity evaluation, physical assessment and AE summary (these

evaluations are part of the source documentation that is filled out at each visit). Grade 3 and 4 non-serious and expected AEs will be reviewed with the P.I. regularly at clinical meetings. All other AEs will be reported to the P.I. at the time they become known and reported as outlined above in Section C.

Each patient research file is audited for completeness. Audits may be conducted by a designated clinical research staff member on an ongoing basis and the results may be presented clinical meetings. Patient's research files are audited for completeness, legibility, and accuracy. Clinical research staff members will review all patient research files for patient recruitment and retention, protocol adherence, follow-up, data quality, and participant risk versus benefit.

Additionally, correspondence with primary physicians and patients is maintained in the patient research file. Copies of email communications and of verbal communications are maintained.

b. *Biannual review with the independent Medical Monitor*:

The Medical Monitor will meet/or conference call with the P.I. or designee, and other members of the clinical team, approximately every 6 months. All patients are reviewed for AEs. Conduct of the study is reviewed for any practice changes and documentation of proper notification of changes as appropriate to the IRB and FDA.

*c. Biannual study audit:*

An independent Study Monitor will be assigned by the CTSO of the FHCRC/UW Division of Oncology and an independent study audit may be performed two times a year (approximately every 6 months). This audit will be conducted on a select percentage of enrolled patients. Patient research files will be formally audited for completeness, accuracy and compliance to protocol. In addition, a certain percentage of all computer data entry will also be reviewed. In addition to the routine visit of the UW IDS pharmacy, the monitor will also confirm the correct randomized product (PSK/Placebo) was dispensed to the patient.

2. Data validity/integrity

Systems to insure data integrity have been put into place to provide multiple checks to data entry. Patient eligibility is initially reviewed by the Research Coordinator/Research Nurse at screening, by the Research MD prior to enrollment in select cases, approved by the P.I. before enrollment, and biannually by the Independent Study Monitor of a certain percentage of charts. Source documents are initially reviewed by the Research Nurse and/or any clinical research staff member involved with recruitment for completeness.

Clinical laboratory monitoring data is reviewed by a qualified clinician (Research MD, physician extender, Research Nurse and/or P.I.) and any abnormalities are assessed. All patients' records are kept in the patient's research file and reviewed at biannual monitoring visits. All toxicity scoring is done by a study clinician.

Data is taken from the patient's source documentation, the patient's research file, and entered into a database which links data to patient by a Unique Patient Identifier and is accessible by password code only. Data entry is made by the Research Coordinator/designated clinical research staff member and is verified at end of study by the Research Nurse/designated clinical research staff member reviewing source documents and case report forms; and reconciling them to the study database. A percentage of all data entry is reviewed by a clinical research team member and the Independent Study Monitor. At the time of study reports or publications, print outs of all data from the database are reviewed by the P.I.

**Table 2. Operational procedures**

| Study Event | Specific Action | Performed By |
|---|---|---|
| Screen | Screening of interested patients | Research Coordinator/physician extender/Research Nurse/other designated clinical research staff member assigned by PI |
| Eligibility | Initial review w/interested patient | Research Coordinator/physician extender/Research Nurse/other designated clinical research staff member assigned by PI |

| Study Event | Specific Action | Performed By |
|---|---|---|
| | Obtain source documents to determine eligibility | Research Coordinator/Research Nurse/other designated clinical research staff member assigned by PI |
| | 2nd review of eligibility with final approval from the PI | Research MD/P.I. |
| | Biannual review | Independent Study and Medical Monitors |
| Consent | Obtain Informed Consent | P.I/ Research MD/physician extender |
| Monthly Visits | Blood draw/ Urine Collection | Research Nurse (including nurses from CRC)/physician extender |
| | Physical assessment/toxicity evaluation | P.I/ Research MD/physician extender |
| | Vaccination | Research Nurse (including nurses from CRC)/physician extender |
| | Monitor for immediate reactions | Research Nurse (including nurses from CRC), Study Physician |
| Toxicity Monitoring | Monitoring of AEs with patient at each visit | Research MD/P.I./physician extender |
| | Review of non serious and grade 2 expected AEs regularly | P.I. |
| | Biannual review of documentation of AEs | Independent Study and Medical Monitors |
| Report of serious adverse events | See sections B and C of DSMP | Research Nurse/P.I./Physician Extender/Research MD/ Research Coordinator |
| Immunologic Monitoring-Research data | Performed at specified timepoints for each patient | Immunologic Monitoring Lab Technologist(s) |
| | Review of immunologic data | PI /Research MD |
| | Computer entry of immunologic data | Research Coordinator/physician extender/other designated clinical research staff member assigned by PI |
| Study review with P.I. | Regularly scheduled meeting to report study status/evaluate eligibility, patient recruitment and retention, protocol adherence, and AE evaluations. Daily on an as needed basis for all unexpected grade 3-5 AEs | P.I./Research MD/Physician Extender/Research Nurse/Research Coordinator |
| Study Review by Medical Monitor | Biannual evaluation of patient recruitment and retention, protocol adherence, AEs and participant risk versus benefit. | Medical Monitor, P.I., Research MD, Research Nurse, and Research Coordinator |
| Twice yearly chart audits | Biannual evaluation of patient recruitment and retention, protocol adherence, AEs and participant risk versus benefit. Validation of computer data entry from the source document. | Independent Study Monitor w/ wrap up meeting with the P.I./Study physician/physician extender/Research Nurse/Research Coordinator(s) |
| Assurance of Data Accuracy and Integrity | Validation of computer data entry for clinical data | Research Nurse and Research Coordinator(s) |

| Study Event | Specific Action | Performed By |
|---|---|---|
| | Validation of data entry for immunologic monitoring-research data | Independent Study and Medical Monitors/P.I. |
| Annual reports to: | | |
| IRB | | PI with Research Coordinator(s)/Research Nurse |
| FDA | | PI with Research Coordinator(s)/Research Nurse |
| CRC | | PI with Research Coordinator(s)/Research Nurse |
| NCCAM | | PI with Research Coordinator(s)/Research Nurse |