**SPONSOR: Duke University**

**TITLE: A Phase II randomized study to evaluate the immunologic and antitumor activity of concurrent VRP-HER2 vaccination and Pembrolizumab for patients with advanced HER2-overexpressing breast cancer**

**IND NUMBER: 14932**

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**Duke IRB# Pro00100093**

**NCT03632941**

# CONTENTS

# 1.0 TRIAL SUMMARY

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|---|---|
| Abbreviated Title | Concurrent VRP-HER2 vaccination and Pembrolizumab for patients with advanced HER2+ breast cancer |
| Trial Phase | Phase II |
| Clinical Indication | Advanced HER2+ breast cancer receiving Trastuzumab and Pertuzumab |
| Trial Type | Randomized Phase II Study |
| Type of control | None |
| Route of administration | VRP-HER2 vaccine– intramuscular, Pembrolizumab - intravenous |
| Trial Blinding | None |
| Treatment Groups | Safety Arm: VRP-HER2 vaccine + Pembrolizumab (n=3)<br>Arm A: VRP-HER2 vaccine (n=12)<br>Arm B: Pembrolizumab alone (n=12)<br>Arm C: VRP-HER2 vaccine + Pembrolizumab (n=12) |
| Number of trial subjects | 39 |
| Estimated enrollment period | 18 months |
| Estimated duration of trial | 30 months |
| Duration of Participation | 24 months |
| Estimated average length of treatment per patient | 12 weeks |

# 2.0 TRIAL DESIGN

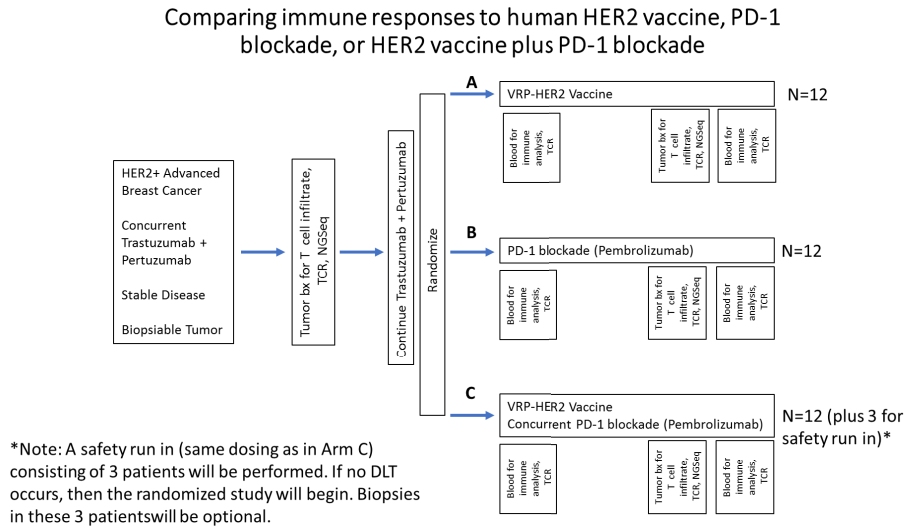
## 2.1 Trial Design

There will be an initial Safety Arm (n=3) during which subjects will receive the VRP-HER2 immunizations plus pembrolizumab and, if there is no dose limiting toxicity* in the Safety Arm, then subjects will be stratified by hormone receptor status (ER and/or PR + versus negative) and randomized 1:1:1 into 3 arms (n=12 per arm) as per the diagram in Section 2.2. Specifically, subjects with metastatic HER2-overexpressing breast cancer receiving trastuzumab and pertuzumab** will continue these antibodies. They will undergo a biopsy of their tumor and peripheral blood draw for immune cell analyses and be assigned to the applicable arm of the study. Arm A will consist of the VRP-HER2 immunizations; Arm B will consist of pembrolizumab; Arm C will consist of the VRP-HER2 immunizations plus pembrolizumab. Tumor biopsies and peripheral blood draws will be performed following the course of immunizations.

* Dose limiting toxicity is defined as any Grade 2 or higher immediate hypersensitivity reactions or neurological toxicity, other Grade 3 or 4 allergic or major organ toxicity, or any other Grade 3 or higher toxicity attributable to study treatment (VRP-HER2 vaccine or pembrolizumab).

** For the concurrent Trastuzumab and Pertuzumab, dosing will be at the discretion of the treating physician.

### 2.2 Trial Diagram




## 3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

### 3.1 Primary Objective(s) & Hypothesis(es)

(1) **Objective:** To determine whether pembrolizumab increases the tumor infiltrating (T cell) and peripheral blood (T cell and antibody) immune response to the VRP-HER2 vaccine.

**Hypothesis:** We hypothesize that HER2 specific T cell responses and anti-tumor immunity induced with HER2 vaccination will be augmented by concurrent anti-PD-1 antibody therapy.

### 3.2 Secondary Objective(s) & Hypothesis(es)

(2) **Objective**: To determine whether the administration of pembrolizumab is safe in patients with recurrent or metastatic HER2+ cancers who are receiving the anti-HER2 vaccine VRP-HER2.

**Hypothesis**: We hypothesize that concurrent immunization with HER2-targeting vaccines and anti-PD-1 antibody therapy will be well tolerated.

### 3.3 Exploratory Objective

(3) **Objective**: To collect preliminary data on the clinical response rates to this concurrent therapy of agents in subjects with assessable disease based on RECIST 1.1 criteria.

**Hypothesis**: We hypothesize that the induction of enhanced anti-tumor T cell responses from concurrent HER2 vaccination and anti-PD-1 antibody therapy will lead to greater clinical efficacy.

## 4.0 BACKGROUND & RATIONALE

### 4.1 Background

HER2 expressing malignancies, while initially responsive to trastuzumab and pertuzumab, eventually progress; yet the HER2 protein continues to be expressed on these tumors suggesting they may be targets for immune therapies. Indeed, it has been demonstrated that a higher percentage of tumor infiltrating lymphocytes (TILs) is associated with better tumor response to trastuzumab in both the adjuvant[1] and neoadjuvant setting in HER2+ breast cancers.

One strategy for increasing the T cell lymphocyte response to tumors is to use vaccines capable of activating T cells against tumor associated antigens such as HER2. We have extensive experience developing vaccines to activate HER2-specific immune responses in patients with HER2 overexpressing malignancies. We vaccinated patients with HER2 overexpressing breast cancer with dendritic cells loaded with HER2 protein or peptide[2] or vaccines based on HER2 protein plus adjuvants[3] and reported the induction of anti-HER2-specific antibodies and T cell responses. In an attempt to develop a vaccine capable of inducing more effective T and B cell responses, we generated alphaviruses modified to express tumor antigens such as HER2. These vectors have tropism for dendritic cells and direct high expression of the HER2 antigen within dendritic cells. In preclinical models, we observed that a vaccine based on an attenuated Venezuelan Equine Encephalitis alphavirus

encoding HER2, could induce both cellular and humoral immune responses capable of controlling HER2 expressing malignancies. We completed Phase I testing of this vaccine called VRP(HuHER2-ECD+Tm) encoding the extracellular (ECD) and transmembrane (TM) domains of HER2 (subsequently referred to as VRP-HER2) alone and in conjunction with HER2 targeted therapies (mainly trastuzumab) to patients with locally advanced or metastatic HER2+ cancer. Our data from this trial, presented at the 2015 annual ASCO meeting[4], demonstrates induction of HER2 specific T cell responses and detectable anti-HER2 antibodies in the serum. Clinically, the VRP-HER2 vaccine was well tolerated with no dose-limiting toxicities related to the vaccine. Our clinical follow-up to date reveals a median overall survival (OS) that has not been reached after greater than 35 months of follow-up in this heavily pre-treated metastatic population[4]. As the results from the CLEOPATRA study have established trastuzumab + pertuzumab based therapy as the standard of care for first line treatment of metastatic HER2+ disease, demonstrating a progression free survival (PFS) of 18.5 months[5], we are seeking to augment the HER2 specific and anti-tumor immunity in these patients through a randomized Phase II study of VRP-HER2 vaccination with or without anti-PD-1 therapy with pembrolizumab.

Previous biomarker studies have demonstrated that PD-1+ TILs are associated with poor prognosis in HER2 positive breast cancer[6]. In preclinical studies, higher T cell expression of the checkpoint molecule PD-1 (programmed death-1), was associated with greater trastuzumab benefit. Combining trastuzumab with anti-PD-1 and anti-PD-L1 antibodies showed greater tumor regression in mouse models of HER2+ mammary tumors[7]. It has also recently been demonstrated in breast cancer bearing mice that a combination regimen with an anti-PD-1 antibody and a multipeptide vaccine (derived from breast cancer antigens, including neu (HER2)) prolonged the vaccine-induced progression-free survival period and increased the median survival by nearly three-fold when compared with vaccine alone. This research also demonstrated that PD-1 blockade enhances breast cancer vaccine efficacy by altering both CD8 T cell and DC components of the tumor microenvironment[8]. In our own (unpublished) preclinical data, vaccination with a viral vector against a HER family receptor and anti-PD-1 antibody led to tumor regression and sustained control.

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on pembrolizumab (MK-3475).

### 4.1.1 Pharmaceutical and Therapeutic Background

#### 4.1.1.1 The need for new therapies for HER2+ metastatic breast cancer and other HER2 overexpressing malignancies

Metastatic breast cancer continues to account for more than 400,000 deaths yearly with HER2+ breast cancers representing a significant fraction. Significant clinical benefit has been

achieved with the anti-HER2 antibody trastuzumab (Herceptin), but resistance eventually develops[1]. Lapatinib (Tykerb), a small molecule inhibitor of the tyrosine kinases of HER2 and epidermal growth factor receptor type 1 (EGFR), enhances time to progression to 8.4 months, when given with the cytotoxic capecitabine, compared with 4.4 months with capecitabine monotherapy[2].

Since the time of the original FDA approval of our Phase I VRP-HER2 trial, the landscape of HER2 breast cancer management has changed due to several recently approved HER2 targeted therapies. Currently, first line therapies for HER2+ breast cancer are regimens containing trastuzumab with or without pertuzumab[5]. In the second line setting, the current approved therapies are: T-DM1[3] and Lapatinib[9]. These changes in approved regimens have occurred within the last few years due to the results of large randomized controlled trials[3,5,9]. With the approval of these HER2 targeted therapies, many HER2+ patients will continue on subsequent HER2 directed therapies after progressing on trastuzumab +/- pertuzumab. Nonetheless, additional therapies are clearly needed because only 20% are progression-free at one year. Because these tumors continue to overexpress HER2, there is great interest in other approaches for targeting HER2 such as "cancer vaccines" that activate anti-HER2 T cells and antibodies.

We propose to institute our study in the first line setting with the goal of maximizing the immune and clinical response to first line therapy and HER2 vaccination.

#### 4.1.1.2 Immunotherapy targeting HER2 in breast and other tumors

Immunotherapy for malignancies such as breast cancer with cancer vaccines is a promising strategy[10]. A number of vaccine studies for breast cancer have now been reported in which HER2 has served as the vaccine target and where clinical and immunologic activity has been reported[11] (reviewed in reference 10). Immunization of breast cancer patients with therapeutic cancer vaccines increases the frequency of breast cancer antigen-specific T cells[11]. HER2 is a tumor antigen against which antigen-specific CD8+ cytolytic and CD4+ helper T cell responses have been activated in breast cancer patients by vaccines utilizing various peptide fragments of HER2[12-15]. In a pilot clinical trial, we tested the safety of a vaccine consisting of autologous dendritic cells (DC) pulsed with the HER2 extracellular domain (ECD) E75 peptide in patients with advanced HER2 overexpressing malignancies[16]. This was followed by a Phase I clinical trial of vaccines consisting of DC loaded with E75 and the HER2 intracellular domain protein (ICD) in 7 breast cancer patients with no evidence of disease, but predicted to have more than a 30-50% risk of recurrence. Cardiac function was maintained in all patients[17]. Delayed-type hypersensitivity (DTH) reactions at the injection site occurred in 6/7 patients and HER2 specificity was detected by cytokine flow cytometry or ELISPOT in 5 patients. At more than 5 years of follow-up, 6/7 had detectable anti-ICD antibodies. One

patient experienced a resectable pulmonary recurrence at 4 years from their study immunizations. All patients are alive and disease-free at 4.6-6.7 years of follow-up.

More recently, we performed a clinical trial in which we immunized women (n = 12) with metastatic, trastuzumab-refractory HER2-overexpressing breast cancer every 2 weeks with dHER2, a recombinant, truncated version of the HER2 protein including the extracellular domain (ECD) and part of the intracellular domain (ICD) along with the adjuvant AS15, a liposomal formulation containing QS21, MPL and CpG, while treating with concomitant lapatinib (1250mg/day)[18]. This regimen was well tolerated, having no cardiovascular adverse events or significant declines in LVEF. HER2-specific antibody responses were detected in all patients following immunization. Importantly, several patients had received trastuzumab just prior to study initiation and still had detectable anti-HER2 antibody levels for several weeks after initiating the vaccinations. These data suggest that administering a HER2 targeting vaccine during anti-HER2 therapy is well tolerated. This data is consistent with the findings of Disis et al, that vaccinations against HER2 during trastuzumab therapy are safe and immunogenic[19].

#### 4.1.1.3 Rationale for assessing HER2-specific antibodies

An emerging concept about how cancer targeting vaccines may work includes consideration of the impact of anti-HER2 antibodies on signaling pathways critical for breast cancer growth. We hypothesize that, in addition to the classical immunologic mechanisms (such as antibody dependent cytotoxicity and complement fixation), antibodies against receptors critical to tumor cell viability and growth could also have clinical benefits. This concept has been documented in clinical trials of HER2-based peptide vaccines. Montgomery et al reported that antibodies induced by HER2 peptide vaccination could affect HER2 signaling in *in vitro* assays[20]. This work was extended by our collaborator, Neil Spector, MD who demonstrated that antibodies induced by experimental HER2 vaccines (VIA) could synergize with a small molecule inhibitor of HER2 to induce apoptosis of HER2+ cell lines in vitro[18]. We observed the ability of the antibodies, identified in the serum of patients on our own clinical trial of ECD and ICD loaded dendritic cells, to bind to HER2 overexpressing cells lines (data not shown). Importantly, the serum from the immunized patients caused growth inhibition of HER2-overexpressing cell lines in vitro as shown in Figure 1 below. The magnitude of the effect varied, but in some patients, it was similar to the effect of trastuzumab (Herceptin). These data suggested we should perform additional studies to document the role of HER2-specific antibodies on HER2-overexpressing breast cancer.

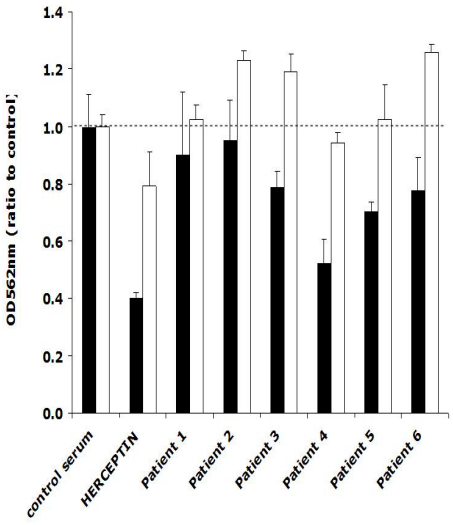



FIGURE 1. Proliferation of tumor cells expressing HER2 is inhibited by serum from patients vaccinated with a HER2-pulsed DC vaccine: BT474 (HER2+; solid bars) or MDA-MB-231 (HER2-; open bars) tumor cells were cultured with patients' serum (1:20 dilution). Trastuzumab (1 µg/ml) was used as a positive control and normal donor serum as a negative control. An MTT assay was performed on day 6 by adding MTT and incubating for 3 h at 37°C followed by lysing cells with DMSO at 37°C for 5 minutes. Proliferation (Absorbance at 562nm) was measured and ratios to negative control are shown.

#### 4.1.1.4 Rationale for targeting PD-1/PD-L1 signaling

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an

overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) is now approved in the US in melanoma, non-small cell lung cancer, head and neck squamous cell carcinoma, classical Hodgkin Lymphoma, urothelial carcinoma, microsatellite instability high cancer, and gastric cancer. Refer to the Investigator's Brochure for Preclinical and Clinical data.

### 4.1.2 Preclinical and Clinical Trial Data

#### 4.1.2.1 Development of recombinant vectors that induce HER2-specific antibodies (HER2-VIA).

In order to further study the role of antibodies induced against HER2, we first sought to improve our ability to activate anti-HER2 immune responses. Because the proteins used in our dendritic cell (DC) vaccine study are no longer available, and because autologous DC vaccines are complicated to generate, we have been developing novel recombinant adenoviral vectors and alphavirus replicon particles expressing HER2 for our next studies. One disadvantage of adenovirus is the frequent development of neutralizing antibodies that limit repeated immunizations. One solution is to use alphavirus-like replicon particles (VRP)[21], based on attenuated Venezuelan equine encephalitis (VEE) virus, which are especially attractive because they highly express heterologous proteins, target expression to dendritic

cells, and induce robust humoral and cellular immune responses to the vectored gene products [22-33]. Foreign genes can be inserted in place of the VEE structural protein gene region in the cDNA plasmid, and an RNA transcript from such a plasmid, when introduced into cells, will replicate and express the heterologous genes as shown schematically in Figure 2. This self-amplifying replicon RNA will direct the synthesis of large amounts of the foreign gene product within the cell, typically reaching levels of 15-20% of total cell protein[22]. The replicon RNA can be packaged into VRP by supplying the structural protein genes of VEE in trans (Figure 3).

**Figure 2. VEE Genome Organization and Replication Strategy**

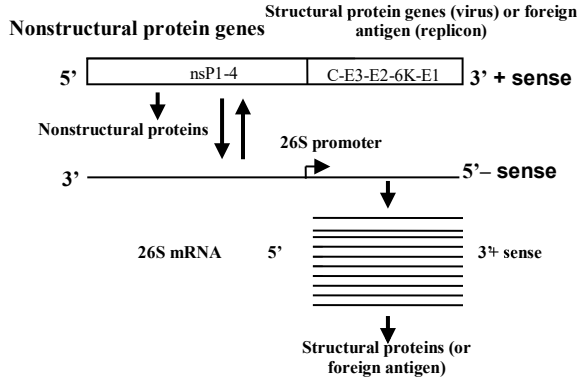
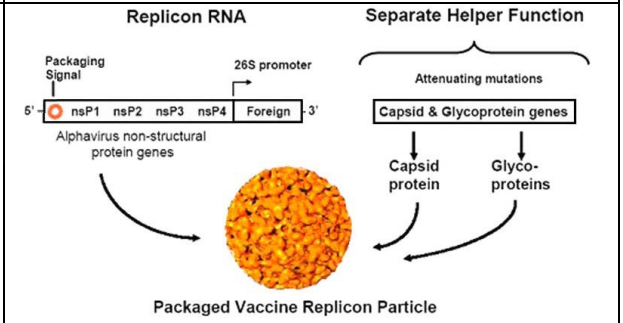



**Figure 3. Replicon Particle Vaccine and Packaging System**




Regarding their efficacy, VRP confer protection in animal models against a variety of diseases that require humoral and/or cellular responses for protection (including viral infections and breast cancer) [21-44].

Regarding the safety of VRP, because the replicon RNA does not contain the structural genes for VEE, it is a single-cycle, propagation-defective RNA and replicates only within the cell into which it is introduced. The "split helper" system greatly reduces the chance of an intact genome being regenerated by RNA-RNA recombination and, as an independent and additional layer of safety, two attenuating mutations are incorporated in the glycoprotein helper. In addition, because alphaviruses and alphavirus replicons replicate in the cytoplasm without any DNA intermediate forms, the VRP vaccine vector system avoids the concerns of chromosomal integration. The safety of the VRP system has been studied with a variety of vaccines, at doses up to 1 x $10^9$ IU, in many thousands of rodents (including immunocompromised mice) and over 100 nonhuman primates (rhesus and cynomolgus macaques), with no evidence of acute or chronic illness. GLP Toxicology studies in rabbits using doses of at least 1 x $10^8$ IU and up to 1 x $10^9$ IU of VRP, showed only local inflammatory reactions and local reactogenicity was

negligible in rabbits after intramuscular injection. There was no evidence of systemic, hematopoietic, immune system or other organ toxicity related to the vaccine.

In regards to the clinical safety and efficacy of VRP based vectors, we performed a phase I clinical trial of a novel alphavirus vector encoding the tumor-associated antigen CEA (VRP-CEA(6D), AVx701) in metastatic cancer patients[44]. Cohorts of 3-6 patients with advanced CEA- expressing malignancies who had progressed on prior therapies were enrolled at successive dosage levels ($4x10^7$, $1x10^8$, $4x10^8$ IU) of VRP-CEA(6D) given as 4 IM injections every 3 weeks. Dose escalation occurred if DLT occurred in <33% of patients in a given dosage level. Following establishment of safety, an additional cohort of 14 patients received VRP-CEA(6D) at the maximum tolerated dose (MTD). Treg levels and CEA-specific T cell and antibody responses were measured before and after immunization. Patients had colorectal cancer (n=23), appendiceal (1), pancreas (n=1), lung (n=2), and breast (n=1) cancer, a performance status of 80-100%, and had failed a median of 4 prior chemotherapeutic regimens. DLT was not reached and the highest dose tested ($4x10^8$ IU) was determined to be the maximal feasible dose. Immunizations were well tolerated with 6 grade 3 events, all related to tumor progression, and no grade 4 events. Patients generally possessed higher blood $CD4^{pos}CD25^{high}FoxP3^{pos}$ Treg cells compared to normal donors. CEA-specific T cell and antibody responses following VRP-CEA(6D) vaccination were observed regardless of patients' Treg level. There were two patients with stable disease and one with a CR of a small liver lesion. With a median follow-up of 13 months among surviving patients, 2 of 4 patients without immune response and 9 of 11 patients with immune response are alive. We concluded that activation of CEA-specific T cells and functional antibodies is possible using VRP-CEA(6D) despite high Treg levels in this heavily pre-treated patient population. In this study, the most consistent activation of T cell and antibody responses was reported at dose level of $4 \times 10^8$ IU.

Regarding construction of the vector for the current proposal, we have previously generated a replicon construct containing the full length human HER2. In a preclinical study using mice transgenic for the HER2-*neu* protein (in which mice develop spontaneous breast tumors that are uniformly lethal), a group of 10 mice were vaccinated 3 times with VRP expressing HER2 prior to tumor development. Control animals vaccinated with VRP expressing the influenza HA antigen (as a control) were not protected and succumbed to lethal tumor burden between 130-200 days. In contrast, mice vaccinated with the VRP expressing HER2 were completely protected. No tumor masses were detected in these animals for the entirety of the study (data not shown).

In order to address potential concerns about oncogenicity of the full length HER2 protein, we generated a similar VRP construct consisting of the extracellular (ECD) and transmembrane (TM) domains of HER2. This VRP, AVX901 or VRP-HER2, was used to immunize C57BL/6 mice and demonstrated activation of HER2 ECD-specific T cells and antibodies (Figure 4).

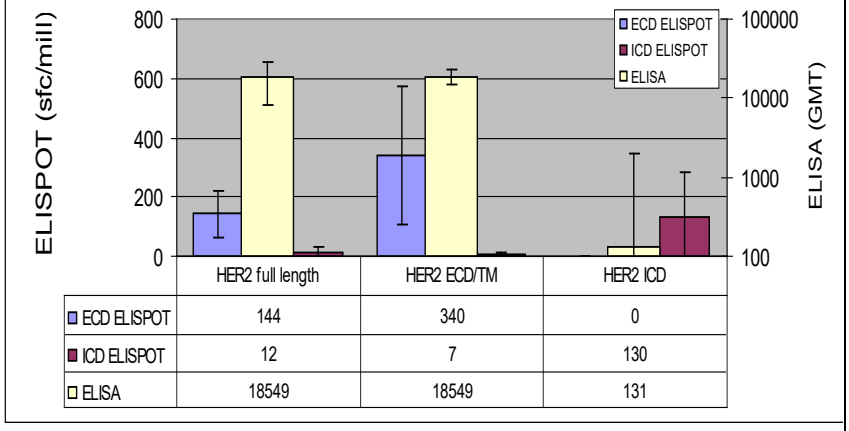



Figure 4. HER2 ECD and ICD-specific T cell and antibody responses of C57BL/6 mice vaccinated with VRP-HER2 full length, VRP-HER2 ECD-TM(VRP-HER2) and VRP-HER2 ICD. Mice received 2 vaccinations of 1x10^7 particles into the bilateral footpads on d0 and d21. Splenocytes and serum were harvested from euthanized mice on d35 for analysis in Interferon-gamma ELISPOT and anti-HER2 ELISA assays.

We demonstrated that the antibodies present in sera after immunization with VRP-HER2 possessed complement dependent cytotoxicity (Figure 5), but also had antiproliferative activities (Figure 6) against a HER2-expressing cell line.

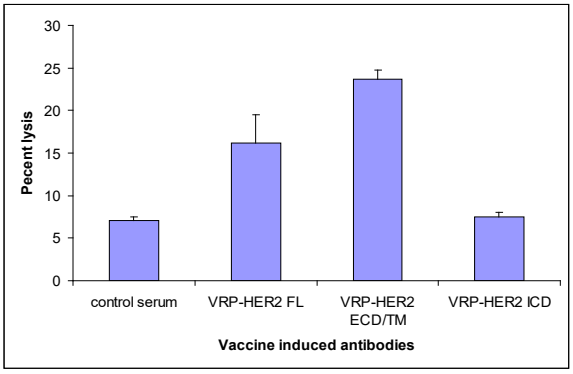



Figure 5. Vaccine induced antibodies from the serum of mice vaccinated with VRP-HER2 full length (FL) and VRP-HER2 ECD/TM (VRP-HER2) mediate complement dependent lysis of the HER2+ human breast tumor line BT474, but not a HER2 neg. control cell line (data not shown). Serum was diluted 1:100 and coincubated with target cells at 37°C for 1h; rabbit serum (source of complement) was added. After 2.5 h, cytotoxicity was measured using the CytoTox 96 Non Radioactive Cytotoxicity Assay. Percent lysis is shown. Errors bars represent standard deviation.

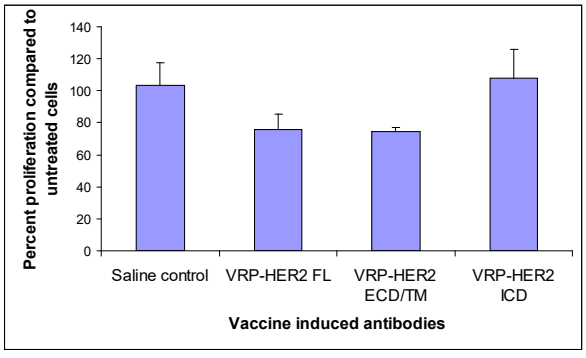



Figure 6. Vaccine induced antibodies (VIA) from the serum of mice vaccinated with VRP-HER2 full length (FL) and VRP-HER2 ECD/TM (VRP-HER2) inhibit proliferation of HER2+ human breast tumor line SKBR3, but not a HER2 neg. control cell line (data not shown). Cells were plated in 96-well plates and allowed to adhere overnight at 37oC. 1:20 VIA were added to the cultured cells and incubated for at 37°C for 72 hr. Cell proliferation was measured by MTT assay.

We have now completed the enrollment on the Phase 1 clinical trial of VRP-HER2 in 22 patients with advanced HER2+ disease. Patients enrolled on this trial primarily had metastatic

HER2+ breast cancer and were heavily pretreated, with an average of 4.5 prior lines of therapy. Vaccination with VRP-HER2 was well tolerated in both cohorts. There were no dose-limiting toxicities in either cohort. The majority of the adverse events (AEs) were either Grade 1 or 2. There were no grade 4 or greater AEs in either cohort. There were four Grade 3 AEs recorded consisting of hyponatremia and increased LFTs, though these were felt unrelated to VRP-HER2 vaccination. There was no decrease in cardiac ejection fractions of any enrolled patients after completing the VRP-HER2 vaccination course. Initial immune analysis of cohort 1, T cell responses specific for HER2 were detected by ELISPOT. Also, cohort 1 demonstrated that the VRP-HER2 vaccine induced anti-HER2 antibodies that were detected by ELISA. In cohort 1 (n=4), the median PFS was 1.9 months and the median OS has not been reached after a median of 35 months of follow-up. In cohort 2 (n=18), there has been one partial response (PR) and there are seven patients with continued stable disease (SD) at time of manuscript submission. The median PFS for cohort 2 is 2.0 months and the median OS has not been reached after a median follow-up of 6.5 months. In cohort 2, concurrent anti-HER2 therapies included: TDM-1 (6/18), trastuzumab/ pertuzumab (5/18), trastuzumab/lapatinib (3/18), single agent trastuzumab (2/18) and single agent lapatinib (1/18).

## 4.2 Rationale

### 4.2.1 Rationale for the Trial and Selected Subject Population

HER2 expressing malignancies, while initially responsive to trastuzumab, pertuzumab, and TD-M1, eventually progress; yet the HER2 protein continues to be expressed on these tumors suggesting they may be targets for immune therapies. Indeed, It has been demonstrated that a higher percentage of tumor infiltrating lymphocytes (TILs) is associated with better tumor response to trastuzumab in both the adjuvant[1] and neoadjuvant setting in HER2+ breast cancers.

One strategy for increasing the T cell lymphocyte response to tumors is to use vaccines capable of activating T cells against tumor associated antigens such as HER2. We have had extensive experience developing vaccines to activate HER2-specific immune responses in patients with HER2 overexpressing malignancies. We vaccinated patients with HER2 overexpressing breast cancer with dendritic cells loaded with HER2 protein or peptide[2] or vaccines based on HER2 protein plus adjuvants[3] and reported the induction of anti-HER2-specific antibodies and T cell responses. In an attempt to develop a vaccine capable of inducing more effective T and B cell responses, we generated alphaviruses modified to express tumor antigens such as HER2. These vectors have tropism for dendritic cells and direct high expression of the HER2 antigen within dendritic cells. In preclinical models, we observed that a vaccine based on an attenuated Venezuelan Equine Encephalitis alphavirus encoding HER2, could induce both cellular and humoral immune responses capable of controlling HER2 expressing malignancies. We completed phase I dose escalation testing of

this vaccine called VRP(HuHER2-ECD+Tm) encoding the extracellular (ECD) and transmembrane (TM) domains of HER2 (subsequently referred to as VRP-HER2) alone and in conjunction with HER2 targeted therapies (mainly trastuzumab) to patients with locally advanced or metastatic HER2+ cancer. Our data from this trial, presented at the 2015 annual ASCO meeting[4], demonstrates the induction of HER2 specific T cell responses and detectable anti-HER2 antibodies in the serum. Additionally, the magnitude of immune response was not impaired by prior anti-HER2 therapy. Clinically, the VRP-HER2 vaccine was well tolerated with no dose-limiting toxicities related to the vaccine. Our clinical follow-up to date of greater than 26 months in this heavily pre-treated metastatic population has still not met median OS[4]. As the results from the CLEOPATRA study have established that trastuzumab + pertuzumab based therapy as the standard of care for first line therapy for metastatic HER2+ disease, demonstrating a PFS of 18.5 months[5]. Based on these results we plan to open a Phase II study of VRP-HER2 vaccine given concurrently with pembrolizumab in patients receiving first line trastuzumab and pertuzumab for metastatic HER2+ breast cancer.

Previous biomarker studies have shown that PD-1+ TILs are associated with poor prognosis in HER2 positive breast cancer[6]. In correlative preclinical studies, higher T cell expression of the checkpoint molecule PD-1 (programmed death-1), was associated with greater trastuzumab benefit. Combining trastuzumab with anti-PD-1 and anti-PD-L1 antibodies showed greater tumor regression in mouse models of HER2+ mammary tumors[7]. It has also recently been demonstrated in breast cancer bearing mice that a combination regimen with an anti-PD-1 antibody and a multipeptide vaccine (derived from breast cancer antigens, including neu (HER2)) prolonged the vaccine-induced progression-free survival period and increased the median survival by nearly three-fold when compared with vaccine alone. This research also demonstrated that PD-1 blockade enhances breast cancer vaccine efficacy by altering both CD8 T cell and DC components of the tumor microenvironment[8]. In our own (unpublished) preclinical data, vaccination with a viral vector against a HER family molecule followed by anti-PD-1 antibody led to tumor regression and sustained control.

### 4.2.2 Rationale for Dose Selection/Regimen/Modification

In our previously discussed Phase I clinical trial of the novel alphavirus vector encoding the tumor-associated antigen CEA (VRP-CEA(6D), AVx701) in metastatic cancer patients (44). DLT was not reached and the highest dose tested ($4x10^8$ IU) was determined to be the maximal feasible dose. Immunizations were well tolerated with 6 grade 3 events, all related to tumor progression, and no grade 4 events. Patients generally possessed higher blood $CD4^{pos}CD25^{high}FoxP3^{pos}$ Treg cells compared to normal donors. CEA-specific T cell and antibody responses following VRP-CEA(6D) vaccination were observed regardless of patients' Treg level. There were two patients with stable disease and one with a CR of a small liver lesion. With a median follow-up of 13 months among surviving patients, 2 of 4 patients without immune response and 9 of 11 patients with immune response are alive. We concluded

that activation of CEA-specific T cells and functional antibodies is possible using VRP-CEA(6D) despite high Treg levels in this heavily pre-treated patient population. Because in this previous study, the most consistent activation of T cell and antibody responses was reported at dose level of 4 x $10^8$ IU, we plan to use the same dose in this current study.

In our recently completed study of VRP-HER2 in advanced HER2+ breast cancer patients, vaccination with VRP-HER2 4 x $10^8$ IU was well tolerated by all enrolled patients. There were no dose-limiting toxicities in either cohort (cohort 1: VRP-HER2 vaccine alone, cohort 2L VRP-HER2 vaccine + anti-HER2 therapy) in this study. The majority of the adverse events (AEs) were either Grade 1 or 2. There were no grade 4 or greater AEs in either cohort. There were four Grade 3 AEs recorded consisting of hyponatremia and increased LFTs, though these were felt unrelated to VRP-HER2 treatment. There was no decrease in cardiac ejection fractions of any enrolled patients after completing the VRP-HER2 vaccination course. In cohort 1, T cell responses specific for HER2 were detected by ELISPOT. Also in cohort 1, vaccine induced anti-HER2 antibodies were detected by ELISA. In cohort 1 (n=4), the median PFS was 1.9 months and the median OS has not been reached after a median of 35 months of follow-up. In cohort 2 (n=18), there has been one partial response (PR) and there are seven patients with continued stable disease (SD) at last data collection. The median PFS for cohort 2 is 2.0 months and the median OS has not been reached after a median follow-up of 6.5 months. In cohort 2, concurrent anti-HER2 therapy was well tolerated and concurrent anti-HER2 therapies included trastuzumab/ pertuzumab in five of the enrolled patients.

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

We therefore **hypothesize** that HER2 specific T cell responses and anti-tumor immunity induced with HER2 vaccination will be augmented by concurrent anti-PD-1 antibody therapy and will lead to greater clinical efficacy. ***We propose a randomized clinical trial with a safety run in to evaluate the immunologic and clinical activity of concurrent VRP-HER2 vaccination with pembrolizumab administration to patients with HER2-overexpressing***

***breast cancer***. As patients with advanced HER2+ tumors typically remain on trastuzumab + pertuzumab therapy, this study will enroll patients who have been stable on trastuzumab + pertuzumab and plan to continue this therapy during the trial.

### 4.2.3 Rationale for Endpoints

#### 4.2.3.1 Efficacy Endpoints

(1) **Primary Objective:** To determine whether pembrolizumab increases the tumor infiltrating (T cell) and peripheral blood (T cell and antibody) immune response to the VRP-HER2 vaccine.

**Hypothesis:** We hypothesize that HER2 specific T cell responses and anti-tumor immunity induced with HER2 vaccination will be augmented by concurrent anti-PD-1 antibody therapy.

(2) **Secondary Objective**: To determine whether the administration of pembrolizumab is safe in patients with recurrent or metastatic HER2+ cancers who are receiving the anti-HER2 vaccine VRP-HER2.

**Hypothesis**: We hypothesize that concurrent immunization with HER2-targeting vaccines and anti-PD-1 antibody therapy will be well tolerated.

(3) **Objective**: To collect preliminary data on the clinical response rates to this concurrent therapy of agents in subjects with assessable disease based on RECIST 1.1 criteria.

**Hypothesis**: We hypothesize that the induction of enhanced HER2-specific T cell responses from concurrent HER2 vaccination and anti-PD-1 antibody therapy will lead to greater clinical efficacy.

#### 4.2.3.2 Biomarker Research

<u>Primary Translational Objectives:</u>

(1) To determine whether concurrent anti-PD1 therapy (pembrolizumab) and VRP-HER2 vaccination increases circulating and tumor infiltrating, HER2 specific T cells.

(2) To determine whether anti-PD1 therapy (pembrolizumab) increases the percent of TIL density (intra-tumoral and at the advancing tumor edge) between pre- and post-pembrolizumab.

Secondary Translational Objectives:

(1) To determine whether concurrent anti-PD1 therapy (pembrolizumab) and VRP-HER2 vaccination increases the evenness and richness of T-cell receptor clonality between Pre-Pembrolizumab and post-Pembrolizumab.

(2) To determine whether concurrent anti-PD1 therapy (pembrolizumab) and VRP-HER2 vaccination results in generalized activation of intra-tumoral CD8+ and Th1 T cell responses in enrolled patients

Translational Hypothesis:

We hypothesize that:

(1) Administration of concurrent anti-PD1 therapy (pembrolizumab) and VRP-HER2 vaccination will result in activation of a more potent HER2-specific T cell response in peripheral blood and in tumor infiltrating T cells than vaccination alone.

(2) Administration of concurrent anti-PD1 therapy (pembrolizumab) and VRP-HER2 vaccination will enhance the antitumor activity of vaccine activated HER2-specific T cells.

1. ELISPOT: Peripheral blood for immune analysis will be drawn at screening/baseline, cycle 1 Day 1 (C1D1), C3D1, C5D1, week 24, and 6 months post-study completion. The PBMC will then be cryopreserved, and batched for subsequent immune analysis including ELISPOT and/or multi-parameter flow cytometric analysis. For planned ELISPOT analysis, PBMCs will be stimulated with peptide pools of the HER2 antigen (ICD and ECD peptides) as well as positive and negative controls. In the ELISPOT assay, a T-cell response (against HER2) will be considered positive if the mean number of spots in six wells with antigen exceeds the number of spots in six control wells by a magnitude of 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of p=0.05 by the t-test. In this trial of HER2-positive (either Stage IV or locally recurrent Stage III) invasive breast cancer patients, anti-HER2 INFg ELISPOT responses in Arm 1 patients receiving the VRP-HER2 vaccine, trastuzumab, pertuzumab, and pembrolizumab will be compared with the anti-HER2 INFg ELISPOT responses in Arm 2 patients who are receiving Pembrolizumab, trastuzumab, and pertuzumab but not VRP-HER2 vaccine. Post-study analysis will evaluate for changes in the HER2-specific T cell responses Selected from the post-immunization time point that yields the largest magnitude of HER2 specific T cells and compare this peak level of HER2 specific T cells with the baseline magnitude of HER2 specific T cells. Univariate analysis will compared the difference in this maximal difference anti-HER2 INFg ELISPOT response between the Arm 1 patients and the Arm

2 patients. Differences between study arms will be evaluated by Student t-test with significant difference defined with a p-value ≤ 0.05. Exploratory analyses will evaluate the association between the induction of HER2 specific T cell responses and the clinical endpoints of progression free survival and overall survival.

2. Serum will be collected at screening/baseline, C1D1, C3D1, C5D1, week 24, and 6 months post-study completion. Collected serum will be analyzed for HER2-specific antibodies and used for immune studies. Antibody responses to HER2 will be measured by ELISA. Absorbance will be read at 450 nM, and control signal will be subtracted from protein signal. HER2 antibody titer will be defined as the serum dilution that has an absorbance that is twice the negative control value for the same dilution. Positive results may be confirmed by Western blot analysis and additional immune assays. The presence of anti-HER2 antibody responses detected by ELISA in Arm 1 patients receiving VRP-HER2 vaccine, trastuzumab, pertuzumab, and pembrolizumab will be compared with the anti-HER2 antibody responses in Arm 2 patients receiving Pembrolizumab, trastuzumab, and pertuzumab but not VRP-HER2 vaccine. It is recognized that Trastuzumab binds to domain IV on the HER2 molecule, while Pertuzumab binds to domain II. As such, for our ELISA testing we will perform ELISA evaluation against multiple peptides sequences derived from previously recognized HER2 epitopes that will include but will not be limited to HER2 domains II and IV. Post-study analysis will compare the change in anti-HER2 antibody responses from baseline, through study treatment, and post-study treatment in each arm. Univariate analysis will compare the difference in anti-HER2 antibody responses at each time point between Arm 1 patients and the Arm 2 patients. Differences between study arms will be evaluated by Student t-test with significance defined as a p-value ≤ 0.05. Exploratory analyses will evaluate the association between the anti-HER2 antibody responses and clinical survival endpoints of PFS and OS.

3. T cell receptor sequencing will be performed on the tumor tissue collected pre- and post-trial therapy. TCR sequencing will also be performed on blood collected prior to initiating pembrolizumab treatment and at C5D1 and week 24 of study and out to 6 months post-study to assess for changes in clonality of the T cell response. In this TCR analysis, we will evaluate only the AA sequences for the CDR3 regions of each TCR Beta chain, as our prior experience suggests that small DNA sequencing errors can lead to challenges in DNA data alignment. To evaluate TCR clonality we will utilize Shannon entropy for each sample. Shannon entropy will be calculated on the clonal abundance of all productive TCR sequences in the dataset. Shannon entropy will then be normalized to the range [0–1] by dividing Shannon entropy by the logarithm of the number of unique productive TCR sequences in the dataset. This normalized entropy value will then be inverted (1 − normalized entropy) to produce our clonality metric. In addition, as this is a fairly homogenous population we will also evaluate for the presence of induced unique consensus T cell populations resulting from this therapy identified from very high-level

summaries of clone populations in both treatment arms, and compare differences in these T cell populations between Arm 1 and Arm 2. In our interrogation of these dominant TCR clones we will specifically evaluate for changes in the TCR clones that make up the top 5% most common clones. As an exploratory evaluation to identify HER2 specific TCR we will select the patients with the highest HER2 ELIspot response to VRP-HER2 vaccination. In these patients, cytokine flow cytometry will be used to separate HER2 responsive T-cells. We will then collect these HER2 responsive T cells and perform TCR sequencing. The TCR sequencing will be performed in conjunction with Adaptive Biotech. *The second correlative analysis evaluating for HER2 specific T-cells was not included in the original proposal and budget and as such we will be pursuing additional funding mechanisms to support this work.*

4. Tumor biopsies collected pre- and post- trial therapy will be analyzed by IHC for PD-1, PD-L1, and PD-L2 expression as well as changes in expression between these time points. CD3+ TIL infiltration of the biopsied tumors will be assessed at these time points as well evaluating for changes in TIL content with vaccination with or without pembrolizumab. Analysis will be performed for changes in Th1, Th2, and Treg immune response through evaluation of changes in CD4+, CD8+, and FOXP3+ T cell subtypes. Exploratory analyses will evaluate the differences in TIL content between study arms to assess the influence of pembrolizumab on the overall TIL populations.

5. Tumor Biopsies collected pre- and post- trial therapy will also be processed and evaluated by flow cytometry for differences and changes in T cell content and T cell subtype. Specifically, we will evaluate the differences in the presence of INFg+T-bet+CD4+ T cells and INFg+GATA+CD4+ T cells, T cell subtypes recently recognized to influence both the induced HER2- specific cellular immunity and clinical outcomes. In additional, intracellular cytokine staining will be performed to evaluate for changes in Type 1 immunity (INFg, TNFa, and IL-2) versus Type 2 immunity (IL-4, IL-6, and IL-10) in each study arm. Changes in the tumor content of T-bet+ and GATA+ CD4+ T cells between the pre- and post- trial therapy time points will be evaluated in each arm and how the presence or absence of pembrolizumab influences this important population. Exploratory analyses will evaluate for a relationship between difference in the T-bet+ and GATA+ CD4+ T cell populations between the study arms and PFS and OS differences. In additional we will evaluate for the presence of immunosuppressive cell popualtions including regulatory T cells (Treg) and myeloid derived suppressor cells (MDSCs). We will identify Tregs based on CD4+, FOXP3+, CD25+, CD127- staining and identify MDSCs with staining for HLA-DR+, CD11b+ and CD33+. Exploratory analysis will evaluate the correlation between the tumor content of these immunosuppressive immune cell populations and the clinical outcomes observed in each of the arms of this study. *These studies were not included in the original proposal and budget and as such we will be pursuing additional funding mechanisms to support the work detailed in #5.*

6. Gene-expression analysis of RNA isolated from tumor biopsies collected at pre- and post-trial therapy will be performed by real-time PCR for changes in the expression of the following: Granzyme A, Granzyme B, Perforin, EOMES, CXCL9, CXCL10, CD8A, CD4, FOXP3, ICOS, PD-1, PD-L1, PD-L2, and CTLA4. Correlation of these cytokines and surface proteins and induction of HER2-specific immune response in this trial will be exploratory. We have previously created a clinical database for the collection and documentation of prior lines of systemic therapy as well as the duration of such therapy. We will enter the enrolled patients prior lines of therapy into this database for analysis with planned exome sequencing results. Next generation sequencing will be performed by the Duke Sequencing Core. *These studies were not included in the now approved budget and we will pursue additional funding mechanisms to support this work.*

7. Tumor biopsies collected pre- trial therapy will processed for Next-generation sequencing in order to evaluate for the presence of neoantigens. The presence and amount of neoantigens in tumor biopsies will be compared between both study arms to evaluate for equal distribution. Exploratory analyses will then evaluate the relationship between the presence of neoantigens and clinical response as measured by RECIST criteria to pembrolizumab in this study. *These studies were not included in the now approved budget and we will pursue additional funding mechanisms to support this work.*

8. CTCs will be collected from peripheral blood collections at screening/baseline, C1D1, C3D1, C5D1, week 24, and 6 months post-study completion and analyzed for changes in CTC number correlating with treatment response by study arm. In an exploratory analysis CTCs will also be evaluated for surface expression of PD-L1, PD-L2, and CTLA-4 and whether changes in these surface proteins predict response to the pembrolizumab with or without VRP-HER2 vaccination as measured by RECIST criteria on radiographic imaging. *These studies were not included in the now approved budget and we will pursue additional funding mechanisms to support this work.*

9. cfDNA will be collected from peripheral blood collections at screening/baseline, week 24, and 6 months post-study completion and analyzed in all patients for presence of known HER2 mutations. In additional, at these time points in patients who also have HR+ disease, cfDNA will be evaluated for the presence of ESR1 mutations. Exploratory analysis will evaluate if the presence of these mutations significantly affects the clinical response to pembrolizumab with or without VRP-HER2 vaccination as measured by RECIST criteria on radiographic imaging. *These studies were not included in the now approved budget and we will pursue additional funding mechanisms to support this work.*

10. cfRNA will be collected from peripheral blood collections at screening/baseline, week 24, and 6 months post-study completion and analyzed from the peripheral blood of all study

patients and analyzed for the presence of Bmi-1 as a marker of poor prognosis in breast cancer. In addition from the patients with HER2+HR+ breast cancer the presence of cyclin D1 as a marker of endocrine resistance in HR+ patients will be evaluated for. Exploratory analysis will evaluate if the presence of Bmi-1 and cycle D1 affect the clinical response to VRP-HER2 vaccination with or without pembrolizumab as measured by RECIST criteria on radiographic imaging. *These studies were not included in the now approved budget and we will pursue additional funding mechanisms to support this work.*

11. Multiplex array analysis using an "immunosignature" antibody based assay will be performed on a limited number of pre-therapy samples from this randomized trial. Serum obtained at baseline screening will be applied to this "immunosignature" array of 10,000 peptides for the detection of each patient's individual immunosignature. We will perform an exploratory analysis to evaluate if there is a predictive immunosignature for clinical response to the VRP-HER2 vaccine +/- pembrolizumab as determined by RECIST criteria. *These studies were not included in the original proposal and budget and as such we will be pursuing additional funding mechanisms to support the work detailed in #11.*

12. Multicolor IHC analysis will be performed by a collaborator, who has developed this assay. This spatial image analysis approach will be performed on a limited number of pre- and post-therapy tumor tissue samples and can be performed on both frozen and FFPE tissues. For the purposes of this study, paired tumor tissue specimen slides from pre- and post-therapy tumor biopsies will be collected, assigned a study number, and then deidentified. The deidentified paired slides will be sent to our collaborator for formal analysis by 10-color IHC. This analysis will evaluate the immunologic changes in three dimensions in the tumor microenvironment between the pre- and post-therapy samples. In an exploratory analysis, we will evaluate if specific immunologic changes in the tumor microenvironment correlate with clinical response in the selected patients, as determined by RECIST criteria. *These studies were not included in the original proposal and budget and as such we will be pursuing additional funding mechanisms to support the work detailed in #12.*

## 5.0 METHODOLOGY

### 5.1 Entry Criteria

#### 5.1.1 Diagnosis/Condition for Entry into the Trial

Histologically-confirmed breast cancer that is metastatic or locally recurrent (7th Edition of the AJCC TNM System) with HER2/neu overexpression by immunohistochemistry (2+,3+) or FISH+ per ASCO CAP guidelines and receiving trastuzumab plus pertuzumab (determined

by their physician). Patients who are hormone receptor (ER, PR) positive or negative are permitted.

### 5.1.2 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Have undergone treatment with trastuzumab plus pertuzumab (as selected by their attending physician) for at least 3 weeks prior to initiation on this study.

2. Be willing and able to provide written informed consent/assent for the trial. Informed consent will be obtained from the patient prior to performing any study-related procedures, including screening visits. Available CT scans and bone scans performed as standard of care prior to signing consent can be used to fulfill eligibility requirements if they were performed within 4 weeks of the first dose of study drug(s). Available MUGA, Echocardiogram, and EKG performed as standard of care prior to signing consent can be used to fulfill eligibility requirements if they were performed within 8 weeks of the first dose of study drug(s).

3. Resolution of **all toxic side effects** of prior chemotherapy, radiotherapy or surgical procedures to NCI CTCAE (version 5.0) Grade ≤ 1 (with the exception of grade 2 alopecia, grade 2 neuropathy and grade 2 fatigue);

4. Be ≥ 18 years of age on day of signing informed consent.

5. Be willing to provide tissue from a newly obtained core or excisional biopsy of a tumor lesion. *Newly-obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on Day 1. Subjects for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the Sponsor.*

6. Have a performance status of 0 or 1 on the ECOG Performance Scale.

7. Normal cardiac function defined as either a MUGA or ECHO with LVEF in normal institutional range (MUGA 50%; ECHO 55%)

8. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 7 days of treatment initiation.

Table 1 Adequate Organ Function Laboratory Values

| System | Laboratory Value |
|---|---|
| **Hematological** | |
| Absolute neutrophil count (ANC) | ≥1,500 /mcL |
| Platelets | ≥100,000 / mcL |
| Hemoglobin | ≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment) |
| **Renal** | |
| Serum creatinine **OR**<br>Measured or calculated[a] creatinine clearance<br>(GFR can also be used in place of creatinine or CrCl) | ≤1.5 X upper limit of normal (ULN) **OR**<br><br>≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN |
| **Hepatic** | |
| Serum total bilirubin | ≤ 1.5 X ULN **OR** |
| | Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN |
| AST (SGOT) and ALT (SGPT) | ≤ 2.5 X ULN **OR**<br>≤ 5 X ULN for subjects with liver metastases |
| Albumin | ≥2.5 mg/dL |
| **Coagulation** | |
| International Normalized Ratio (INR) or Prothrombin Time (PT)<br><br>Activated Partial Thromboplastin Time (aPTT) | ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants<br>≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants |
| [a]Creatinine clearance should be calculated per institutional standard. | |

9. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

10. Female subjects of childbearing potential (Section 5.6.2) must be willing to use an adequate method of contraception as outlined in Section 5.6.2 – Contraception, for the course of the study through 120 days after the last dose of study medication.

Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

11. Male subjects of childbearing potential (Section 5.6.2) must agree to use an adequate method of contraception as outlined in Section 5.6.2- Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

12. Ability to return to Duke University Medical Center for adequate follow-up as required by this protocol.

### 5.1.3 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if any of the following criteria are met:

1. Patients in this study, may not receive cytotoxic chemotherapy targeted small molecule therapy, or radiation therapy in the 3 weeks before the first infusion of Pembrolizumab, during the injection period for VRP-HER2 and infusion period for Pembrolizumab or for at least 2 weeks after booster immunization with VRP-HER2 (Arm 1) or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.

    - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

2. Patients may have received prior radiation including for brain metastases.

3. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.

4. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. Prior history of autoimmune thyroiditis or vitiligo is permitted.

5. Has a known history of active TB (Bacillus Tuberculosis)

6. Hypersensitivity to pembrolizumab or any of its excipients.

7. Has had a prior anti-cancer monoclonal antibody (mAb) within 3 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 3 weeks earlier. Exceptions include trastuzumab, pertuzumab.

8. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.

9. Has known active CNS metastases and/or carcinomatous meningitis. Participants with previously treated brain metastases may participate provided they are radiologically stable, i.e. without evidence of progression for at least 4 weeks by repeat imaging (note that the repeat imaging should be performed during study screening), clinically stable and without requirements of steroid treatment for at least 14 days prior to the first dose of study treatment.

10. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

11. Has history of (non-infectious) pneumonitis that required steroids or active, non-infectious pneumonitis.

12. Has an active infection requiring systemic therapy or systemic use of antimicrobials within 72 hours prior to the first study treatment

13. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

14. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

15. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.

16. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.

17. Hypersensitivity to pembrolizumab or any of its excipients.

18. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

19. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

20. Has received a live vaccine within 30 days of planned start of study therapy.

    *Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.*

### 5.2 Trial Treatments

The treatment to be used in this trial is outlined below:

Table 2. Trial Treatment – Safety Arm, Arm A, Arm B, and ARM C

| Drug | Dose/Potency | Dose Frequency | Route of Administration | Regimen/Treatment Period | Use |
|---|---|---|---|---|---|
| Safety Arm | | | | | |
| Pembrolizumab | 200 mg | Q3W | IV infusion | Day 1 of each 3 week cycle x 5 cycles | Experimental |
| VRP-HER2 | 4 x $10^8$ IU | Q2W | IM injection | Cycle 1: Day 1 and Day 15<br>Cycle 2: Day 8<br>Booster – Cycle 5, Day 1 | Experimental |
| Arm A | | | | | |
| VRP-HER2 | 4 x $10^8$ IU | Q2W | IM injection | Cycle 1: Day 1 and Day 15<br>Cycle 2: Day 8<br>Booster – Cycle 5, Day 1 | Experimental |
| Arm B | | | | | |
| Pembrolizumab | 200 mg | Q3W | IV infusion | Day 1 of each 3 week cycle x 5 cycles | Experimental |

| Drug | Dose/Potency | Dose Frequency | Route of Administration | Regimen/Treatment Period | Use |
|---|---|---|---|---|---|
| Arm C | | | | | |
| Pembrolizumab | 200 mg | Q3W | IV infusion | Day 1 of each 3 week cycle x 5 cycles | Experimental |
| VRP-HER2 | 4 x $10^8$ IU | Q2W | IM injection | Cycle 1: Day 1 and Day 15<br>Cycle 2: Day 8<br>Booster – Cycle 5, Day 1 | Experimental |

Trial treatment should begin on the day of randomization or as close as possible to the date on which treatment is allocated/assigned.

### 5.2.1 Dose Selection/Modification

#### 5.2.1.1 Dose Selection – VRP-HER2

Because 4 x $10^8$ IU VRP was previously given in our prior vaccine study of VRP-HER2, and because of manufacturing considerations, we will use 4 x $10^8$ IU VRP-HER2 for the present study.

VRP-HER2 is formulated at a concentration of 5.2 x $10^8$ IU/mL (2.6 x $10^8$ IU per 0.5 mL). Two vials will be needed for each injection for 4 x $10^8$ IU dose. Prior to injection, the appropriate vials should be removed from the freezer and allowed to thaw at controlled room temperature (20-25°C, 68-77°F) for at least 20 minutes and not more than 30 minutes, after which it should be kept at 2-8°C (35-46°F). The vaccine is stable for at least 4 hours after removal from the freezer when kept refrigerated at 2-8°C (35-46°F). The thawed vial should be swirled and then, using aseptic technique, the pharmacist should withdraw the appropriate volume from the appropriate vial using a 1-mL syringe.

The vaccine should be injected as soon as possible (but within 2 hrs of preparation) using a 1 to 1/2 inch, 20 to 25 gauge needle. If the vaccine cannot be injected immediately, the syringe should be stored at 2-8°C (35-46°F) for up to 2 hours.

All injections of vaccine should be given as a volume of 0.77 mL by intramuscular injection in the upper arm after preparation of the site with alcohol. Either arm may be used for the initial injection. Subsequent vaccinations should be rotated between arms (e.g., injections 1 and 3 in right arm, then injections 2 in left arm). If for any reason a patient is not able to

receive one of the scheduled vaccinations in their upper arm, the vaccination may be administered by intramuscular injection in the subject's thigh at the discretion of the investigator. This change in administration will be noted in the CRF. When preparing a dose in a syringe and administering the dose, consideration should be given to the volume of solution that may remain in the needle after the dose is administered, to ensure that the full dose specified in the protocol is administered.

#### 5.2.1.2 Dose Selection – Pembrolizumab

The rationale for selection of doses to be used in this trial is provided in Section 4.0 – Background and Rationale.

Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual.

#### 5.2.1.3 Dose Modification (Escalation/Titration/Other) – VRP-HER2

In the prior Phase I study of VRP-HER2 vaccination in advanced HER2+ malignancies, the vaccination was well tolerated with no dose limiting toxicities (Table 3).

Table 3 Adverse events – possibly, probably, and definitely related to VRP-HER2 from Phase 1 protocol.

| **Grade of Adverse Event - VRP-HER2** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Arm** | **1- Mild** | | **2- Mod** | | **3-Severe** | | **4-LifeThr** | | **5-Lethal** | | **Total** |
| | **n** | **n** | **(%)** | **n** | **(%)** | **n** | **(%)** | **n** | **(%)** | **n** | **(%)** | **N** |
| **Non-Hematologic Adverse Events** | | | | | | | | | | | | |
| General disorders and administration site conditions | | | | | | | | | | | | |
| Fatigue | 1 | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 5 |
| | 2 | 0 | *(0%)* | 1 | *(6%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 18 |
| Fever | 1 | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 5 |
| | 2 | 2 | *(11%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 18 |
| Injection site reaction | 1 | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 5 |
| | 2 | 2 | *(11%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 18 |
| Malaise | 1 | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 5 |
| | 2 | 1 | *(6%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 18 |
| Infections and infestations | | | | | | | | | | | | |
| Rash pustular | 1 | 1 | *(20%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 5 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 18 |
| Musculoskeletal and connective tissue disorders | | | | | | | | | | | | |
| Musculoskeletal and connective tissue disorder - Other, specify | 1 | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 5 |
| | 2 | 1 | *(6%)* | 1 | *(6%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 18 |
| Pain in extremity | 1 | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 5 |
| | 2 | 5 | *(28%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 18 |
| Renal and urinary disorders | | | | | | | | | | | | |
| Urinary retention | 1 | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 5 |
| | 2 | 1 | *(6%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 0 | *(0%)* | 18 |
| Summary | | | | | | | | | | | | |
| **Maximum Non-Hematologic AE** | **1** | **1** | ***(20%)*** | **0** | ***(0%)*** | **0** | ***(0%)*** | **0** | ***(0%)*** | **0** | ***(0%)*** | **5** |
| | **2** | **7** | ***(39%)*** | **2** | ***(11%)*** | **0** | ***(0%)*** | **0** | ***(0%)*** | **0** | ***(0%)*** | **18** |
| **All Adverse Events** | | | | | | | | | | | | |
| Summary | | | | | | | | | | | | |
| **Maximum Overall AE** | **1** | **1** | ***(20%)*** | **0** | ***(0%)*** | **0** | ***(0%)*** | **0** | ***(0%)*** | **0** | ***(0%)*** | **5** |
| | **2** | **7** | ***(39%)*** | **2** | ***(11%)*** | **0** | ***(0%)*** | **0** | ***(0%)*** | **0** | ***(0%)*** | **18** |

Dose modifications:

Any subject that experiences a Dose Limiting Toxicities (DLT) related to the VRP-HER2 immunization will not receive any further injections of study drug. (See section below for the definition of a DLT)

For grade 2 toxicity (other than fever, myalgias, arthalgias, or fatigue) related to the VRP-HER2 immunizations, dosing in an individual patient must be held until toxicity resolves to grade 1 or less, but no dose adjustments will be made.

For grade 1 or less toxicity, no modifications are required.

- If 0 or 1 Dose Limiting Toxicities (DLT) occur in the Safety Arm, this dose of VRP-HER2 will be used in the randomized portion of the study.
- If 2 or more DLT occur in the Safety Arm, then 6 more patients will be accrued into the safety cohort at dose level -1 consisting of VRP-HER2 at dose $2 \times 10^8$ IU intramuscularly, given every 2 weeks for a total of three doses and one additional booster dose at C5D1.
- If 0 or 1 patients experience DLT at dose level -1, then this dose will be chosen for the randomized portion of the study.

- If 2 or more subjects experience DLT at dose level -1 in either cohort, the study will be held and redesigned.

DLTs will be collected and reviewed by the PI as they occur and entered into the study database for assessment at Day 42.

Dosing:

| **Dose level** | **Dose of VRP-HER2** |
|---|---|
| Dose level 1 | $4 \times 10^8$ IU |
| Dose level -1 | $2 \times 10^8$ IU |

Any occurrence of a grade 4 or greater toxicity will prompt the study sponsor and investigator to conduct a thorough evaluation of the available safety information to justify a decision to continue enrolling new subjects into the study.

Definition of Dose-limiting Toxicity (DLT)

Dose limiting toxicity is defined as any Grade 2 or higher immediate hypersensitivity reactions or neurological toxicity, other Grade 3 or 4 allergic or major organ toxicity, or any other Grade 3 or higher toxicity attributable to study treatment (VRP-HER2 vaccine or pembrolizumab).

#### 5.2.1.4 Dose Modification (Escalation/Titration/Other) – Pembrolizumab

Table 4: **Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab**

**General instructions:**

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

| Immune-related AEs | Toxicity grade or conditions (CTCAEv5.0) | Action taken to pembrolizumab | irAE management with corticosteroid and/or other therapies | Monitor and follow-up |
|---|---|---|---|---|
| Pneumonitis | Grade 2 | Withhold | • Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper | • Monitor participants for signs and symptoms of pneumonitis<br>• Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment<br>• Add prophylactic antibiotics for opportunistic infections |
| | Grade 3 or 4, or recurrent Grade 2 | Permanently discontinue | | |
| Diarrhea / Colitis | Grade 2 or 3 | Withhold | • Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper | • Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus).<br>• Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. |
| | Grade 4 | Permanently discontinue | | |

| | | | | |
|---|---|---|---|---|
| | | | | • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. |
| AST / ALT elevation or Increased bilirubin | Grade 2 | Withhold | • Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper | • Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable |
| | Grade 3 or 4 | Permanently discontinue | • Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper | |
| Type 1 diabetes mellitus (T1DM) or Hyperglycemia | Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure | Withhold | • Initiate insulin replacement therapy for participants with T1DM<br>• Administer anti-hyperglycemic in participants with hyperglycemia | • Monitor participants for hyperglycemia or other signs and symptoms of diabetes. |
| Hypophysitis | Grade 2 | Withhold | • Administer corticosteroids and initiate hormonal replacements as clinically indicated. | • Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency) |
| | Grade 3 or 4 | Withhold or permanently discontinue[1] | | |
| Hyperthyroidism | Grade 2 | Continue | • Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate | • Monitor for signs and symptoms of thyroid disorders. |
| | Grade 3 or 4 | Withhold or permanently discontinue[1] | | |
| Hypothyroidism | Grade 2-4 | Continue | • Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care | • Monitor for signs and symptoms of thyroid disorders. |

| | | | | |
|---|---|---|---|---|
| Nephritis and Renal dysfunction | Grade 2 | Withhold | • Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper. | • Monitor changes of renal function |
| | Grade 3 or 4 | Permanently discontinue | | |
| Myocarditis | Grade 1 or 2 | Withhold | • Based on severity of AE administer corticosteroids | • Ensure adequate evaluation to confirm etiology and/or exclude other causes |
| | Grade 3 or 4 | Permanently discontinue | | |
| All other immune-related AEs | Intolerable/ persistent Grade 2 | Withhold | • Based on type and severity of AE administer corticosteroids | • Ensure adequate evaluation to confirm etiology and/or exclude other causes |
| | Grade 3 | Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis | | |
| | Grade 4 or recurrent Grade 3 | Permanently discontinue | | |

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

**NOTE:**
For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

### 5.2.2 Timing of Dose Administration

Trial treatment with pembrolizumab and/or VRP-HER2 vaccine should be administered according to the study calendar after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0).

In the Safety Arm and in Arms A and C, timing of VRP-HER2 vaccine doses will be on Cycle 1 - Day 1, Cycle 1 - Day 15, and Cycle 2 – Day 8. A VRP-HER2 booster vaccination will be given on Cycle 5, Day 1. This timing for VRP-HER2 is based on a Q2 weekly schedule established in our recently completed trial.

In the Safety Arm and Arms B and C, pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. Although every effort should be made to target infusion timing to be as close to 30 minutes as possible, given the variability of infusion pumps, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

### 5.2.3 Concurrent HER2 targeted therapy: trastuzumab plus pertuzumab

Patients receiving concurrent trastuzumab plus pertuzumab will receive the same doses of their concurrent treatment. Trastuzumab and Pertuzumab will be administered under the direction of the patients attending physician. VRP-HER2 vaccinations will be given on the same day as trastuzumab plus pertuzumab if possible, but may be given between trastuzumab plus pertuzumab doses. Pembrolizumab infusions will be given on the same day as trastuzumab plus pertuzumab if possible, but may be given between trastuzumab plus pertuzumab doses.

### 5.2.4 Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigator, and subject will know the treatment administered.

### 5.3 Randomization or Treatment Allocation

There will be an initial safety arm (n=3), and if this combination therapy is without DLT, then patients will be stratified by hormone receptor status (ER and/or PR + (defined as ≥1%) versus negative) and randomized 1:1:1 into 3 arms (up to N=12 in each arm (ARM A, B, C) by the study statistician using a random number generator or table.

### 5.4 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

#### 5.4.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 7.9.

#### 5.4.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Antineoplastic systemic chemotherapy or biological therapy. Endocrine therapy and anti-HER2 therapies are permitted.
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab

- Radiation therapy
    - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications which are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

## 5.5 Rescue Medications & Supportive Care

### 5.5.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 5.2.1 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

- **Pneumonitis**:
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
    - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
    - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.
- **Diarrhea/Colitis**:

  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

    - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
    - For **Grade 2 diarrhea/colitis,** administer oral corticosteroids.
    - For **Grade 3 or 4 diarrhea/colitis,** treat with intravenous steroids followed by high dose oral steroids.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**
    - For **T1DM** or **Grade 3-4** Hyperglycemia
        - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
        - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.
- **Hypophysitis**:
    - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than

4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:

  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**:
  - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
    - Treat with IV or oral corticosteroids
  - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
  - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Renal Failure or Nephritis**:
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.

- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

  The Table below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 5 Infusion Reaction Treatment Guidelines

| NCI CTCAE Grade | Treatment | Premedication at subsequent dosing |
|---|---|---|
| Grade 1<br>Mild reaction; infusion interruption not indicated; intervention not indicated | Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. | None |
| Grade 2<br>Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs | **Stop Infusion and monitor symptoms.**<br>Additional appropriate medical therapy may include but is not limited to:<br>IV fluids<br>Antihistamines<br>NSAIDS<br>Acetaminophen<br>Narcotics<br>Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.<br>If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose.<br>**Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.** | Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with:<br><br>Diphenhydramine 50 mg po (or equivalent dose of antihistamine).<br><br>Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic). |
| Grades 3 or 4<br><br>Grade 3:<br>Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates)<br><br>Grade 4:<br>Life-threatening; pressor or ventilatory support indicated | **Stop Infusion.**<br>Additional appropriate medical therapy may include but is not limited to:<br>IV fluids<br>Antihistamines<br>NSAIDS<br>Acetaminophen<br>Narcotics<br>Oxygen<br>Pressors<br>Corticosteroids<br>Epinephrine<br><br>Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.<br>Hospitalization may be indicated. | No subsequent dosing |

| NCI CTCAE Grade | Treatment | Premedication at subsequent dosing |
|---|---|---|
| | **Subject is permanently discontinued from further trial treatment administration.** | |
| Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration. | | |

- **Management of VRP-HER2 Vaccine Reactions**: Reactions have not been observed in prior study; however, like other vaccines, they could theoretically occur within minutes to as long as a day after an injection.

  Treatment of Toxicity for VRP-HER2 vaccine: Bronchospasm, stridor, wheezing, respiratory depression (Respiratory Rate < 8), cardiac arrhythmia, generalized urticaria, systolic Blood Pressure ≤ 80mm Hg, angioedema, shock, or loss of consciousness

  1. Remain at patient bedside
  2. Have another nurse notify physician
  3. Administer Normal Saline (NS) at KVO, if hypotensive then give NS 500ml bolus
  4. Start oxygen for dyspnea, stridor, wheezing or respiratory depression at 2 liters/nasal cannula, initiate continuous pulse oximetry and call respiratory therapist (RT)
  5. Give diphenhydramine 50 mg IVP** x 1 (Intravenous Push)
  6. Give methylprednisolone 125mg IV** x 1 (Intravenous Infusion)
  7. Give epinephrine 0.3mg (auto-injector) x 1 IM (Intramuscular) x **OR** epinephrine 0.3mg (1:1000) SQ (Subcutaneous) x 1 (may repeat x 1 in 5 minutes)
  8. If no response to above interventions within 5 minutes or patient condition worsens, call a code 911 or:
     At Duke: x115 (Duke)
  9. Vital signs with pulse oximetry every 2 minutes until patient is stable, then every 5 minutes for 30 minutes and then every 15 minutes for 1 hr or as the patient's condition requires

  **If no IV access or IV access lost, may give these agents IM as appropriate.

- **Discontinuation of VRP-HER2 injections Required for:**

  (1) Life threatening anaphylactic reactions or Grade 4 toxicity attributed to the injections.

(2) Patients with an NCI CTCAE (version 5.0) ≥ Grade 2 decrease in ejection fraction, during the vaccination period may not receive any further vaccine (VRP-HER2).

Patients with a ≥ Grade 2 decrease in ejection fraction in the period thirty days after the last vaccination should continue to be monitored every 4 weeks for at least 16 weeks.

(3) Initiation of radiotherapy or chemotherapy or new hormonal therapy.

Note: If patients experience progression prior to receiving all 3 immunizations and the booster, they may continue to receive VRP-HER2 at their physician's discretion.

Note: If a subject in the safety cohort becomes safety non-evaluable (i.e., is removed prior to completion of the assigned vaccine schedule for the following reasons: refusal of therapy, noncompliance, intercurrent illness and physician discretion), the subject will be replaced.

- **Criteria for Evaluating Cardiac Adverse Events in Patient receiving concurrent trastuzumab**

(1) Patients with an NCI CTCAE (version 5.0) Grade 2 or greater LVEF decrease, during the vaccination period may not receive any further vaccine (VRP-HER2).

Patients with a Grade 2 or greater LVEF decrease in the period thirty days after the last vaccination should continue to be monitored every 4 weeks for at least 16 weeks.

(2) Patients will be assessed for left ventricular ejection fraction (LVEF) within 8 weeks prior to initiation of study drug and will receive a MUGA or ECHO at week 8 of the study. If a patient has completed their vaccinations and has a clinical or standard of care indication for an ECHO/MUGA before week 8 and there is a Grade 2 or greater LVEF decrease, we will stop their HER2 targeted therapy. Any patient found to have a Grade 2 or greater LVEF decrease while still within the 8 weeks of this study should continue to be monitored every 4 weeks for at least 16 weeks. After the cardiac echo/MUGA follow-up as directed above, patients further cardiac monitoring will be at the discretion of their attending physician. We will collect the LVEF data, obtained by the patients attending physician, for up to 6 months after completion of vaccination.

Patients on study will be monitored by their treating clinical team for cardiac adverse events every 3 months according to labeling recommendations in patients that continue to receive trastuzumab (including after completion or discontinuation of vaccine injections and/or pembrolizumab infusions).

## 5.6 Diet/Activity/Other Considerations

### 5.6.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

### 5.6.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

(3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

(1) practice abstinence[†] from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential

will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

### 5.6.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 7.9.2.

### 5.6.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

## 5.7 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.5 – Other Procedures.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression

*Note*: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved.

- Adverse experience that is determined by the principal investigator or the patients physician to warrant study discontinuation for safety reasons
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Protocol Flow Chart). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.6). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

### 5.7.1 Discontinuation of Study Therapy after CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared.

## 5.8 Subject Replacement Strategy

If any patient in the safety cohort is inevaluable for toxicity (for example, does not complete all study therapy and 30 day follow-up), then they will be replaced. No study replacements are planned during the randomization phase.

## 5.9 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
4. Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

# 6.0 TRIAL FLOW CHART

## 6.1 Study Flow Chart

| Cycle Days → | Screen[3] | Cycle 1 | | | Cycle 2 | | | Cycle 3 | | | Cycle 4 | | | Cycle 5 | | | 30-Day Safety Visit | Week 24 and 6-Mo. Follow-up[11] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Procedure / Test ↓** | | **D1** | **D8** | **D15** | **D1** | **D8** | **D15** | **D1** | **D8** | **D15** | **D1** | **D8** | **D15** | **D1** | **D8** | **D15** | | |
| Informed Consent | X | | | | | | | | | | | | | | | | | |
| History / Physical / ECOG | X | X | | X[1] | X[2] | X[1] | | X | | | X[2] | | | X | | | | X |
| Collection of AEs | X | X | | X[1] | X[2] | X[1] | | X | | | X[2] | | | X | | | X[10] | X |
| Serum Pregnancy (HCG) | X[4] | | | | | | | | | | | | | | | | | |
| CBC with diff | X[5] | X | | | X[2] | | | X | | | X[2] | | | X | | | | X |
| Chemistries, LFTs, TSH | X[5] | X | | | X[2] | | | X | | | X[2] | | | X | | | | X |
| Coags (PT/INR, aPTT) | X | X | | | | | | | | | | | | | | | | |
| EKG | X[6] | | | | | | | | | | | | | | | | | |
| MUGA or ECHO | X[6] | | | | | | | | | | | | | X[7] | | | | |
| Tumor biopsy | X | | | | | | | | | | | | | X | | | | |
| Blood draw for Immune Monitoring | X | X | | | | | | X | | | | | | X[8] | | | | X[12] |

| Cycle Days → | Screen | Cycle 1 | | | Cycle 2 | | | Cycle 3 | | | Cycle 4 | | | Cycle 5 | | | 30-Day Safety Visit | Week 24 and 6-Mo. Follow-up[I] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Procedure / Test ↓** | | **D1** | **D8** | **D15** | **D1** | **D8** | **D15** | **D1** | **D8** | **D15** | **D1** | **D8** | **D15** | **D1** | **D8** | **D15** | | |
| CT Scan of C/A/P; Bone scan if needed | X[6] | | | | | | | | | | | | | X[9] | | | | X[9] |
| VRP-HER2[1] | | X | | X[1] | | X[1] | | | | | | | | X | | | | |
| Pembrolizumab[1] | | X | | | X[2] | | | X | | | X[2] | | | X | | | | |

Note: Study activities may be performed +/-3 days from designated day.

[1]VRP-HER2 is to be administered only to subjects in Arms A, C, and the Safety Arm; Subjects in Arm B (pembrolizumab alone) do not need to return to clinic for assessments on C1D15 or C2D8. Subjects in Arm C and the Safety Arm will return for all study visits.

[2]Pembrolizumab is to be administered only to subjects in Arms B, C, and the Safety Arm; Subjects in Arm A (VRP-HER2 alone) do not need to return to clinic for assessments on C2D1 or C4D1. Subjects in Arm C and the Safety Arm will return for all study visits.

[3]Unless otherwise noted below, screening assessments are to take place within 28 days prior to C1D1. Refer to Section 7.1.1 for all screening assessment timelines.

[4]Serum pregnancy test must be performed and determined negative within 72 hours of the first dose of study drug(s).

[5]CBC with differential, Chemistries including LFTs tests are to be completed within 7 days of the first dose of study drug(s). (Refer to Table 6 of Section 7.4 for the complete list of labs to be assessed for screening)

[6]Available CT scans and bone scans performed as standard of care prior to signing consent can be used to fulfill eligibility requirements if they were performed within 4 weeks of the first dose of study drug(s). Available MUGA, Echocardiogram (ECHO), and EKG performed as standard of care prior to signing consent can be used to fulfill eligibility requirements if they were performed within 8 weeks of the first dose of study drug(s).

[7]The second ECHO or MUGA Scan will be performed at week cycle 5 day 1 or at end of study visit, whichever is completed first. The same cardiac imaging modality should be used consistently for an individual patient throughout the study.

[8]Treatment phase concludes at week 14. However, if subjects go off study before week 14 a blood draw for immune monitoring will be performed. A research biopsy will be performed at the discretion of the investigator.

[9]The results of available CT scans and bone scans performed as standard of care will be obtained at approximately cycle 5 day 1 +/- 7 days and then during follow-up every 6 weeks for 1 year, followed by every 9 weeks.

[10]AE assessments will be conducted in person or by phone approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should be followed and recorded.

[11]During the follow-up phase, we will collect survival data every 12 weeks through contacting the patients (in person, by email, or phone call), chart review, and/or by contacting their local oncologist to determine when subjects have any late toxicities, have progression of disease, or are no longer living. If the subject has progressive disease they do not need to be followed after the time of progression.

[12]At investigators discretion, peripheral blood (up to 90 ml) for immune monitoring will be obtained every three months for one year.

# 7.0 TRIAL PROCEDURES/ASSESSMENTS

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator. Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

## 7.1 Screening period

During the Screening Period, subjects are consented and screened for the study. Informed consent must be obtained before initiation of any screening procedure that is performed solely for the purpose of determining eligibility for this study. Evaluations performed as part of routine care before informed consent can be considered as screening evaluations if done within the defined screening period, and if permitted by the local Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) policies. Study eligibility is based on meeting all of the inclusion criteria and none of the exclusion criteria before the first dose of study drug on Cycle 1 Day 1.

### 7.1.1 Screening Procedures

The following study procedures must be done within 28 days prior to Cycle 1 Day 1:

- Demographics
- Medical and cancer history
- Physical examination including Height, Vital signs and weight
- Concomitant medications
- ECOG performance status
- EKG (assessment performed up to 8 weeks prior to C1D1 for SOC can be used)
- TSH
- Adverse event assessment (review of baseline symptoms)
- Tumor assessment (CT and/or bone scans performed up to 4 weeks prior to C1D1 for SOC can be used)
- MUGA or echocardiogram (assessments performed up to 8 weeks prior to C1D1 for SOC can be used)
- Blood draw for immune monitoring
- Tumor biopsy will be performed following informed consent and screening procedures and within 6 weeks prior to Cycle 1 Day 1.

The following study procedures must be done within 7 days prior to Cycle 1 Day 1:

- CBC with differential
- Chemistries including liver function tests (LFTs)*

The serum pregnancy test for women of childbearing potential must be completed (and result must be negative) within 72 hours of the first dose of study drug(s).

Subject eligibility is determined using lab results obtained up to 7 days prior to Cycle 1 Day 1. Any laboratory assessments repeated on Cycle 1 Day 1 must meet eligibility requirements. The Screening Period ends upon receipt of the first dose of study drug or final determination that the subject is ineligible for the study.
*See 7.4 Laboratory Procedures/Assessments

### 7.1.2 Assignment of Subject ID Number

Patients will be identified with the following code system:

Safety Arm: VRP-HER2-**0S1-001**, -002, -003…-00x

Arm A: VRP-HER2-**A-001**, -002, -003…-00x

Arm B: VRP-HER2-**B-001**, -002, -003…-00x

Arm C: VRP-HER2-**C-001**, -002, -003…-00x

## 7.2 Treatment Period

During the Treatment Period, subjects will receive the assigned study treatment as per section 5.2. Dose modification guidelines are described in 5.2.1.

The following study procedures are to be completed as follows:

- History including concomitant medications and adverse events assessment and Physical examination including vital signs and ECOG performance status each visit (C1D1, C1D15, C2D1, C2D8, C3D1, C4D1, C5D1)
  - Arm A (VRP-HER2) will require visits C1D1, C1D15, C3D1, C2D8, C5D1
  - Arm B (pembrolizumab) will require visits C1D1, C2D1, C3D1, C4D1, C5D1
  - Arm C and Safety Arm (pembrolizumab + VRP-HER2) will require all visits listed above
- CBC with differential (Each cycle day 1 – excluding Arm A: C2D1, C4D1)
- Chemistries including LFTs and TSH (Each cycle day 1 – excluding Arm A: C2D1, C4D1)
- Blood draw for immune assays visit (C1D1, C3D1, C5D1)
- MUGA scan or echocardiogram (C5D1 or at end of treatment, whichever comes first)
- Tumor biopsy (C5D1)

### 7.3 Post-Treatment Period (week 24 and 6-month follow-up)

- History including concomitant medications and adverse events assessment and Physical examination including vital signs and ECOG performance status each visit
- CBC with differential
- Chemistries including LFTs and TSH
- Blood draw for immune assays visit

### 7.4 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided in the table below.

Table 6 Laboratory Tests

| Hematology | Chemistry | Other |
|---|---|---|
| Hematocrit | Albumin | Serum β-human chorionic gonadotropin (β-hCG)† |
| Hemoglobin | Alkaline phosphatase | |
| Platelet count | Alanine aminotransferase (ALT) | PT (INR) |
| WBC (total and differential) | Aspartate aminotransferase (AST) | aPTT |
| Red Blood Cell Count | Lactate dehydrogenase (LDH) | |
| Absolute Neutrophil Count | Carbon Dioxide ‡ | Free tyroxine (T4) |
| Absolute Lymphocyte Count | *($CO_2$ or biocarbonate)* | Thyroid stimulating hormone (TSH) |
| | Uric Acid | PK |
| | Calcium | |
| | Chloride | Blood for correlative studies |
| | Glucose | |
| | Phosphorus | |
| | Potassium | |
| | Sodium | |
| | Magnesium | |
| | Total Bilirubin | |
| | Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)* | |
| | Total protein | |
| | Blood Urea Nitrogen | |

## 7.5 Other Procedures

### 7.5.1 Withdrawal/Discontinuation

When a subject discontinues or is withdrawn prior to trial completion, all applicable activities scheduled for the final Week 24 trial visit should be performed at the time of discontinuation. If subject withdraws prior to cycle 5, a post-treatment research biopsy will be performed at the discretion of the investigator. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.9 - Assessing and Recording Adverse Events. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.6) and then proceed to the Follow-Up Period of the study (described in Sections 7.7 and 7.8).

## 7.6 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted either in person or by telephone approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

## 7.7 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 6 weeks (42 ± 7 days) by radiologic imaging to monitor disease status. After 1 year, the imaging time point will occur every 9 weeks (± 7 days). Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study. At investigators discretion, blood draws (up to 90 ml) for immune monitoring will be obtained every three months for one year.

## 7.8 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

### 7.9 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Progression of the cancer under study is not considered an adverse event.

All adverse events that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

From the time of treatment allocation/randomization through 30 days following cessation of treatment, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.9.3.1. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

Adverse events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy

etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

### 7.9.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

### 7.9.2 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

### 7.9.3 Immediate Reporting of Adverse Events to the Sponsor and to Merck

#### 7.9.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Merck's product and/or VRP-HER2 that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is an other important medical event

- **Note:** In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.
    - Is a new cancer (that is not a condition of the study);
    - Is associated with an overdose.

For the time period beginning when the consent form is signed until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (reference Section 7.9.3.3 for additional details), that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (reference Section 7.9.3.3 for additional details), whether or not related to the Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified

in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck Global Safety.

All subjects with serious adverse events must be followed up for outcome.

**SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220**

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

#### 7.9.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.9.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

#### 7.9.3.3 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined in this section will not be reported to Merck as described in Section 7.9.3- Immediate Reporting of Adverse Events to the Sponsor and to Merck, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study

Hospitalization related to convenience (e.g.transportation issues etc.) will not be considered a SAE.

### 7.9.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 5.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 7 Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

| | | |
|---|---|---|
| **V5.0 CTCAE Grading** | **Grade 1** | **Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.** |
| | **Grade 2** | **Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.** |
| | **Grade 3** | **Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.** |
| | **Grade 4** | **Life threatening consequences; urgent intervention indicated.** |
| | **Grade 5** | **Death related to AE** |
| **Seriousness** | A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that: | |
| | †**Results in death**; or | |
| | †**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or | |
| | †**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or | |
| | †**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or | |
| | †**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or | |
| | **Is a** new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); **or** | |
| | **Is an overdose** (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor and to Merck within 2 working days.. | |
| | **Other important medical events** that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †). | |
| **Duration** | Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units | |
| **Action taken** | Did the adverse event cause Merck product to be discontinued? | |

<table>
<tr><td>Relationship to Merck Product</td><td colspan="2">Did Merck product cause the adverse event? The determination of the likelihood that Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information.<br><br>The following components are to be used to assess the relationship between Merck product and the AE; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):</td></tr>
<tr><td></td><td>Exposure</td><td>Is there evidence that the subject was actually exposed to Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?</td></tr>
<tr><td></td><td>Time Course</td><td>Did the AE follow in a reasonable temporal sequence from administration of Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?</td></tr>
<tr><td></td><td>Likely Cause</td><td>Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors</td></tr>
</table>

| | | |
|---|---|---|
| **Relationship to Merck Product (continued)** | **The following components are to be used to assess the relationship between the test drug and the AE: (continued)** | |
| | **Dechallenge** | Was Merck product discontinued or dose/exposure/frequency reduced?<br>If yes, did the AE resolve or improve?<br>If yes, this is a positive dechallenge. If no, this is a negative dechallenge.<br>(Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Sponsor's product; or (3) the trial is a single-dose drug trial); or (4) Sponsor's product(s) is/are only used one time.) |
| | **Rechallenge** | Was the subject re-exposed to Merck product in this study?<br>If yes, did the AE recur or worsen?<br>If yes, this is a positive rechallenge. If no, this is a negative rechallenge.<br>(Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Sponsor's product(s) is/are used only one time).<br>NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY MERCK PRODUCT, OR IF REEXPOSURE TO MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL. |
| | **Consistency with Trial Treatment Profile** | Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding Merck product or drug class pharmacology or toxicology? |
| The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements. | | |
| **Record one of the following** | **Use the following scale of criteria as guidance (not all criteria must be present to be indicative of Merck product relationship).** | |
| **Yes, there is a reasonable possibility of Merck product relationship.** | There is evidence of exposure to Merck product. The temporal sequence of the AE onset relative to the administration of Merck product is reasonable. The AE is more likely explained by Merck product than by another cause. | |
| **No, there is not a reasonable possibility of Merck product relationship** | Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of Merck product is not reasonable OR the AE is more likely explained by another cause than the Merck product. (Also entered for a subject with overdose without an associated AE.) | |

### 7.9.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

### 7.9.6 Definitions for Reporting Adverse Events – VRP-HER2 Vaccine

The definitions in this section have been adapted from the Code of Federal Regulations (CFR) and the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practices (GCPs). Please refer to the complete regulations and guidelines for additional details regarding these definitions.

**Adverse Event (AE) [ICH GCP]:** An Adverse Event (AE) shall mean any untoward medical occurrence whether thought to have been caused by the Study Drug or not.

**Serious Adverse Event (SAE) [21 CFR 312.32]:** Serious Adverse Event (SAE) shall mean any adverse event which is fatal, life-threatening, disabling or incapacitating, requires in-patient treatment or prolongs existing hospitalization, is a congenital anomaly in the off-spring of the patient or which may require intervention to prevent the previously stated outcomes.

- A **life-threatening** adverse experience.
    - The subject was at immediate risk of death from the reaction as it occurred.
    - Does not include a reaction that, had it occurred in a more severe form, could have led to death.
- **In-patient hospitalization or prolongation of existing hospitalization.** Hospitalizations do not include:
    - Preplanned (prior to the study) hospital admissions unless the hospitalization is prolonged.
    - Planned admissions (as part of a study – e.g. routine biopsies).
    - 23 hour re-hospitalizations.
    - Hospitalization for elective procedures.
    - Emergency room visits.
- A **persistent or significant disability/incapacity**.
    - The event resulted in a substantial disruption of the subject's ability to conduct normal life functions.
- **A congenital anomaly/birth defect.**
- Important medical events that may not result in death, be life-threatening, or require hospitalization, but based upon appropriate medical judgment may jeopardize the patient or subject, and may require medical or surgical intervention to prevent one of the outcomes listed above.
- **Death**

**Unexpected Adverse Event (UAE) [21 CFR 312.32]:** Any adverse drug experience or adverse reaction, the specificity or severity of which is not consistent with the applicable product information (e.g., the current Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product); or if the drug label is not required or available, the specificity or severity of which is

not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

**Related/Associated [ICH GCP]:** There is a reasonable possibility (more likely than not) that the event may have been caused by the drug, device or research. Determining the possible cause of an event includes assessing temporal relationships, dechallenge/rechallenge information, association (or lack of association) with underlying diseases, and the presence (or absence) of a more likely cause.

### 7.9.7 Procedures for Reporting – VRP-HER2 Vaccine

All adverse events that are identified from the time of written informed consent through 30 days post study drug stop should be recorded on the CRFs.

Serious Adverse Events (SAEs) must ALSO be recorded on the Duke Cancer Institute Safety SAE Report Form and reported within 24 hours of learning of an event. The form should be completed as much as possible but should not be held until all information is available. Additional information and/or corrections may be submitted as they are obtained. All SAEs must be followed through resolution or stabilization.

SAEs should be reported by fax to the following:

a. Duke Site PI: Micheal Morse, MD
Address: DUMC Box 3233, Durham, NC 27710
Telephone: (919) 681-3480
Fax: (919) 681-7970
E-mail: michael.morse@duke.edu

b. Duke Breast Oncology Research Office
Address: DUMC Box 2965, Duke South White Zone, Room 2592, Durham, NC 27710
Phone: (919) 660-1278
Fax: (919) 681-7335

- Each notification shall be made as soon as possible and in no event later than 15 calendar days after Duke University's initial receipt of the information. Each written notification may be submitted on the Duke Cancer Institute Safety SAE Report Form or in a narrative format. In each written IND safety report, Duke University shall identify all safety reports previously filed with the IND concerning a similar adverse experience, and shall analyze the significance of the adverse experience in light of the previous, similar reports.

- Duke University shall also notify the FDA by telephone or by fax of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days after the initial receipt of the information.
- Follow-up information to a safety report shall be submitted as soon as the relevant information is available.

Additionally, adverse events will be reported to the FDA in an annual report according to annual report requirements. Events will be reviewed and reported to the Duke IRB according to local IRB guidelines.

# 8.0 STATISTICAL ANALYSIS PLAN

## 8.1 Statistical Analysis Plan Summary

### 8.1.1 Primary Endpoint

The primary endpoint for this study is to determine whether pembrolizumab increases tumor infiltrating (T cell) and the peripheral blood (T cell and antibody) immune response to the VRP-HER2 vaccine.

T cell immunologic response (IR) will be measured by ELISPOT and/or cytokine flow cytometry assay for HER2 specific T cell responses, and ELISA for anti-HER2 antibody responses. In the ELISPOT assay, a T-cell response will be considered positive if the mean number of spots in six wells with antigen exceeds the number of spots in six control wells by a magnitude of 10 and the difference between single values of the six wells containing antigen and the six control wells is statistically significant at a level of p=0.05 by the t-test. We propose a 3-arm trial; Her2-vaccine, PD-1 blockade, and Her2+PD-1, with n=12 patients randomized to each arm. If 5 or more patients in each arm exhibit a T-cell response the therapy will be considered efficacious. Each arm is based on an exact single stage phase II trial with randomization to achieve representativeness of patients in each arm under the assumption that 10% response is too low and 50% or higher response rates is indicative of further study (alpha=0.05 and 90% power). There is no planned statistical comparison across the arms for the primary outcome, T-cell response. We will report results in aggregate by arm and by hormone receptor status. Samples will also be archived for future TCR Sequencing to identify changes in clonality of the T cell response.

Antibody responses to HER2 will be measured by an ELISA assay. HER2 antibody titer will be defined as the serum dilution which has an absorbance that is twice the negative control value for the same dilution. Positive results may be confirmed by Western blot analysis and additional immune assays.

### 8.1.2 Secondary Endpoints

The secondary endpoint for this study is safety of concurrent VRP-HER2 vaccine and pembrolizumab.

Since the experimental arm is a novel combination, we will enroll a preliminary safety study of 3 patients receiving the combination of VRP-HER2+pembrolizumab. If there are no toxicities related to study drug therapy in these patients, monitored continually according to the upper half of the table below, then we will proceed with the randomization across the 3 arms. In addition, we will incorporate toxicity monitoring within the randomized VRP-HER2+pembrolizumab arm so that it can be stopped in the event of too much toxicity. The toxicity monitoring is based on 20% or lower toxicity as acceptable and 33% or higher toxicity as unacceptable. The stopping rule indicates that for each patient number achieved in enrollment the arm should stop if the number of toxicities exceeds the corresponding number in the table.

| Patient # | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Max # of toxicities | - | 1 | 2 | 2 | 2 | 2 |
| Patient # | 7 | 8 | 9 | 10 | 11 | 12 |
| Max # of toxicities | 3 | 3 | 3 | 4 | 4 | 4 |

## 9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

### 9.1 Investigational Product - Pembrolizumab

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Clinical Supplies will be provided by Merck as summarized in Table 8.

Table 8 Product Descriptions

| Product Name & Potency | Dosage Form |
|---|---|
| Pembrolizumab 50 mg | Lyophilized Powder for Injection |
| Pembrolizumab 100 mg/ 4mL | Solution for Injection |

### 9.2 Packaging and Labeling Information - Pembrolizumab

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

### 9.3 Clinical Supplies Disclosure - Pembrolizumab

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

### 9.4 Storage and Handling Requirements - Pembrolizumab

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

### 9.5 Returns and Reconciliation - Pembrolizumab

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

### 9.6 Investigational Product – VRP-HER2 Vaccine

The investigator must maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

VRP-HER2 is supplied as a sterile, clear solution in a 2 mL single-dose vial. The vaccine is provided at 5.2 x $10^8$ IU/mL (2.6 x $10^8$ IU per 0.5 mL, and contains phosphate buffered saline, pH 7.3, human serum albumin, sodium gluconate and sucrose as a cryopreservative). Each vial contains an extractable vaccine volume of 0.5 mL. The product should be stored at –80 ± 10°C.

### 9.7 Packaging and Labeling Information – VRP-HER

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

### 9.8 Clinical Supplies Disclosure – VRP-HER2

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

### 9.9 Returns and Reconciliation – VRP-HER2

Unless other arrangements are agreed in writing, all unused vaccine should be returned to H. Kim Lyerly, MD at the completion of the clinical study.

## 10.0 ADMINISTRATIVE AND REGULATORY DETAILS

### 10.1 Confidentiality

All subject data will be identified by a subject identification number and the subject's initials only, to protect the subject's privacy. The data will be blinded accordingly in all data analysis. However, in compliance with federal guidelines regarding the monitoring of clinical studies, it is required that the investigator permit a representative of the FDA that portion of the subject's medical record that is directly related to the study. This will include all relevant study documentation including medical histories, to verify eligibility, laboratory test results to verify transcription accuracy, X-ray reports, admission, discharge summaries for hospital/outpatient admissions while the subject is on-study, and autopsy reports for deaths occurring during the study. As part of the required content of informed consent, the subject must be informed that

his, medical chart may be reviewed by a representative of the FDA. Should access to the medical record require a separate waiver or authorization, it is the investigator's responsibility to obtain such permission from the subject in writing before the subject is entered into the study.

### 10.2 Compliance with Financial Disclosure Requirements

All study personnel will be required to comply with Financial Disclosure Requirements while participating in the administration and oversight of this phase II clinical trial.

### 10.3 Compliance with Law, Audit and Debarment

Research Monitor

The Research Monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside the study about the research. The Research Monitor shall have the authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the Monitor's report. Research Monitors shall have the responsibility to promptly report their observations and findings to the IRB or other designated official.

This clinical research study will be monitored both internally by the PI and institutionally by the Duke Cancer Institute (DCI). In terms of internal review the PI will continuously monitor and tabulate adverse events. Appropriate reporting to the Duke University Medical Center IRB will be made. If an unexpected frequency of Grade III or IV events occur, depending on their nature, action appropriate to the nature and frequency of these adverse events will be taken. This may require a protocol amendment, dose de-escalation, or potentially closure of the study. The PI of this study will also continuously monitor the conduct, data, and safety of this study to ensure that:

- Interim analyses occur as scheduled;
- Stopping rules for toxicity and/or response are met;
- Risk/benefit ratio is not altered to the detriment of the subjects;
- Appropriate internal monitoring of AEs and outcomes is done;
- Over-accrual does not occur;
- Under-accrual is addressed with appropriate amendments or actions;
- Data are being appropriately collected in a reasonably timely manner.
- DCI review and monitoring of this protocol occurs in accordance with the NCI-approved Data and Safety Monitoring Plan. Briefly, protocol review begins with an initial review by the Cancer Protocol Committee (CPC), which assesses the ethics and safety of the protocol. Documentation of these assessments will be maintained. Formal, independent monitoring will be conducted by the DCI Monitoring Team after the first

3 subjects are enrolled, followed by annual monitoring of 1-3 subjects until the study is closed to enrollment and subjects are no longer receiving study interventions that are more than minimal risk. DCI Monitoring Team reports and additional data/safety/toxicity reports submitted by the PI will be reviewed by the Safety Oversight Committee (SOC) on an annual basis. Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns. Monitoring visits may also be initiated upon request by DUHS and DCI Leadership, CPC, SOC, a sponsor, an investigator, or the IRB.

Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon request of DUHS and DCI leadership, the CPC, the Safety Oversight Committee (SOC), the sponsor, the Principal Investigator, or the IRB. All study documents must be made available upon request to the DCI Monitoring Team and other authorized regulatory authorities, which may include but is not limited to the National Institute of Health, National Cancer Institute, and the FDA. Every reasonable effort will be made to maintain confidentiality during study monitoring.

### 10.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

### 10.5 Data Management

#### 10.5.1 Case Report Forms

Electronic Case Report Forms (CRF) are used to record study data and are an integral part of the study and subsequent reports. The database used is Medidata RAVE. Most data will be collected from each subject's electronic record. Each Subject's CRF will be signed by the principal investigator at the end of the study, including those removed from the study for any reason. Case Report Forms must be kept current to reflect subject status at each phase during the course of the study. Subjects are not to be identified on case report forms by name; appropriate coded identification and subject initials must be used. The investigator must keep a separate log of subject names, medical record numbers, and subject numbers. This log is subject to FDA inspection.

### 10.5.2 Maintenance of Study Documentation

The following will be maintained:

a. Electronic Case Report Forms - must be kept accurate, and up-to-date.

b. Electronic Medical Records and/or Shadow Chart - substantiates the data entered on the case report forms for all required tests and evaluation procedures and verifies that the subject has signed an informed consent to enter the study.

c. Subject Exclusion Record - which should reflect the reason any subject was screened and found ineligible for the study.

d. Regulatory Documents - including protocol, FDA Form 1572, CVs, IRB correspondence, IRB approval/renewals and IRB approved consent form.

e. Adverse Experience Report Form - which should explain any serious or unexpected adverse experiences.

All study documentation pertaining to the conduct of the study must be kept on file by the investigator for a minimum of two years.

# 11.0 APPENDICES

## 11.1 ECOG Performance Status

| Grade | Description |
|---|---|
| 0 | Normal activity. Fully active, able to carry on all pre-disease performance without restriction. |
| 1 | Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work). |
| 2 | In bed <50% of the time. 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 | In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours. |
| 4 | 100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair. |
| 5 | Dead. |
| * As published in Am. J. Clin. Oncol.: *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.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair. | |

## 11.2 Common Terminology Criteria for Adverse Events V5.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

## 11.3 Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria for Evaluating Response in Solid Tumors

RECIST version 1.1* will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

* As published in the European Journal of Cancer:

E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

In addition, volumetric analysis will be explored by central review for response assessment.

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