Phase II Trial of Immune Checkpoint Inhibitor Pembrolizumab with Anti-CD3 x Anti-HER2 Bispecific Antibody Armed Activated T Cells in Metastatic Castrate Resistant Prostate Cancer

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Study Synopsis

Prostate cancer (PC) is the most common cancer in American men. Most of the deaths related to PC are from metastatic disease. Despite recent advances in treatment options, progression eventually occurs and metastatic castrate resistant prostate cancer (mCRPC) remains an incurable disease. Novel therapies are needed to induce long term remission, decrease pain, and improve survival in mCRPC. Sipuleucel T has demonstrated an overall survival (OS) benefit in mCRPC, but given the modest magnitude of benefit and rare anti-tumor responses, better immune therapies are clearly needed [1]. In this protocol, we will combine the novel immune therapy of antibody activated T cells with anti-PD-1 (programed death receptor 1) therapy of pembrolizumab to achieve durable anti-tumor responses in mCRPC patients.

Arming anti-CD3 activated T cells (ATC) with anti-CD3 x anti-HER2 bispecific antibody (HER2Bi) transforms every T cell into a specific cytotoxic T cell directed against prostate cancer. HER2 is overexpressed in about 42-70% of mCRPC cases, and HER2 expression has prognostic significance [1]. Preclinical data revealed that bispecific antibody armed T cells (BATs) kill even tumors that express low levels of HER2; responses and tumor cell kill were noted in a clinical trial in triple negative breast cancer [2]. A phase I study using HER2Bi armed ATC (HER2 BATs) in metastatic breast cancer (MBC) induced immune responses and encouraging survival results with a median OS of 27 months in the HER2 negative group [3]. Immune responses in a phase I clinical trial in mCRPC patients suggest that infusions of HER2 BATs "vaccinate" the patients against their own PC by inducing brisk anti-tumor responses [4]. Seven pretreated patients with mCRPC were enrolled. There were no dose limiting toxicities, and three of seven patients demonstrated significant decreases in their prostate-specific antigen (PSA) levels and relief of bone pain. Immune evaluations of peripheral blood mononuclear cells in two patients before and after immunotherapy showed increases in interferon-gamma (IFN-γ) ELISpot responses and Th_1 serum cytokines. The preclinical and clinical results provide the rationale for development of a phase II trial to determine the anti-tumor efficacy of BATs in mCRPC.

Prostate cancer bone metastases have immunosuppressive characteristics with overexpression of CTLA-4, and increased PD-1 expression, that has been reported in the tumor infiltrating lymphocytes in prostate cancer metastases [5]. Agents such as CTLA-4, PD-1 and PD-L1 inhibitors are rapidly becoming the mainstay of immune therapy in solid tumors; however, a phase III trial of ipilimumab, a CTLA-4 inhibitor, showed no clinical benefit in mCRPC, and preliminary evaluation of PD-1 inhibitors also showed a lack of promising clinical activity. While immune checkpoint inhibitors may not be effective in PC alone, there is data to suggest that they can enhance the anti-tumor effect of targeted therapy [6]. We propose adding a monoclonal antibody inhibitor of PD-1 (pembrolizumab) to BATs therapy in an attempt to overcome immunosuppression and enhance anti-cancer effect.

We will conduct a phase II study in mCRPC patients, to estimate the clinical efficacy of 8 infusions of HER2 BATs (up to 10^{10}/infusion) twice per week for 4 weeks in combination anti-PD-1 therapy with pembrolizumab once every 3 weeks starting 3 weeks before the 1st BATs infusion by assessing the percentage of patients free of clinical progression at 6 months after registration. We hypothesize that infusions of HER2 BATs will induce measurable immunologic changes and

evidence of clinical efficacy and that blocking PD-1 signaling will enhance the T cell mediated killing. We will also estimate the magnitude of change in anti-PC immune functions and markers, after infusions of HER2 BATs in mCRPC and evaluate the magnitude of increase in tumor infiltrating T cells, PD-1 expression, and the Th_1/Th_2 ratio in prostate cancer tumor tissue before and after immunotherapy.

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1.0 OBJECTIVES

1.1 Primary Objectives:

1.1.1 Phase II clinical trial to estimate efficacy. Conduct a phase II study in mCRPC patients, to estimate the clinical efficacy of 8 infusions of HER2 BATs (up to 10^{10}/infusion) twice per week for 4 weeks in combination with pembrolizumab once every 3 weeks starting with one dose 1 week before the 1st BATs infusion, by assessing the percentage of patients free of clinical progression at 6 months after registration.

1.2 Secondary Objectives:

1.2.1 Monitor immune responses. Evaluate phenotype, cytokine profiles and IFN-γ ELISpots, cytotoxicity and antibodies directed at laboratory prostate cancer cell lines for proof of principle of immune system activation and to correlate with clinical outcomes of response, progression free survival (PFS), and overall survival (OS).

1.2.2 Monitor changes in tumor infiltrating T cells, PD-1 expression, and Th_1/Th_2 ratio. Evaluate the magnitude of change in tumor infiltrating T cells, PD-1 expression, and the Th_1/Th_2 ratio in prostate cancer tumor tissue before and after immunotherapy and correlate it with the clinical outcomes of response, PFS, and OS.

2.0 BACKGROUND AND RATIONALE

2.1 Castrate Resistant Prostate Cancer: Prostate cancer (PC) is the most common cancer in American men, and the majority of deaths related to PC are from metastatic disease. Despite current advances, metastatic PC remains an incurable malignancy. Androgen deprivation therapy that suppresses testosterone is effective for a limited period of time, and with the development of the castrate resistant state, the addition of chemotherapy, androgen receptor targeted agents, and immunotherapy have proven overall survival benefit. Despite these advances, metastatic castrate resistant prostate cancer (mCRPC) remains incurable, and novel therapies to achieve a cure continue to be a priority. The modest but statistically significant overall survival (OS) benefit seen with sipuleucel-T provides proof of principle of the potential of immunotherapy in prostate cancer [7].

2.2 HER2 in Prostate Cancer: HER2 expression in 42%-70% of prostate cancers is associated with shorter prostate cancer specific survival and higher relapse rates in those with higher expression of HER2 compared to those who are HER2 negative [1,8]. Serum levels of HER2 were significantly higher in PC patients than controls without cancer and the levels were significantly higher in patients with metastatic disease [9]. Furthermore, in localized disease, higher levels correlated with shorter times to recurrence [9]. HER2 expression in PC cells increases with progression to androgen independence [10,11]. Forced HER2 over-expression in a LAPC-4 PC model confers androgen-independent growth to androgen-dependent PC cells [12]. This reveals the role and significance of HER2 expression in the progression and survival of CRPC**.** Unfortunately, a unique opportunity to evaluate the effects of trastuzumab, an anti-HER2 monoclonal antibody (mAb), in PC patients resulted in early closing of the trial due to poor accrual of HER2+ (≥ 2+) PC patients [13]. Another phase II clinical trial failed to demonstrate any benefit for trastuzumab in CRPC patients suggesting that antibody therapy alone is not potent enough to impact PC progression [14]. In a phase II advanced prostate and renal cancer trial using the bispecific antibody (BiAb) MDX-H210 (15 μg/m^2) IV and granulocyte macrophage colony-stimulating factor (GM-CSF) 5 μg/kg/day subcutaneously for 4 days repeated weekly for 6 weeks, 7 of 20 (35%) patients with prostate cancer had PSA responses (>50% decline in PSA) [15]. Our

approach combines BiAb and activated T cells (ATC) to direct the non-MHC restricted cytotoxicity mediated by activated T cells via independent mechanisms of killing. HER2 BATs induce an immune response even in instances of low receptor HER2 receptor expression (e.g. targeting and cytotoxicity of breast cancer cell lines that are HER2 negative) or if receptors are present but inactivated, conditions that would otherwise impact negatively on treatment outcome with mAb therapy alone.

2.3 HER2 Bispecific Antibody Armed T Cells (BATs): Peripheral blood mononuclear cells (PBMCs) collected with pheresis are activated ex-vivo with OKT3 to generate activated T cells (ATC). These ATC are subsequently "armed" with BiAb that targets CD3 and HER2. Arming ATC with HER2 BiAb (HER2 BATs) makes every T cell into a non-MHC restricted HER2-specific cytotoxic T lymphocyte (CTL) [16]. BATs showed repeated killing, proliferation, and release of several cytokines that induce DC maturation. Dying tumor cells release tumor associated antigens (TAA) that can lead to increased cross-presentation and epitope spreading of TAA by antigen presenting cells. This mechanism, illustrated in **Fig 1**, may vaccinate patients against their own TAA. Preclinical studies showed that HER2 BATs killed prostate, breast, and ovarian cancer cell lines, secreted cytokines, and inhibited tumor growth in mice [17,18,19,20].

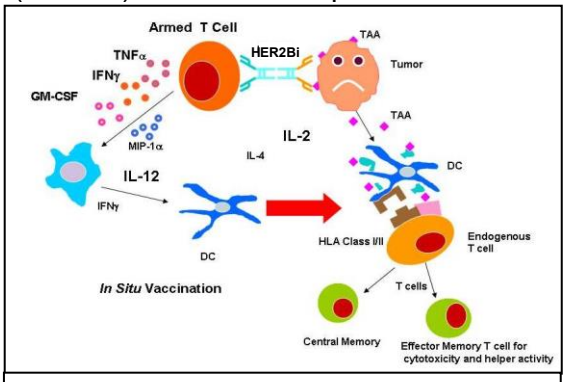


Fig 1: Infusion of targeted T cells induces anti-tumor immune responses. T cells kill tumor cells releasing cytokines and chemokines that induce DC maturation followed by antigen presentation of unknown tumor antigens to "vaccinate" the patient against their own TAA.

2.4 Programmed Cell Death Receptor 1 (PD-1): Immune checkpoint pathways restrict T cell activation and limit immune activation to minimize normal tissue damage. PD-L1 is a receptor expressed by T cells upon activation. PD-1 signaling is activated in T cells in response to PD-L1 or L2 engagement and limits effector T-cell activity in peripheral tissues to limit normal tissue damage. This PD-1/PD-L1/2 pathway is hijacked by cancers to limit anti-tumor immune response and immune surveillance [21]. In prostate cancer bone metastases PD-1 expression is reported to be one of the reasons for resistance to immunotherapies. mAbs blocking PD-1 or PD-L1 have shown efficacy in a variety of cancers. Unfortunately, early phase trials in prostate cancer failed to show clinical benefit using anti-PD-1 treatment alone; however, anti-PD-1 does increase the efficacy of active immunotherapies such as CAR T cells [5]. Combining PD-1 blockade with the specific targeting of HER2 BATs may increase the likelihood of clinical benefit for mCRPC patients. Hence our study design incorporates the therapeutic strategy of PD-1 inhibition and assesses the expression of PD-1 in tumor tissue biopsies pre and post therapy.

2.5 HER2 BATs Target High HER2 SK-BR-3 and Low HER2 SUM 1315 Expression Cell Lines: Preclinical studies showed that HER2 BATs were able to lyse breast cancer cells lines that expressed not only high amounts of HER2 (SK-BR-3, high 3+ expression) but, more importantly, target and lyse cell lines that express little (MCF-7, a negative control for immunohistochemistry) or essentially no HER2 expression (Sum, 1315) [22]. While this is not a major concern based on cell line expression, it is reassuring to know that only a few molecules of the target antigen on the tumor surface are sufficient to allow binding and triggering of specific cytotoxicity (**Fig 2**). This is consistent with the observation that as few as 10-30 TCR-ligand interactions are sufficient to mediate T cell killing [23].

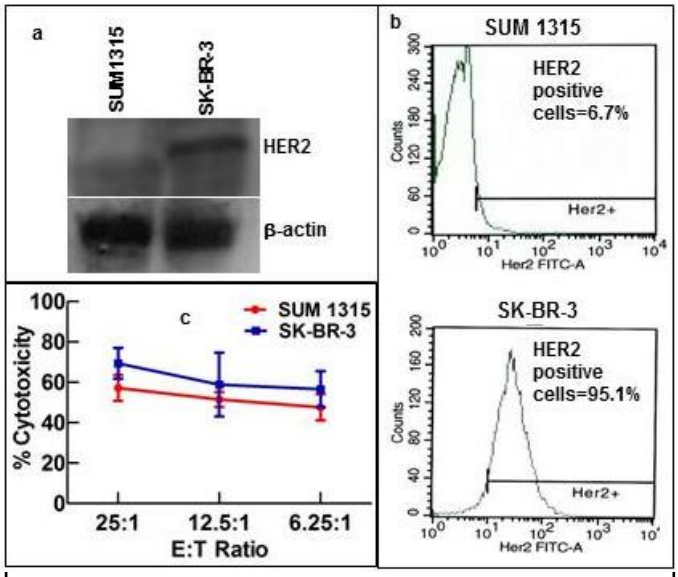


Fig 2. Cytotoxicity remains high in very low HER2 expressing SUM1315. a) Western blot showing HER2 protein expression in low (SUM1315) and high (SK-BR-3) HER2 expressing breast cancer cell lines. **b)** Flow cytometry data showing that 95% cells were HER2 positive in SK-BR-3, while only 6.7% cells were HER2 positive in SUM 1315 cells. **c)** Cytotoxicity remains high in very low HER2 expressing cell line.

may provide a clinical benefit [24,25]. The HER2 BATS infused into MBC patients in the phase I trial were safe and induced immune and clinical responses. BATs infusions induced endogenous immune cells to develop anti-breast cancer CTL. This study provides PROOF of CONCEPT that BATs can vaccinate and provide adoptive transfer of immune cells.

2.6 Phase I Safety, Immune and Clinical Responses in Metastatic Breast Cancer (MBC):

A phase I trial in 23 women with HER2 0-3+ pretreated MBC consisted of 8 infusions of HER2 BATs in combination with IL-2 and GM-CSF to evaluate safety, feasibility, PFS, OS, T cell trafficking and immune responses. The median OS for 8 HER2 3+ patients, 14 HER2 0-2+ patients, and all patients were 57.4, 27.4, and 36.2 months, respectively (**Fig 3**). Targeting HER2 positive and negative tumors induced cytotoxic anti-tumor responses, increases in Th_1 cytokines, and IL-12 serum levels. These robust immune responses suggest that BATs infusions

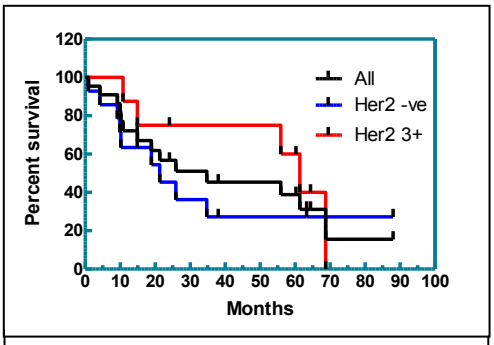
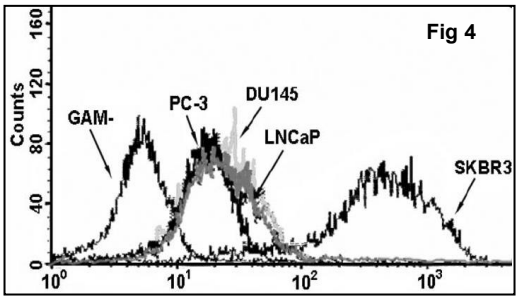


Fig 3. Median OS is 57.4 months for the HER2 3+ group (**red**), 27.4 months for the HER2 0-2+ (**blue**) group, and is 36.2 months for the entire group (**black**).

2.7 Preclinical Studies that Support Proposed Research:

2.7.1 HER2 expression on prostate cancer cells. The prostate cancer cell lines PC-3, DU-145, and LNCaP express easily detectable levels of HER2 (**Fig 4).** SK-BR-3, a breast cancer cell line that exhibits high levels of expression of HER2 is shown as a positive control and an isotype control (goat anti-mouse, GaM) was used as the negative control.



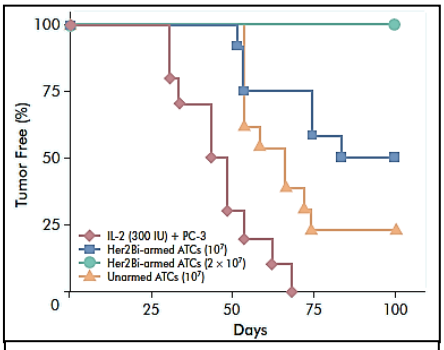


Fig 5. Her2 BATs (Her2Bi-aATC) prevent tumor growth in Winn Assay.

2.7.2 HER2 BATs prevent prostate cancer development in Winn Assay and induce remission in SCID mice. SCID-beige mice were injected subcutaneously in the right rear flank with 10^6 PC-3 tumor cells mixed with IL-2 alone (300 IU), 10^7 unarmed ATC and IL-2, and 1-2 × 10^7 HER2 BATs and IL-2 (n = 8 mice per group). **Fig 5** shows the time to tumor formation in each group. None of the mice in the HER2 BATs treated group developed tumors

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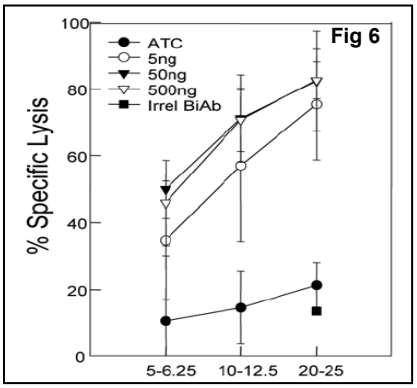
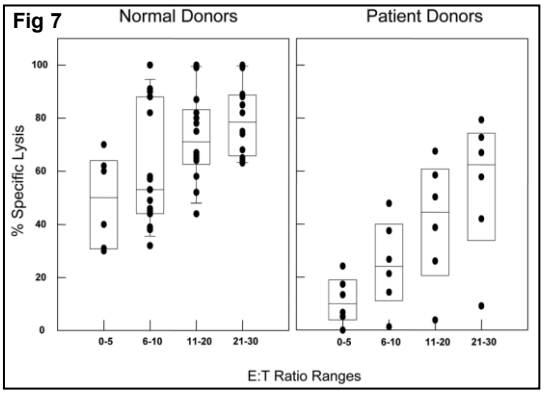
at 100 days when >50% of all other groups showed tumor progression. In a separate experiment, SCID-beige mice with established PC-3 tumors were treated with IL-2, or HER2 BATs (1 or $5x10^7$ cells) and IL-2 twice a week until remission or euthanization due to excessive tumor burden. 8 of 8 mice treated with IL-2 alone were euthanized after 6 weeks. 1 of 8 mice treated with $1x10^7$ HER2 BATs and 2 of 8 mice treated with $5x10^7$ HER2 BATs went into remission lasting 96 and 136 days, respectively [18].

2.7.3 BATs kill PC-3, DU-145, and LNCaP PC cell lines. In ^{51}Cr release cytotoxicity assays, arming of ATC with HER2Bi significantly enhanced lysis of PC-3, DU-145 and LNCaP targets (**Fig 6**). ATC were expanded from PBMC from normal subjects or cancer patients by anti-CD3 activation and expansion in low dose IL-2 as described [22]. After 14 days, ATC were harvested, counted, and armed with HER2Bi or irrelevant (OKT3 x CD20). **Fig 7** shows the cytotoxicity directed at PC-3 line mediated by ATC from 6 cancer patients and 10 normal donors armed with 5, 50 and 500 ng of HER2Bi /10^6 ATC or 50 ng of CD20Bi at E/T intervals of 5-6.25, 10-12.5, and 20-25. The mean and medians for specific cytotoxicity directed at PC-3 by HER2 BATs for each group are represented in box plots at the E:T intervals indicated. Unarmed ATC-mediated specific lysis was significantly lower than BATs in the normal donor (p=0.017) and patient (p=0.04) groups.





2.7.4 BATs secrete cytokines and chemokines when engaging targets cells. Fig 8 shows the specific cytotoxicity directed at PC-3, DU-145, and LNCaP by HER2Bi BATs from 3 normal donors (N1, N2, and N3). Cytokines and chemokines are released from the BATs when the BATs are exposed to PC-3 tumor cells **(Fig 9).** There was an increase in Th_1 cytokines GM-CSF, tumor necrosis factor α (TNFα), and IFNγ by 7-, 54-, and 58-fold, respectively, but not the Th_2 cytokine IL-4 when compared to unarmed ATC or ATC armed with an irrelevant (Irrel) BiAb (anti-CD3 x anti-CD20) [2].

Fig 8

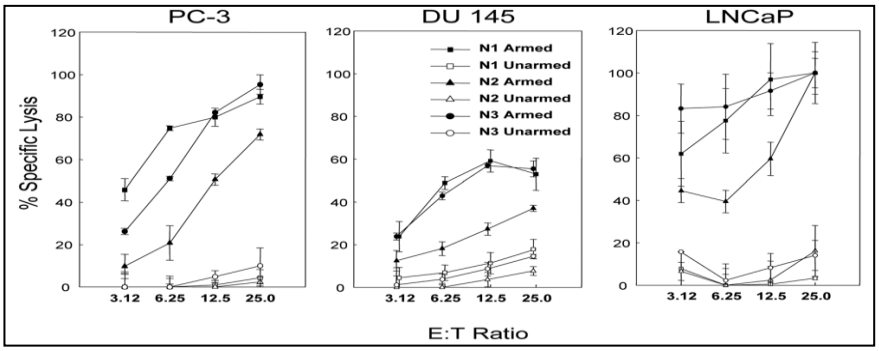
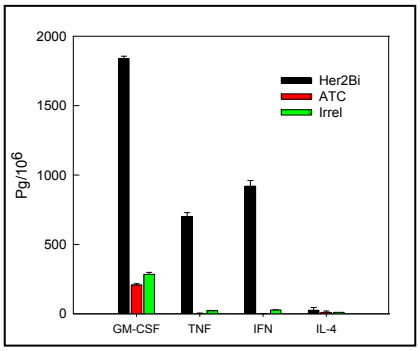


Fig 9



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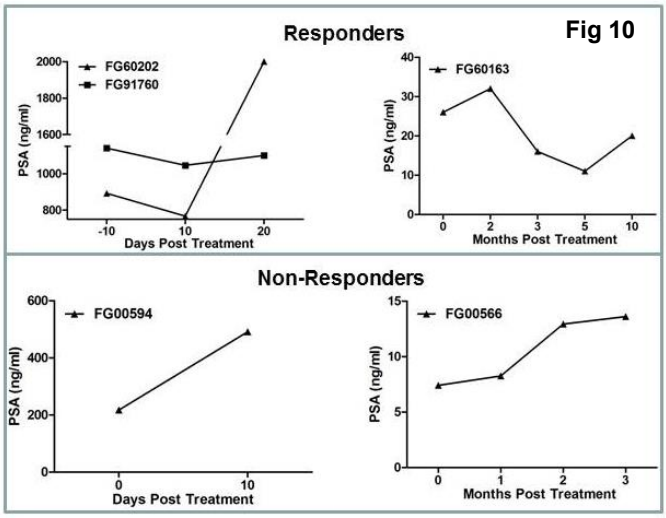
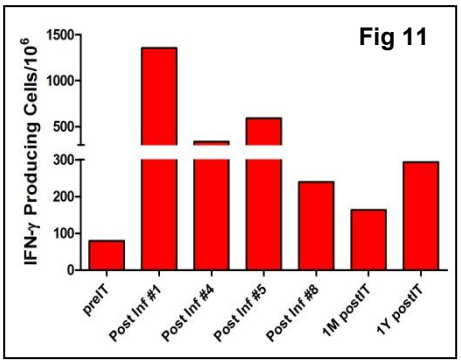
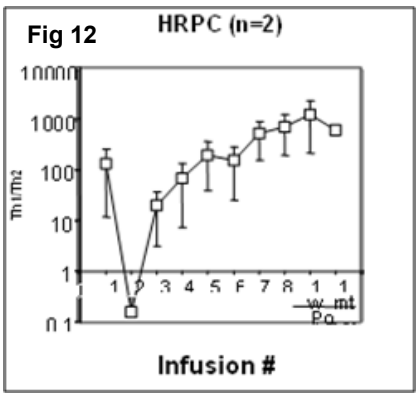
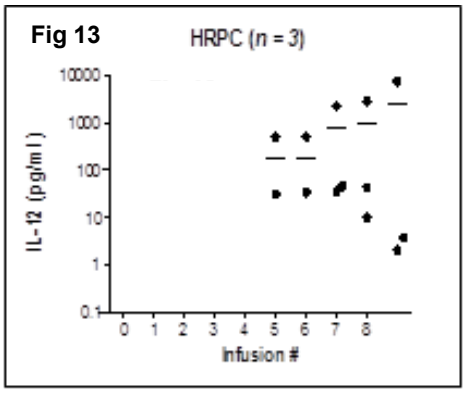
2.8 Phase I HER2 BATs Trial Shows Safety, PSA Responses and Decreased Bone Pain in PC: A phase I dose escalation safety trial was conducted with BATs in patients with mCRPC [4]. The patients received 2.5, 5 or 10 x 10^9 BATs per infusion in combination with low dose interleukin 2 (IL-2) and GM-CSF. All 7 men had bone metastases and 2 had lung and liver involvement. Three patients had progressed after chemotherapy. Expansion was done using a single pheresis. The total doses achieved ranged from 20 -80 billion. There were no dose-limiting toxicities (DLT) at the doses tested. The only clinically relevant toxicity noted was grade 3 rigors requiring meperidine. Overall survival ranged from 68 to 598 days (median 170 days). Three patients experienced a ***decrease in bone pain*** with a concomitant reduction in their pain medications. All infusions were done in the outpatient clinic. One patient who received 40 x 10^9 BATs had a partial response with his PSA decreasing from 32 to 12 that persisted for about 4 months (**Fig 10**). Two other patients had transient decreases in their bone pain and PSA levels. Immune evaluations of fresh peripheral blood mononuclear cells in 3 responding patients before and after immunotherapy showed increases in IFN-γ ELISpot responses (**Fig 11**) and Th_1 serum cytokines. The pheresis products contained a median of 15.1x10^9 (9.4-25) PBMC prior to 11-16 days of culture which yielded a median of 40 x 10^9 (32-139) ATC after culture. There was a median viability of 95% (90-97%), a median **%CD3 of 89** (31-99), a **median %CD4 of 51** (19-75), and a **median % CD8 of 34** (2-62) after culture.





Polarized Th_1/Th_2 and increased levels of IL-12. Serum samples were obtained prior to each infusion and tested for IL-2, IL-4, IL-5, IL-10, IL-12 p70, IL-13, GM-CSF, IFNγ, and TNF-α using the Bio-Plex Protein Array System. The immune response in 2 CRPC patients remained polarized towards a Th_1 response throughout treatment (**Fig 12**). The ratio of type1/type 2 was also highly polarized to a type 1 response (data not shown). Serum levels of IL-12 dramatically increased after the 3rd -4th infusion of BATs (**Fig 13**). Since IL-12 is produced by endogenous monocytes, these data provide evidence of "vaccination" of the endogenous immune system.





In addition, a phase I trial in breast cancer has well established the clinical safety and preliminary efficacy of BATs. The predominant toxicities noted in this study were fever, chills and headaches. The trial enrolled 22 pre-treated metastatic breast cancer patients. The adverse

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event incidence increased with increasing doses of BATs. The incidence of chills was 8.6, 20.8, and 43.1% at dose levels 1, 2, and 3, respectively. The incidence of headaches was 3.1, 8.3, and 19.6% at dose levels, 1, 2, and 3, respectively. All patients with grade 3 chills responded to meperidine. One patient at dose level 3 experienced a grade 4 headache and hypertension and was removed from the study after 3 infusions (65.7 × 109 total aATC). Evidence of clinical and immunologic responses in women with HER2 0/1+ status in the Phase I trials suggests a therapeutic benefit even in the absence of HER2 over-expression and provides encouraging results in HER2 low-negative patients treated with armed activated T cells [16]. The median overall survival (OS) was 36.2 months for all 23 patients (22 evaluable and 1 non-evaluable = 23), 57.4 months for the HER2 3+ patients, and 27.4 months for the HER2 0–2+ patients. BATs infusions induced endogenous cytotoxic T-cell and immunokine responses that persisted up to 4 months [16]. The findings showed that cellular immune responses develop and may augment immune based killing of tumors even in patients who were progressing. One explanation for the encouraging OS in HER2 0–2+ patients could be a polyclonal immune responses that may target residual chemotherapy resistant HER2 positive "cancer stem-like cells" (CSC) that exhibit self-renewal properties and are responsible for cancer relapse. Prior studies also suggest that anti-HER2 reagents may be effective against HER2 positive CSC in tumors that are primarily HER2 negative [16].

2.9 Immune Check Point Inhibitors Enhance EGFR BATs Function: It has been demonstrated that combining ipilimumab, a CTLA-4 immune checkpoint inhibitor, with T cells armed with anti-CD3 x anti-EGFR bispecific antibody (EGFR BATs) can enhance the anti-tumor activity of BATs. In PBMC from healthy individuals, the addition of ipilimumab at the initiation of culture significantly enhanced T cell proliferation ($p = 0.0029$). **Fig 14a** shows the dose titration effect on cytotoxicity in the presence or absence of EGFR BATs, and **Fig 14b** shows increased killing by normal EGFR BATs in the presence or absence of ipilimumab at an E:T of 25:1 for COLO356/FG. **Fig 14c** shows the same experiments for 3 colorectal patients. BATs infusions increased the secretion of chemokines CCL2, CCL3, CCL4, CCL5, CXCL9, and GM-CSF [data not shown]. **Fig 15** shows that higher doses of ipilimumab in the presence of EGFR BATs increase IFN-γ, IL-2R, IL-12, and IL-10 secretion, while reducing IL-10 secretion [6]. These data provide the rationale for combining BATs and an immune checkpoint inhibitor.

Fig 14

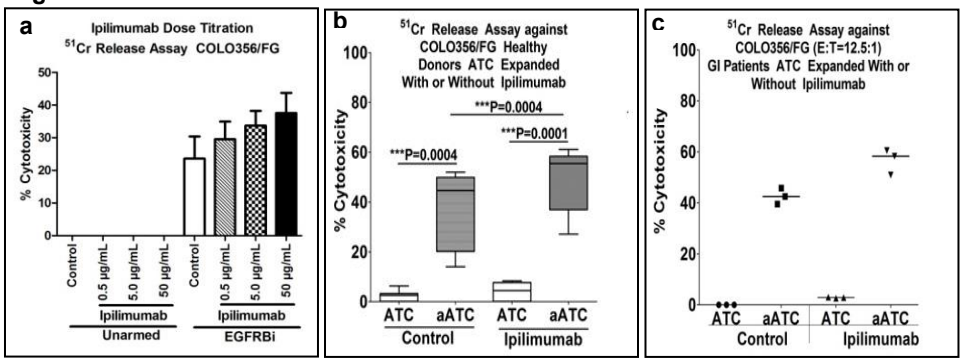
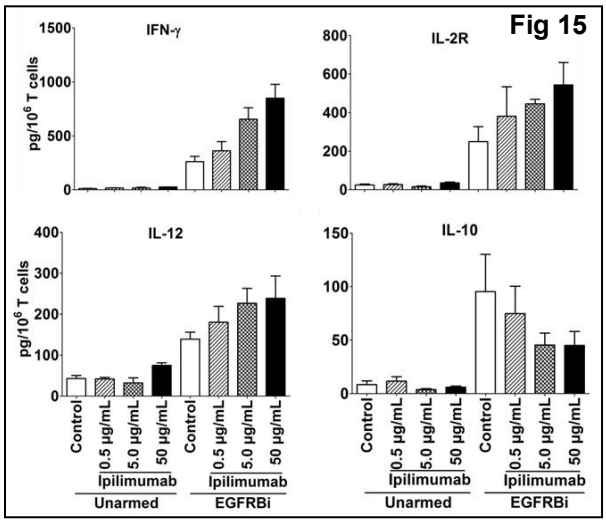


Fig 15



In PBMC from healthy individuals, the addition of ipilimumab at the initiation of culture significantly enhanced T cell proliferation ($p = 0.0029$). Anti-CD3 monoclonal antibody activated T cells (ATC) grown in the presence of ipilimumab showed significantly increased mean tumor-specific cytotoxicity at

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effector:target (E:T) ratio of 25:1 directed at COLO356/FG and Daudi by 37.71% ($p < 0.0004$) and 27.5% ($p < 0.0004$), respectively, and increased the secretion of chemokines (CCL2, CCL3, CCL4,CCL5, CXCL9, and granulocyte-macrophage colony stimulating factor (GM-CSF)) and cytokines (IFN-γ, IL-2R, IL-12, and IL-13), while reducing IL-10 secretion. Expansion of ATC in the presence of ipilimumab significantly improves not only the T cell proliferation but it also enhances cytokine secretion and the specific cytotoxicity of T cells armed with bispecific antibodies.

2.10 Pembrolizumab: 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) has recently been approved in the United States for

the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab as well as in metastatic non-small cell lung cancer. This agent has been evaluated in multiple malignancies and has established safety and efficacy profile.

2.11 Rationale for Dose Selection/Regimen/Modification: An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent pembrolizumab (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 was identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, was the dose and schedule utilized in multiple cohorts 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.

Pharmacokinetic (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 pharmacodynamics (PD) data provides scientific rationale for utilizing a Q3W dosing schedule.

A population PK analysis of pembrolizumab was performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of pembrolizumab 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. 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. **Hence the dose used in this study is 200mg IV every 3 weeks.**

2.12 Summary of Clinical and Preclinical Data: Preclinical testing of HER2 BATs revealed efficacy in binding and killing PC-3, DU-145, and LNCaP cell lines [25,26]. PC-3 cells are low expressors of HER2; despite this cell kill was observed. Anti-CD3 cross-linking leads to T cell activation, proliferation, and cytokine synthesis [27-30]. T cells expanded in IL-2 after anti-CD3 activation exhibited non-major histocompatibility restricted cytotoxicity, and produced tumoricidal cytokines such as IFNγ, TNFα, and GM-CSF [31-41]. HER2 BATs administered with PC-3 (Winn Assay) or injected intratumorally prevented development of, or induced remissions, respectively, in PC-3 tumors in severe combined immunodeficient beige mice [18]. Intravenously administered BATs localized to PC-3 xenografts mediated cytotoxicity toward tumor cells and produced significant delay in tumor of PC-3 tumors, but not in HER2-negative LS174T colon adenocarcinoma xenografts. By flow cytometry analyses, HER2 BATs had a proliferative advantage over unarmed ATCs and persisted in the circulation and tumor tissues longer than unarmed ATCs. The addition of CTLA-4 immune check point inhibitor enhanced T cell proliferation and cytotoxicity in vivo.

These findings suggest that HER2 BATs therapy in combination with anti-PD-1 monoclonal antibody may be an effective, nontoxic, tumor-specific treatment for HER2-positive mCRPC.

3.0 ELIGIBILITY

3.1 Subject Inclusion Criteria: In order to be eligible for participation in this trial, the subject must:

1. Be willing and able to provide written informed consent/assent for the trial
2. Be > 18 years of age on day of signing informed consent
3. Have histologically confirmed prostate adenocarcinoma, with metastases
4. Progression by either PSA, RECIST 1.1 criteria for measurable disease or new areas of metastases on bone scan or symptom progression related to prostate cancer despite castrate levels of testosterone. (Level<50 ng/ml)
5. Be agreeable to continue to maintain castrate levels of testosterone
6. At least 2 weeks should have elapsed since any immunosuppressive therapy
7. At least 4 weeks since prior chemotherapy for metastatic disease or at least 2 weeks since prior androgen targeting agents such as ketoconazole, abiraterone, enzalutamide, etc.
8. Discontinue anti-androgens prior to therapy; at least 6 weeks since last dose of bicalutamide or nilutamide and at least 4 weeks from last dose of flutamide
9. Have adequate bone marrow, renal and hepatic function as deemed by the treating physician to be clinically acceptable
10. Not have concurrent anti-cancer therapy
11. Not have concurrent immunosuppressive therapy or medical condition likely to cause immunosuppression
12. Have life expectancy > 6 months
13. Have a performance status of 0 or 1 on the ECOG/Zubrod Performance Scale
14. Agree to use an adequate method of 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

15. Demonstrate adequate organ function as defined in **Table 1**. All screening labs should be performed within 28 days of registration.

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** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥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** **≤** 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) 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 ≤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.	

3.2 Subject Exclusion Criteria: The subject must be excluded from participating in the trial if the subject:

1. 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 registration.
2. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy (>10 mg of prednisone daily or equivalent steroid doses) or any other form of immunosuppressive therapy within 7 days of registration.
3. Has a known history of active TB (Bacillus Tuberculosis)
4. Has hypersensitivity to pembrolizumab or any of its excipients.
5. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or has not recovered (i.e. ≤ Grade 1 or at baseline) from adverse events due to agents administered earlier. Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.
6. Has received major surgery, subject must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.
7. 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.

8. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to registration and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to registration. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.
9. 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 (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
10. Has known history of, or any evidence of active, non-infectious pneumonitis.
11. Has an active infection requiring systemic therapy.
12. 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.
13. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
14. 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.
15. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.
16. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
17. Has known active Hepatitis B (e.g. HBsAg reactive) or Hepatitis C (e.g. HCV RNA [qualitative/quantitative] is detected).
18. Has received a live vaccine within 30 days of registration. 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.
19. Has history of cardiac or pulmonary impairment either by clinical history or symptoms or cardiac ejection fraction <40%.

4.0 TREATMENT PLAN

4.1 Summary:

Table 2. Schema

Drug	Dose	Route	Timing
Pembrolizumab	200mg	IV	Every three weeks beginning after pheresis and at least 1 week prior to BATs and continuing up to a maximum of9 doses. The interval between pembrolizumab should be a minimum of 21 and a maximum of 42 days. If pembrolizumab cannot be administered for >42 days then it should be discontinued.
BATs	5-10 billion cells	IV	2 times weekly (minimum 42 hours between doses) for 4 weeks beginning at least 1 week after pembrolizumab. BATs therapy should be

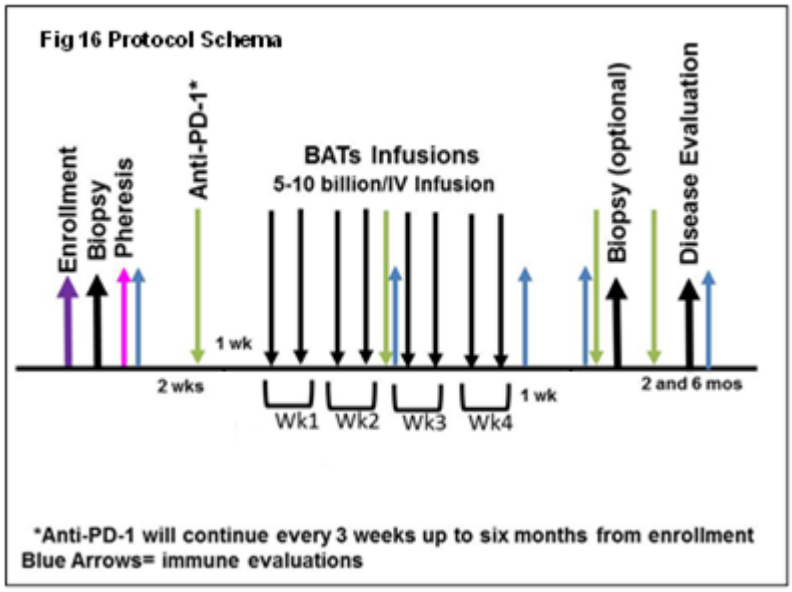
			administered over a maximum of 8 weeks from first dose. Any dose of BATs given after the 8 weeks should be considered under the retreatment criteria in section 5.7.

The clinical protocol will enroll mCRPC patients who are progressing per Prostate Cancer working Group II (PCWGII) criteria, on treatment with androgen suppression therapy [42]. Scientific, regulatory, and FDA approval will be obtained for the clinical trial protocol. The study will be conducted at Karmanos Cancer Institute (KCI) under the direction of Dr. Deol as the Principal Investigator. Dr. Lum at University of Virginia (UVA) will oversee the manufacturing and preparation of BATs product which will be shipped to KCI for patient infusion.

Dr. Lum at UVA is the sponsor of the currently active IND #9985 for the BATs therapy and will be responsible for manufacturing product and conducting quality checks and shipping it to KCI. Dr. Lum will also be responsible for IND reporting and maintenance. Dr. Deol and Dr. Lum will have periodic teleconferences and will communicate and exchange information after every patient is registered.

All pheresis procedures for the patients enrolled will be conducted in KCI pheresis unit. The fresh pheresis product from KCI will be shipped by overnight courier service to UVA GMP laboratory for processing (see section 7.0). Pheresis procedure will be done in accordance with institutional procedures using mononuclear cells (MNC) collection program. Up to a total of 5 circulating blood volumes (CBV) will be processed. Midpoint procedure (after 2 CBVs have been processed) cell count from the collection bag will be done to estimate the final yield with the goal of at least 5 x 10^9 PBMCs, however, this is not a requirement and any collection will be used for generation of activated T cells. HER2 BATs will be prepared by qualified personnel in the Cancer Immunotherapy GMP facility at University of Virginia.

We have selected a dose-range of 5-10 billion HER2 BATs per infusion (a total dose of 40-80 billion BATs) based on the immune and clinical responses and proven safety noted in our prior phase I trials. BATs will be infused twice a week for 4 weeks for a total of 8 doses. Booster doses maybe administered after assessment of primary endpoint. This will be a decision based on extent and duration of patient response and patient willingness. PD-1 inhibitor therapy (MK-3475 2 mg/kg IV every 3 weeks) will start atleast 1 week prior to BATs infusion. This treatment will continue every 3 weeks thereafter until patient continues on study or for a maximum of 9 doses If patient has any grade 3 or 4 toxicity attributable to the PD-1 inhibitor that is sustained for >12 weeksdespite adequate treatment then the immune checkpoint inhibitor will be discontinued. BATs doses can be held or delayed if there is grade 2-4 related toxicity that has not resolved to grade 1 or less at the time of next dose. Maximum duration for BATs administration will be over



8 weeks if hold or delay is necessary. The lead time while the patient is on PD-1 inhibitor will allow assessment for any toxicity and safety concerns before the initiation of BATs. Disease evaluation with scans will occurevery 12 weeks (+/- 1 week) after first pembrolizumab dose for one year, unless progression occurs. The primary endpoint will be the proportion of patients that are free of progression at 6 months. Additional clinical outcome parameters will include time to PSA, skeletal and radiologic progression, time to next systemic therapy intervention, and overall survival (OS) of the patients enrolled on the clinical trial. The response to immune therapy is likely to be delayed and an immediate PSA decline or decrease in metastases cannot be expected. We have hence chosen a 6 month time point to evaluate patients as being progression free or not, to use as a go/no go signal for future development of BATs in mCRPC.

4.2 Activation and Expansion of T cells with OKT3 and IL-2: Pheresis will be performed at KCI, and the fresh pheresis product will be shipped to UVA for processing. Armed ATC are prepared in the Cancer Immunotherapy Laboratory at UVA under BB-IND #9985 sponsored by Dr. Lum. The lymphocytes will be activated with anti-CD3 antibody (OKT3) which cross-links the CD3 receptors on T cells and activates T cells. The ATC will be expanded in the presence of IL-2 up to 14 days. After culture, ATC will be harvested, washed, armed with OKT3 x Herceptin, and cryopreserved in 10% DMSO and 20% protein (albumin or autologous plasma) in liquid nitrogen at the doses for IV infusions. No exogenous IL-2, OKT3, or other culture reagents (e.g. medium components) are present in the final cryopreserved product. Evaluation of cryopreserved versus freshly cultured ATC has shown no differences in viability, phenotype, or function (e.g. lytic activity, cytokine production, response to cytokines, and proliferation) in these cell preparations. The cytotoxicity mediated by ATC armed prior to cryopreservation was equivalent to those armed after thawing of cryopreserved ATC.

4.3 Treatment Regimen with BATs:

4.3.1 HER2 BATs infusions. The BATs infusions will be performed by an Oncology Certified Nurse trained by the sponsor's staff on the SOP for infusing the BATs. An infusaport or any central line will be acceptable for infusing the BATs IV. The Immunotherapy Program Coordinator will deliver and coordinate the timing and site for thawing the frozen product for IV infusions. All appropriate assurances for identification of product, patient, sterility, etc. will be performed during the transfer to the infusion site prior to infusions. Frozen BATs will be thawed and infused into the patient at the bedside per SOPs. The time for BATs infusions will vary from patient to patient, but the dose of BATs will be given over 5-15 min based on the endotoxin level calculations. All patients will be observed for at least 4 hours after an infusion. If stable, patients will be discharged home. Dr. Deol, or other designated coinvestigators will be responsible for the coordination of infusion activities. The protocol will have been approved by the FDA, the KCI Protocol Review Medical Committee, and the Wayne State University IRB and the UVA IRB before any clinical studies begin.

4.3.2 Recommended concomitant medications. Patients will not routinely receive pre-medications for their infusions since earlier patients have not experienced toxicities related to ATC infusions. If there is a history of reactions to ATC or other blood products, then Benadryl 50 mg IV or P.O. will be given as a premedication 30 minutes prior to infusion of cryopreserved cells. Tylenol, 1000 mg, may be given 30 minutes prior to cell infusion to as pre-medications to prevent fever, chills, and pain if there is a history of infusion side effects. Demerol 25-50 mg IV may be used to treat chills. These medications may be repeated every 4-6 hours as needed. Patients will be prehydrated with 250-500 ml of normal saline prior to BATs IV infusions. Hydrocortisone (50-100 mg IV) may be given to control severe reactions to infusions.

4.3.3 Ancillary therapy. Patients should receive full supportive care including transfusion of blood and blood products, antibiotics, and anti-emetics, when appropriate. The reason(s) for treatment, dosage and the dates of treatment should be recorded.

4.3.4 Steroids/Other therapy. With the exception of steroids for adrenal failure, severe hypersensitivity, septic shock, pulmonary toxicity or hormones administered for non-disease-related condition (e.g. insulin for diabetes), steroids will not be administered.

4.4 Treatment with Pembrolizumab: All patients will receive pembrolizumab infusions once every 3 weeks beginning after pheresis and 1 week prior to BATs therapy and ending a maximum of 7 months from start of treatment. Merck will supply the pembrolizumab for this study. Pembrolizumab will be administered at a dose of 200mg IV over 30 minutes, repeated every 3 weeks for a maximum of 9 doses. The interval between pembrolizumab doses should be a minimum of 21 days and a maximum of 42 days. BATs should be started at least 1 week after the first dose of pembrolizumab. Every effort should be made to target infusion timing to be as close to 30 minutes as possible. However, 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).

5.0 POTENTIAL TOXICITY, DOSE MODIFICATION, AND MANAGEMENT

5.1 Pheresis Procedure: Reported toxicity from pheresis is minimal. If patients have adequate IV access then a central catheter may not be necessary. This will be determined with a vein check performed by qualified staff. Patients have a small potential for infection from the placement of central catheters. Use of the anticoagulant citrate dextrose may cause symptoms of mild hypocalcemia, which are controlled with calcium carbonate (TUMS).

5.2 HER2Bi Armed Activated T Cells: Toxicity associated with BATs has been minimal. The reported symptoms have been reversible with cessation of therapy. Most patients treated with BATs experienced no side effects during infusions. Fever and chills were the most common reported reaction. Other rarely reported affects included mild gastrointestinal and neurologic symptoms. Toxicity associated with reinfusion of cryopreserved cells is generally attributable to the DMSO preservative. These include fever, nausea and fatigue. No one has died due to a cell-related toxicity.

5.3 Pembrolizumab (MK-3475) Dose Modification: There are no changes in dose for pembrolizumab. Treatment can be held for specific toxicities as per Table 3 below. Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per **Table 3** below. See Section 5.5 for supportive care guidelines, including use of corticosteroids.

Table 3. Dose Modification Guidelines for Drug-Related Adverse Events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	3-4	Permanently discontinue (see exception below)[a]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure	Resume pembrolizumab when patients are clinically and metabolically stable
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism		Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
Infusion Reaction	2[b]	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication
	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[c]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

[a] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

[b] 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 pre-medicated for the next scheduled dose; Refer to **Table 4** – Infusion Treatment Guidelines for further management details.

[c] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

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. The reason for interruption should be documented in the patient's study record.

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 registration 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 11.5.

5.4.2 Prohibited concomitant medications. Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- 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 registration 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 PI.

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 5.3 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. Thyroid function (TSH, free/total

T3 and free/total T4 will be checked at baseline and check TSH at dose # 3, 6 and 9 of pembrolizumab. 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. If symptoms are noted or if thyroid function is abnormal further evaluation and appropriate treatment is required.

- 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.

Stevens Johnson Syndrome, Toxic Epidermal Necrolysis, and immune myocarditis have been reported with pembrolizumab therapy. If any of these conditions occur please treat with corticosteroids and supportive care and discontinue therapy permanently.

5.5.2 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.

Table 4 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 4. Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 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 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.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. 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. **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: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: 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) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.6 Toxicity Grading: The NCI CTEP CTCAE version 4.0 Toxicity Tables and 0-4 Grading Scale will be used.

5.7 Retreatment Criteria: Patients removed from study due to toxicities other than cell-based toxicity may be re-entered upon complete resolution of toxicity, providing the toxicity appears to be unrelated to therapy. Retreatment criteria are the same criteria required for initial treatment. Patients re-entered into the study will continue with their therapy at the same point at which it was discontinued. The patient may also be retreated at the same dose of BATs if they have a prolonged clinical response and are deemed to be candidates for retreatment per the treating physician and approved by Dr. Deol and Dr. Lum.

5.8 Criteria for Removal from Protocol: Patients will be removed from protocol for the following:

- Grade 4 non-hematologic treatment related toxicity.

- Initiation of other therapy.
- Patient decision to discontinue therapy.
- When deemed in the best medical interest of the patient by the physician.
- Protocol violation: Unexplained delay in delivery of treatment.

6.0 DRUG FORMULATION

6.1 Pembrolizumab/MK-3475/Keytruda (Merck): 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 [44]. It is available in 100mg vials. This medication will be provided by Merck for this study.

Pembrolizumab (MK-3475/Keytruda) is a programmed death receptor-1 (PD-1)-blocking antibody indicated for the treatment of:

- patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor.
- patients with metastatic NSCLC whose tumors express PD-L1 as determined by an FDA-approved test and who have disease progression on or after platinum-containing chemotherapy.

6.1.1 Dosage and administration.

- Administer 200 mg as an intravenous infusion over 30 minutes every 3 weeks (maximum dose of 200 mg per infusion).
- Dilute prior to intravenous infusion.

6.1.2 Preparation and administration.

Reconstitution of KEYTRUDA for Injection (Lyophilized Powder):

- Add 2.3 mL of Sterile Water for Injection, USP by injecting the water along the walls of the vial and not directly on the lyophilized powder (resulting concentration 25 mg/mL).
- Slowly swirl the vial. Allow up to 5 minutes for the bubbles to clear. Do not shake the vial.

Preparation for Intravenous Infusion:

- Visually inspect the solution for particulate matter and discoloration prior to administration. The solution is clear to slightly opalescent, colorless to slightly yellow. Discard the vial if visible particles are observed.
- Dilute KEYTRUDA injection (solution) or reconstituted lyophilized powder prior to intravenous administration.
- Withdraw the required volume from the vial(s) of KEYTRUDA and transfer into an intravenous (IV) bag containing 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Mix diluted solution by gentle inversion. The final concentration of the diluted solution should be between 1 mg/mL to 10 mg/mL.
- Discard any unused portion left in the vial. Storage of Reconstituted and Diluted Solutions
- The product does not contain a preservative. Store the reconstituted and diluted solution from the KEYTRUDA 50 mg vial either:

- At room temperature for no more than 6 hours from the time of reconstitution. This includes room temperature storage of reconstituted vials, storage of the infusion solution in the IV bag, and the duration of infusion.
- Under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of reconstitution. If refrigerated, allow the diluted solution to come to room temperature prior to administration. Store the diluted solution from the KEYTRUDA 100 mg/4 mL vial either:
- At room temperature for no more than 6 hours from the time of dilution. This includes room temperature storage of the infusion solution in the IV bag, and the duration of infusion.
- Under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of dilution. If refrigerated, allow the diluted solution to come to room temperature prior to administration. Do not freeze.

Administration:

- Administer infusion solution intravenously over approximately 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter.
- Do not co-administer other drugs through the same infusion line.

6.1.3 Dosage forms and strengths.

- For injection: 50 mg lyophilized powder in a single-use vial for reconstitution
- Injection: 100 mg/4 mL (25 mg/mL) solution in a single-use vial (3)

6.1.4 Dose modifications. Withhold KEYTRUDA for any of the following:

- Grade 2 pneumonitis
- Grade 2 or 3 colitis
- Grade 3 or 4 endocrinopathies
- Grade 2 nephritis
- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than 3 and up to 5 times upper limit of normal (ULN) or total bilirubin greater than 1.5 and up to 3 times ULN
- Any other severe or Grade 3 treatment-related adverse reaction
- Resume KEYTRUDA in patients whose adverse reactions recover to Grade 0-1.
- Permanently discontinue KEYTRUDA for any of the following:
- Any life-threatening adverse reaction (excluding endocrinopathies controlled with hormone replacement therapy)
- Grade 3 or 4 pneumonitis or recurrent pneumonitis of Grade 2 severity
- Grade 3 or 4 nephritis [see Warnings and Precautions (5.2)]
- AST or ALT greater than 5 times ULN or total bilirubin greater than 3 times ULN for patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT
- increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week
- Grade 3 or 4 infusion-related reactions
- Inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks
- Persistent Grade 2 or 3 adverse reactions (excluding endocrinopathies controlled with hormone replacement therapy) that do not recover to Grade 0-1 within 12 weeks after last dose of KEYTRUDA

- Any severe or Grade 3 treatment-related adverse reaction that recurs

6.1.5 Contraindications. None.

6.2 OKT3: This is a murine IgG2a monoclonal antibody directed at human CD3 commercially available from OrthoBiotech, Raritan, NJ. It is purchased in vials containing 5 mg/5 ml of reconstituted bacteriostatic water. OKT3 is used to activate T cells for growth and for heteroconjugation with Herceptin® to produce anti-CD3 x anti-HER2 bispecific antibody.

6.3 Anti-HER2/neu Monoclonal Antibody-Herceptin (Trastuzumab, Genentech, Inc., CA): Herceptin is a humanized murine monoclonal antibody directed at HER2/neu, and is commercially available in multi-dose vials containing 440 mg of drug. The binding characteristics and its ability to mediate cytotoxicity towards MCF-7 and SK-BR-3 (breast cancer cell lines and ovarian cancer cell lines) have been well documented in our preclinical studies.

6.4 Anti-CD3 (OKT3) x anti-HER2/neu (Herceptin) Heteroconjugated Bispecific Monoclonal Antibody: See section 7.3 on the production of anti-CD3 x anti-HER2 per GMP conditions. IND #9985 was approved by the FDA that specifies the production of bispecific antibody, sterility testing, and the standard operation procedures for arming of activated T cells.

6.5 Labeling, Packaging, Storage and Return of Clinical Supplies:

6.5.1 Investigational product. 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 5**.

Table 5. Product Descriptions

Product Name & Potency	Dosage Form
Pembrolizumab 100 mg/ 4mL	Solution for Injection

6.5.2 Packaging and labeling information. Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

6.5.3 Clinical supplies disclosure. 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.

6.5.4 Storage and handling requirements. 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.

6.5.5 Returns and reconciliation. 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.

7.0 PREPARATION OF HER2Bi ARMED ACTIVATED T CELLS

7.1 Facility: Qualified personnel who are familiar with procedures which minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of immunotherapeutic agents in a self-contained protective environment. Cells will be generated in the UVA Cancer Immunotherapy Laboratory under FDA #BB-IND 9985 with standard operating procedures and master batch records (SOPs and MBRs) for growing, splitting, harvesting, arming of ATC, cryopreservation, and infusion of BATs.

The fresh pheresis product from KCI will be shipped by overnight courier service to UVA GMP laboratory for processing.

Shipping Address for Pheresis Product:
University of Virginia
GMP Facility
Aurbach Medical Research Building
Suite 2339D
450 Ray C. Hunt Dr.
Charlottesville, VA 22903

Please notify Archana Thakur by phone or email when samples are sent:
Tel # 434-243-1397
E-mail: AT2FX@virginia.edu

7.2 Activation, Culture, and Freezing of BATs. Lymphocytes are obtained by pheresis and cultured in RPMI 1640 media (Biowhittaker or Gibco), containing low dose IL-2, OKT3, and human serum. Cells will be cultured for a maximum of 14 days in media to which no additional OKT3 will be added when the cultures are expanded.

7.3 Preparation of Anti-CD3 x Anti-HER2 Bispecific Antibody: The specific details for the production, purification, and quality control testing are part IND #9985 (already active).

7.4 Arming of ATC with HER2Bi: The harvested ATC will be counted and a dose of 50 ng of HER2Bi per million ATC will be added to the solution and incubated for 1 hour at 4° C. The BATs will be washed, counted, and resuspended in the final solution that will be cryopreserved in aliquots specific for each infusion.

7.5 Testing of HER2Bi Armed-ATC: Aliquots of 24 hour cell culture supernatants will be evaluated for IFN-γ and TNF-α secretion by ELISA in the presence or absence of a known HER2 positive breast and ovarian cancer lines.

7.6 Cytotoxicity Assay: Cytotoxicity is measured in a 20 hour 51^{Cr} -release assay. Tumor cells are plated in a flat-bottomed microtiter plate and incubated at 37°C. The targets are washed and labeled the next day with 51^{Cr} at 37°C. The wells containing tumor cells will be washed and armed or unarmed ATC will be plated and incubated for 20 hours at 37°C. The next day, the

supernatants harvested from the microtiter wells will counted and the percent specific lysis will be calculated [22].

7.7 Quality Assurance of ATC Cell Product: Lists of suppliers of monoclonal antibodies, heteroconjugation reagents, and culture reagents will be maintained as well as lot numbers used and supplier provided documentation of sterility and documentation that all reagents are free of endotoxin and mycoplasma. The final bag of cells to be administered will be tested for anaerobic and aerobic bacteria, fungus, and mycoplasma, and determined to be endotoxin free. Records of all quality control measures will be maintained by Dr. Lum's laboratory.

7.8 Transfer of HER2 BATs to KCI: Cryopreserved ATC units will be transferred to the KCI Stem Cell Lab. (Please refer to the QCP # 14-v2 for the full description of the transfer procedure). The Immunotherapy facility at UVA will notify the KCI Stem Cell Lab that HER2 BATs will be shipped. After matching the stored product to the KCI patient information and determining the date of shipment, the vapor shipper is prepared 24 hours before shipment by filling its cavity with liquid nitrogen and allowing the liquid nitrogen to soak into the shipper's absorbent overnight. The Activated T Cell Unit Product Packing Log (Log-26) will be used to document the dry shipper's ID number, the time the charging was initiated, and the internal temperature of the dry shipper. On the day of shipment via overnight courier service, the requested ATC unit(s) are located and removed from long term storage. A supplemental ATC Unit label is attached with a plastic tie tag stating "Properly Identify Intended Recipient and Product", "For Use by Intended Recipient Only", "Leukoreduction Filters Should Not Be Used", and "Do Not Irradiate". The released ATC unit(s) are removed from storage and placed in dry shipper on the Activated T Cell Unit Product Packing Log (Log-26). The temperature probe is extended into the center holding canister. Both the shipper and shipping container are labeled with separate labels that include the Infusion center contact person and telephone number as well as ATC recipient Unique Identifier. The contents Label has the following statements: "Non – Hazardous", "Medical Specimen, Handle with Care", "Do not X – ray", "Do not Open", "Extremely Low Temperature (-150ºC or Colder)" ,"Perishable", "Transfer to permanent storage as soon as possible". The dry shipper's weight is recorded on Log-26 Activated T Cell Unit Product Packing Log. The UVA Immunotherapy facility will arrange the shipment through the FedEx courier service. The KCI Stem Cell Lab will verify the dry shipper maintained the temperature of < -150°C by completing Log-27 Activated T Cell Unit Shipment, Receipt and Inspection form and faxing it to the UVA Immunotherapy facility for documentation. Also at the time of return of the dry shipper, temperature data from the data logger will be downloaded and analyzed for archival storage according to OP-07 *ShipsLOG Data Logger for Liquid Nitrogen Vapor Dry Shipments* or OP-08 *MVE Data Logger for Liquid Nitrogen Dry Shipments.*

8.0 REGISTRATION AND REQUIRED DATA

8.1 Pre-Study: All patients registered will be evaluated and staged prior to initiation of therapy. Eligibility criteria will be assessed as stated in Section 3.0. Baseline studies will be completed within 28 days prior to registration. The principal investigators may allow exceptions for not retesting due to delays in obtaining approvals (all exceptions will be documented).

8.2 Informed Consent: The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

8.2.1 General informed consent. Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion. A copy of the signed and dated consent form should be given to the subject before participation in the trial. The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature. Specifics about a trial and the trial population will be added to the consent form template at the protocol level. The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor requirements.

Table 6. Tests and Observations	Pre –Registration[1]	Pheresis	Pembro	Week 1		Week 2		Pembro	Week 3		Week 4		1-2 wks Post #8	2 mos after last BATSinfusion	4 mos after last BATSinfusion	6 mos after last BATSinfusion	Follow up
				BAT #1[2]	BAT #2	BAT #3	BAT #4		BAT #5	BAT #6	BAT #7	BAT #8					
History and Physical[3]	X		X			X		X					X	X	X	X	
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Performance Status[3]	X		X			X		X					X	X	X	X	
CBC diff platelets	X		X					X					X	X	X	X	
Metabolic Panel[4] w/ Mg + Phosphorus	X		X					X					X	X	X	X	
Thyroid function[4]	X		X					X					X	X	X	X	
PSA	X		X					X					X	X	X	X	
HBsAg B & HCV RNA and HIV, PT/PTT, testosterone	X																
Urinalysis & CrCl[4]	X																
Immune Studies[5]		X		X[9]				X					X	X	X	X	
EKG	X																
MUGA or ECHO	X												X				
CT or PET/CT and bone scan[6]	X		X										X	X		X	
Tumor Measurements[7]	X		X										X	X		X	
Pheresis[8]		X															
PD-1 inhibitor/Pembro			X[10]					X					X	X	X	X	
BATs Infusion				X	X	X	X		X	X	X	X					
Safety Assessment[12]				X	X	X	X		X	X	X	X					
Tumor Biopsy		X[11]											X				
Follow Up [13]																	X

1. All tests performed 28 days prior to registration
2. Patient should receive BATs #1-8 infusions twice per week for 4 weeks (+/- 2 days but minimum 42 hours between doses. Maximum duration 8 weeks
3. History, Physical, and Performance Status: To be performed by one of the protocol investigators or their designee
4. Metabolic tests are required prestudy and for every cycle of pembrolizumab and will include electrolytes, BUN, Creatinine , AST, ALT, total Bilirubin, calcium, albumin, alkaline phosphatase and glucose. TSH, free/total T3 and T4 will be conducted at baseline. TSH needed at doses 3, 6 and 9 of pembrolizumab. (Free/total T3 and T4 needed only if TSH is abnormal).
5. Immune evaluation assessment (± 1 week)
6. CT, MRI or PET/CT and bone scan: To be done every 12 weeks from day 1 of pembrolizumab upto 1 year or until disease progression. (+/-1 week window)
7. Tumor evaluation will be done with the discretion of the PI according to the table above
8. T-cells will be collected by pheresis and shipped to Dr. Lum's UVA GMP facility where the cells will be cultured & armed
9. Prior to BATs infusion (-3 days to day of infusion)
10. 1 week prior to BATs and every 3 weeks after. A minimum of 21 days and maximum of 42 days interval between pembrolizumab doses is permitted.
11. Before starting Pembrolizumab (optional archival and fresh tissue acceptable, as feasible)

12. Vital signs prior to pembrolizumab dose and vital signs on day of BATs will be done pre-infusion , , immediately post infusion and 1, 2 and 4 hours after infusion.
13. Patients will be followed every 3 months for the first two years for progression and OS and every 6 months after that for progression and survival. Based on clinical situation determined by treating physician, pt might have scans, blood tests and optional immune testing

.

9.0 CRITERIA FOR EVALUATION

9.1 Tumor Measurements: All tumor measurements will be measured in centimeters prior to initiation of therapy. These measurements should be done according to RECIST 1.1 criteria [45]. The Prostate Cancer Clinical Trials Working Group (PCWG2) criteria will also be used to determine a response in non-measurable disease [42]. PCWG2 recommends a two-objective paradigm in metastatic CRPC: (1) controlling, relieving, or eliminating disease manifestations that are present when treatment is initiated and (2) preventing or delaying disease manifestations expected to occur. PSA decline and changes in imaging will also be reported. An estimate of overall objective and subjective response will be made and recorded at the end of treatment, 2 months after the first infusion. Since it is expected that immune activity would lead to tumor flare, the restaging CTs may be repeated 3 months later based on immune-related response criteria (irRC), and the designation of CR, PR, SD, or PD will be made at that time [46].

9.2 Response Definitions: Response will be defined by using the irRC criteria, or the RECIST 1.1 criteria with the allowance of an initial mild PD due to the nature of immunologic therapies as above.

9.2.1 Complete response (CR). Disappearance of all clinical evidence of active tumor for a minimum of eight (8) weeks. The patient must be free of all symptoms. All measurable, evaluable and non-evaluable lesions and sites must be assessed.

9.2.2 Partial response (PR). At least a 30% decrease in the sum of the target lesions compared to the baseline sum. No lesion may increase in size and no new lesions may appear.

9.2.3 Progressive disease (PD). For measurable disease, an increase of 20% in the sum of the target lesions or unequivocal progression of non-target lesions.

9.2.4 Time to progression in patients with bone metastases. Majority of the patients with prostate cancer have bone metastases which are not considered measurable sites of disease. Progression in patients with bone metastases will be defined as either the appearance of a minimum of 2 new lesions on bone scan which are related to metastatic disease per the judgement of the treating physician, or the occurrence of a new skeletal related event. Skeletal-related event is defined as occurrence of new pathologic bone fractures (vertebral or nonvertebral), spinal cord compression, requirement of surgery or radiation therapy to bone metastases (including the use of radioisotopes), or a requirement to change antineoplastic therapy to treat bone pain or other related symptoms.

9.2.5 Stable disease (SD). Neither sufficient shrinkage to qualify for PR nor sufficient increase/change to qualify for PD.

9.2.6 Response evaluable patients. All patients registered on the protocol and completing a minimum of one dose of pembrolizumab and one dose of BATs therapy followed by clinical, and radiologic or PSA assessment of disease status.

9.2.7 Toxicity evaluable patients. All patients registered on the protocol and starting therapy with protocol medication will be considered toxicity evaluable.

9.2.8 Follow up /Overall survival. Overall survival will be measured from date of registration to death or last follow up. After treatment is discontinued for any reason patients will be followed every 3 months for progression and survival.

10.0 CRITERIA FOR REMOVAL OF PATIENTS FROM STUDY

10.1 Disease Progression: Patients with progressive disease will be removed from study .

10.2 Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, the patient shall be withdrawn from treatment. In this event:

10.2.1 Document reasons for withdrawal and record whether this action was patient or physician directed.

10.2.2 The patient will remain technically "on-study" although treatment has been discontinued. The patients will be followed for PFS, OS, and immune responses.

10.3 Unexpected or Life-Threatening Toxicity:

10.3.1 Direct questions regarding drug therapy to the Principal Investigator.

10.3.2 Reporting requirements and procedures depend upon: (1) whether agents are suspected of causing toxicity, (2) whether possibility of such toxicity was reported in the consent form, or manufacturer's literature (Published Toxicity), (3) the severity or grade of the toxicity.

10.3.2.1 Expected toxicities. Grade 4 myelosuppression: Report only as part of regular data submission. All other toxicities, Grades 4 and 5: Written Report to Principal and Co-Investigators, within 10 working days. All other toxicities, Grades 1 to 3: Report only as part of regular data submission.

10.3.2.2 Unexpected toxicities. Unexpected toxicities are toxicities that are not listed in the toxicity management section of the protocol, the consent form, or the manufacturer's package insert. All unexpected toxicities will be reported to Principal and Co-Investigators, within 15 working days.

11.0 REPORTING ADVERSE REACTIONS

11.1 Investigators are required to notify the **FDA and IRB** of all serious and unexpected adverse drug reactions. Copies of all safety reports will be sent to both Dr. Deol and Dr. Lum (the IND Sponsor at UVA).

11.2 All reactions in a "reportable" category must be reported unless it is documented in the medical record chart that treatment is definitely **not** responsible for the toxicity.

11.3 Serious and unexpected adverse reactions will be reported to the following sources within the stated time frame.

11.3.1 FDA. Written IND safety report within 7 calendar days. The SAE will be reported by telephone within 3 working days. SAE reports will be included in annual reports.

11.3.2 All participating investigators. Written IND safety report within 7 calendar days.

11.3.3 IRB. Written report within 10 working days.

11.4 Procedure for calling the FDA is as follows. Dr. Lum will be responsible for notifying the FDA:

11.4.1 The research nurses or PI will call for ADRs.

11.4.2 The PI or his designee will call for regulatory/protocol issues.

11.4.3 The medical reviewer at the FDA TBN, M.D.:

ATTN: To be named

U.S. Food and Drug Administration
Center for Biologic Evaluation and Research
10903 New Hampshire Avenue
WO71, G112
Silver Spring, MD 20993-0002

11.4.4 Document as completely as possible, and send copies to:
Patient's chart
FDA correspondence binder
Protocol binder
Lawrence G. Lum, M.D., D.Sc.

- Email: lgl4f@virginia.edu
- Phone: 434-243-1375
- Cell phone: 313-410-0797

11.5 Reporting Guidelines for Pembrolizumab SAE: Pembrolizumab will be provided by Merck.

11.5.1 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.

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 11.5.4.

The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

11.5.2 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)

11.5.3 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 that results from intercourse with a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur in partners of subjects from the time of treatment allocation/randomization through 120 days following cessation of Merck'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)

11.5.4 Immediate reporting of adverse events to the Sponsor and to Merck

A serious adverse event is any adverse event occurring at any dose or during any use of Merck's product 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 that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety using the Medwatch form 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, 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.

11.5.5 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:

- an overdose of Merck product, that is not associated with clinical symptoms or abnormal laboratory results.
- 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.

11.5.6 Protocol-specific exceptions to serious adverse event reporting. Efficacy endpoints as outlined in this section will not be reported to Merck. 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.

11.5.7 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 4.0. 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.

12.0 CORRELATIVE TESTING

12.1 Monitor Immune Responses: Estimate the magnitude of change in anti-PC immune functions and markers in the serum after BATs infusions in mCRPC.

12.1.1 Sample delivery. Immune function testing samples will be sent to Dr. Lum's laboratory at UVA. Please call the KCI PK Core lab for immune function specimen pickup. Do not leave the blood overnight. A total of approximately 60ml of blood will be drawn at each of the designated points - one 10 ml red top tube and five 10 ml green or lavender top tubes containing anti-coagulant.

Shipping Address for Immune Evaluations:
University of Virginia
West Complex MSB 7191
1300 Jefferson Park Ave
Charlottesville, VA 22903

Please notify Archana Thakur by phone or email when samples are sent:
Tel # 434-243-1397
E-mail: AT2FX@virginia.edu

12.1.2 Background. In the phase I clinical trial in MBC patients, infusion of HER2 BATs induced endogenous CTLs against breast cancer cell lines, Th_1 cytokines in the serum. After 4 infusions of HER2 BATs, MBC patients develop a Th_1-type skewing of the serum cytokine pattern, consisting of significant levels of IL-2, IL-7, IL-12, IFN-γ, TNF-α, and GM-CSF. Although some of these responses may directly result from BATs functions *in vivo*, other responses, such as IL-12 production, cannot be attributed to T cells but rather reflect

secretion of IL-12 by endogenous monocytes that are responding to stimulation. Based on these data, BATS may have the short-term direct benefit of tumor lysis and the long-term benefits of longer lasting endogenous anti-tumor activity [3]. The studies show that IgG2a-depleted PBMC exhibited high levels of breast cancer specific cytotoxicity and IFN-γ EliSpots after immunotherapy; evidence for the development of endogenous anti-tumor activity [47]. Moreover, we predict that evidence of endogenous immune activity will correlate with improvements in clinical outcomes.

12.1.3 Strategy. We will sequentially test the patient's lymphocytes for phenotype, cytotoxicity directed PC cell lines, and antibodies directed at PC cell lines and selected cytokines in the serum at designated time points in the protocol. Specific cytotoxic responses will be measured with IFN-γ ELISpot and ^{51}Cr release assays. Serum will be tested for the Th_1/Th_2 serum cytokine profiles and serum antibodies directed at PC cells using a cell based ELISA as described [48,49]. Baseline serum and cells will be aliquoted from the pheresis. Blood draws (4 green top tubes containing approximately 40 ml of anti-coagulated blood and 1 red top tube containing 8 ml of serum) will be collected pre therapy, **pre-BATs** infusion, **after the 4th** infusion, approximately **1-2 weeks**, **2 months** (90 days ± 7 days), **4 months** (120 days ± 7 days), **6 months** (180 days ± 7 days) and **optionally every 6 months** thereafter (180 days ± 7 days) after the last infusion**.** Optional immune evaluations (IEs) will be done if there continues to be immune response at 6 month intervals until the responses have disappeared. Patient immune function data will be compared to their baseline to evaluate changes induced by BATs. IFN-γ ELISpots will be considered positive if they change ≥2 fold above a baseline with ≥30 ELISpots/10^6 cells plated.

12.2. Monitor Changes in Tumor Infiltrating T cells, PD-1 Expression, and Th_1/Th_2 Ratio:
These will be considered exploratory biomarkers to assess the rationale for future combinations with immune checkpoint inhibitors. Tumor infiltrating cells (TILs) have shown to be surrogates of improved outcome in metastatic solid tumor malignancies. In advanced breast cancer the induction of tumor infiltrating lymphocytes was noted to correlate closely with improved OS [50]. The TILs were analyzed and quantified in 10% increments in metastatic breast cancer trials. Tumor tissue biopsies will be obtained pre and post therapy and evaluated for TILs. In addition PD-1 expression on the TILs will be assessed by immunohistochemistry (IHC).

12.2.1 Biopsy collection. Every attempt should be made to obtain archival tissue for testing on this study. Tumor Biopsies pre and post therapy are optional on this study and are highly encouraged. If patient consents to biopsy, these will be obtained prior to beginning treatment with pembrolizumab or BATs. Post therapy biopsy will be obtained 1-2 weeks after the last BATs infusion.

Shipping Address for tissue samples:
University of Virginia
West Complex MSB 7191
1300 Jefferson Park Ave
Charlottesville, VA 22903

Please notify Archana Thakur by phone or email when samples are sent:
Tel # 434-243-1397
E-mail: AT2FX@virginia.edu

12.2.2 Rationale. In this aim we will determine the spectrum of biomarkers to identify the T cells subpopulations, activation status of T cells, immune suppressive cells and immune inhibitory molecules (PD-1/PD-L1) in the tumor samples. The analysis of host–tumor interactions and immune reaction within the tumor may provide information. Fukunaga et al [51] showed that the presence of tumor infiltrating CD4+ and CD8+ lymphocytes provide a good indicator of patient's outcome after surgical treatment. Another study analyzing 599 early-stage (stage I and II) colorectal cancers showed that tumors with low cytotoxic (CD8) T cells had poor prognosis, while patients with Th_1 polarized, highly cytotoxic T cells in the center and the invasive margin of the tumor experienced a very low level of recurrence [52]. These studies suggest that the immune landscape of the tumor may provide useful prognostic information.

12.2.3 Strategy. The paraffin blocks will be processed by Dr. Lum's laboratory. In the past year, we developed immunohistochemical staining for cytoplasmic or extracellular IFN-γ and IL-10 as well as CD3 (Thakur, unpublished data) in paraffin sections (**Fig 17**). Original biopsies will be stained for CD3+, CD4+, CD8+, CD127+, cytoplasmic IFN-γ (Th_1 representative cytokine) and IL-10 (Th_2 representative cytokine), T_{regs}, M2-macrophages, MDSC, PD-1/ PD-L1 in the tumor sections. We will calculate the ratio of CD4/CD8, and the ratio of Th_1/Th_2 to evaluate potential clinical correlates.

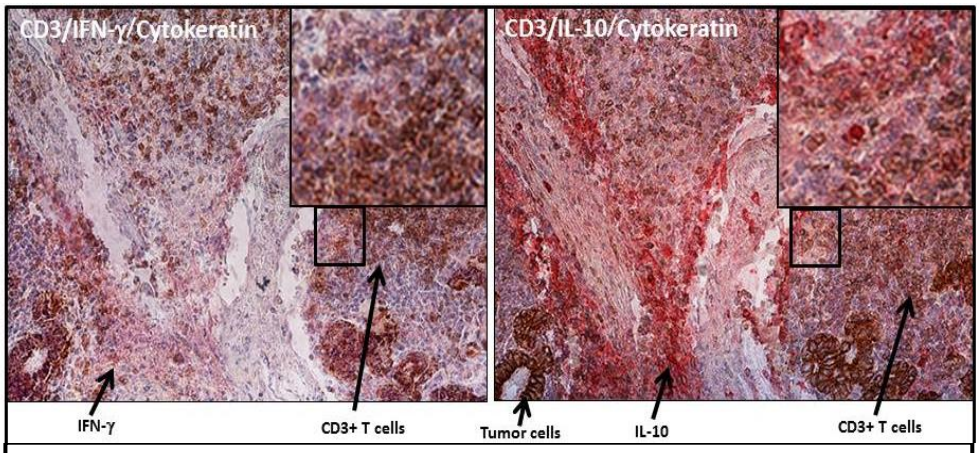


Fig 17. Over lay staining for CD3, IFN-γ, IL-10 in breast cancer. Insets show zoomed sections mapped in blue rectangles. Left panel shows cytokeratin stained breast cancer cells, IFN-γ secreting brown stained CD3+ T cells (pink surround cells). The right panel shows cytokeratin positive breast cancer with T cell secreting IL-10 (inset). Stromal or tumor producing IL-10 (indicated by arrow)

12.2.4 Immunohistochemical staining. Tissue microarray sections will be incubated with mAbs against CD3, CD4, CD8, IFN-γ, IL-10, PD-1, and PD-L1. Staining will be carried out on paraffin embedded resected tumor tissue sections by staining for Th_1 using anti-CD3/anti-IFN-γ anti-CD8/Granzyme B and for IL-10 expression, slide will be stained for anti-CD19/ anti-IL-10 and anti-CD68/anti-IL-10 antibody cocktails followed by incubation with enzyme-conjugated polymer backbone coupled to secondary antibodies, and color development using 3,3′-diaminobenzidine (DAB) as substrate. Hematoxylin-counterstained sections will used for the analysis. For double staining, HRP Polymer anti-Mouse secondary antibody and AP Polymer anti-Rabbit secondary antibody will be used (GBI Labs Polink DS-MR-Hu A1 Kit). Color will be developed using DAB Chromogen and GBI Permanent Red Chromogen followed by counterstain with hematoxylin. Isotype-matched mouse monoclonal antibodies will be used as negative control. Slides will be analyzed using a Panoramic Digital Scanner and Viewer (Caliper Life Sciences). The

density will be recorded as the number of positive cells per unit tissue surface area.

13.0 STATISTICAL CONSIDERATIONS

13.1 Clinical Trial: This is a phase II study in mCRPC patients with a single arm two-stage design. The primary objective is to study the clinical efficacy. The primary endpoint is the proportion of patients achieving clinical progression-free interval of 6 months from study registration. The clinical progression is defined by PCWG II criteria and will be monitored by clinical evaluation, monthly PSA monitoring and radiologic assessment at 3 and 6 months, and every 6-month interval thereafter.

Sample Size/Power calculation: The study will use Simon's two-stage design to test the null hypothesis of 20% or less versus the alternative hypothesis of more than 20% patients achieving progression free time of $\geq$ 6 months. Our expected percentage is 40%. With 5% Type I error and 80% power, the Simon's two-stage minmax design requires a total of 33 patients and has a probability of early termination of 0.716 if the treatment is not promising. The PASS12 was used for sample size calculation [53]. At the first stage, 18 evaluable patients will be enrolled. The trial will be terminated if 4 or fewer patients are progression free at 6 months. If the trial goes on to the second stage, 15 more patients will be enrolled. It the total number of progression free at 6 months is 11 or higher, the null hypothesis will be rejected. The treatment will be considered promising with more than 20% clinical progression free at 6 months. The secondary objective is to estimate the OS with KM method.

The accrual rate at KCI is about 2 per month. The 18 patients in stage I will require 9 months to accrue and 6 months to follow up for primary endpoint. If the trial proceeds to stage II, 8 months accrual for 15 additional patients and 6 months follow up will result in study duration of 29 months.

13.2 Immune Function Testing: For those aforementioned quantitative immune response variables (Section 12.1), we will produce summary statistics (including means, medians, and standard deviations) pre- and post-BATs treatment. Subsequent analyses will compare the immune response variables (after a suitable transformation, if necessary) pre- and post-treatment using a paired t-test (or Wilcoxon sign ranked test if the data are not approximately normally distributed after log transformation). To explore whether immune responses associate with clinical responses, the association between the baseline of each biomarker and clinical endpoints (such as response, or OS) will be analyzed using logistic regression for binary endpoints and Cox regression for time to event endpoints. Analyses will adjust for other covariates such as age and tumor stage.

Power calculation: For this correlative endpoint, we have at least 18 patients (or 33 patients if the trial proceeds to stage II). For one-sample Student t test a sample size of 18 pairs achieves 80% power to detect a median to large effect of 0.69 using a two-sided paired t-test with significance level of 0.05. All the raw p values will be reported together with False Discovery Rate (FDR) adjusted p values for multiple testing [54].

13.3 IHC Testing: For the qualitative IHC measurements, we will produce summary statistics (including frequency table and percentage of positivity). The McNemar test will be used for testing protein positivity concordance pre- and post-BATs treatment. To explore whether protein positivity associate with clinical responses, the association between the baseline of each biomarker and clinical endpoints (such as response, or OS) will be analyzed using logistic regression for binary endpoints and Cox regression for time to event endpoints. Analyses will adjust for other covariates such as age and tumor stage.

Power calculation: For this objective we estimate that about 23 patients (~70% have quality tissue materials for IHC) will have baseline IHC measurements. About 16 patients will have paired pre- and post- treatment IHC measurements. The sample size will decrease if the trial stops at stage I. In that case, only descriptive analysis will be provided for biomarkers. For power calculation, the primary analysis is the Fisher's exact test between the baseline marker status (positive vs. negative) and clinical response. Assuming 40% response and 10% protein positives in non-responder, a sample size of 23 achieves 80% power to detect a large effect of 60% difference with a two sided test at significance level of 0.05. All the raw p values will be reported together with False Discovery Rate (FDR) adjusted p values for multiple testing [54].

13.4 Statistical Monitoring Plan for Toxicity: Data and Safety Monitoring Committee (DSMC) will examine the overall safety data monthly. Frequency and severity of all toxicities will be tabulated and summarized for review by PI and the DSMC. Interim safety analysis will be performed after first 5 and at the completion of first stage of the Simon's two-stage design (18 subjects) on patients who received at least one cycle of treatment.

Interim safety analyses will be focused on two criteria separately: definitely treatment-related (pembrolizumab or BATs) deaths; and grade 4 treatment-related AEs that persisted ≥72 hours despite adequate treatment and management. Two-sided 90% exact binomial confidence intervals (CIs) will be calculated.

Definitely treatment-related deaths: We assume that rare fatal events should not exceed 1%. If the lower CI (LCI) exceeds 1%, the study will be suspended. This corresponds to ≥ 1+ (1 or more) out of 5 and ≥ 2+ out of 18 subjects in the interim safety analyses.

Definitely treatment-related grade 4 SAEs: We assume that rate should not exceed 10%. If the LCI exceeds 10%, the accrual will be suspended. This corresponds to ≥ 3+ (3 or more) out of 5 and ≥ 5+ out of 18 subjects in the interim safety analyses.

If the LCI exceeds the pre-defined levels, study accrual will be halted and the study DSMC will meet to assess the safety of the drug regimen. The committee could decide to reconsider the treatment and/or modify the protocol before proceeding or to stop the study permanently.

14.0 CLINICAL DATA SAFETY MONITORING

14.1 Recruitment: To address concerns related to decreased minority recruitment in local and national protocols, we will take specific steps to enhance the awareness in the Detroit Metro region and the State of Michigan to recruit minority groups for this study. The location of KCI provides a unique opportunity to recruit African Americans to the protocol. Our previous accrual records also indicate that we have been successful in recruitment to this patient population with a track record of about 40% African American patients enrolled on prostate cancer protocols.

14.2 Informed Consent: Patients will be enrolled after signed written informed consent is obtained following a detailed review of the study design, schedule and side effects and discussion of all therapeutic alternatives, with the investigator/s in a consultation visit of approximately 1 hour with a follow up and reinforcement of the risks and benefits with the research nurse coordinator. The consent forms are signed in the presence of one of the investigators and/or the Nurse Coordinator. The patients are given the consent forms after the consult and asked to write down any questions after reading the consent forms and to bring the questions to the signing meeting.

14.3. Data Safety and Monitoring Plan: Patient safety will be monitored by the PI and protocol data manager(s) on a monthly basis. The safety of protocol participants will be reviewed and adherence to stopping rules will be monitored. Adherence to the protocol, i.e., protocol violations, data completeness and integrity will also be reviewed. Overall assessment of accrual, toxicities and responses will be done at this time to determine whether significant benefits or risks are occurring that would warrant study closure. A monthly summary will be provided to the Karmanos Cancer Institute Data and Safety Monitoring Committee and will be reviewed quarterly. One month prior to anniversary date of the IRB original approval, a yearly summary report of trial activities will be made to all participating co-investigators and the Karmanos Cancer Institute Protocol Review and Monitoring Committee. This report will include the number of patients, the number of patients treated, a summary of all adverse events reported to date, a specific list of serious adverse events requiring immediate reporting and any significant developments that may affect the safety of the participants or ethics of the study.

14.3.1 Meetings: Scheduled meetings will be held as necessary and at least monthly, on the activity of the protocol. These meetings will include the protocol investigators, data managers, and key personnel involved with the conduct of the protocol.

14.3.2 Evaluations: During these meetings the investigators will discuss matters related to:

- Safety of protocol participants (Adverse Event reporting)
- Validity and integrity of the data
- Enrollment rate relative to expectation of target accrual, characteristics of the participants
- Retention of participants, adherence to the protocol (potential or real protocol violations)
- Data completeness on case report forms and complete source documentation

14.3.3 Completed data and safety monitoring reports: Reports of these regular investigator meetings will be kept on file in the office of the Clinical Trials Core. The data manager assigned to the clinical trial will be responsible for completing the report form. The completed reports will be reviewed and signed off by the Principal Investigator (PI) or by one of the Co-investigators in the absence of the PI. The signed off forms will then be forwarded to the Quality Manager, Clinical Trials Office for review of completeness and processing with the Karmanos Cancer Institute Data and Safety Monitoring Committee.

14.3.4 Data Safety Monitoring Committee: The Barbara Ann Karmanos Cancer Institute, Data and Safety Monitoring Committee will meet on a monthly basis to review the Serious Adverse Event forms and Data and Safety study specific reports that have been filed.

14.3.5 Retention of records: All documentation of adverse events, records of study drug receipt and dispensation, and all IRB correspondence will be retained for a minimum of 2 years after the investigation is completed.

14.3.6 Protocol activation: This protocol will not be activated until the Karmanos Cancer Institute Protocol Review Committee, the Wayne State University IRB, and the US Food and Drug Administration have approved the initiation of this protocol.

An internal Quality Control Coordinator (QCC) will be monitoring the clinical protocol being conducted under FDA-approved IND studies initiated at the WSU/Karmanos Cancer

Institute. A CTO study monitor will review all required study documents against source documents for the study as well the SOPs for the manufacturing of EGFRBi and activated T cells. A Study Monitor will report to the CTO and the Data Safety Monitoring Committee of the KCI. The Study Monitor must be familiar with the protocol, the investigational product, written informed consent, Standard Operating Procedures (SOPs), Good Clinical Practices (GCPs), and Applicable Regulatory Requirements. Monitoring will be performed annually.

15.0 REFERENCES

1. Nishio Y, Yamada Y, Kokubo H, et al. Prognostic significance of immunohistochemical expression of the HER-2/neu oncoprotein in bone metastatic prostate cancer. *Urology*. 2006;68(1):110-115.
2. Davol PA, Gall JM, Grabert RC, et al. Infusions of T cells armed with anti-CD3 x anti-HER2/neu bispecific antibody modulate in vivo patient immune responses in phase I clinical trials for breast and hormone refractory prostate cancers. *Blood*. 2004;104(11):379a.
3. Lum LG, Thakur A, Zaid Al-Kadhimi, et al. Targeted T cell Therapy in Stage IV Breast Cancer: A Phase I Clinical Trial. Clin Cancer Res. 2015 May 15; 21(10): 2305–2314.
4. Vaishampayan U, Thakur A, Rathore R, et al. Phase I Study of Anti-CD3 x Anti-HER2 Bispecific Antibody in Metastatic Castrate Resistant Prostate Cancer Patients. P*rostate Cancer*. 2015;2015:285193
5. Hillerdal V, Essand M. Chimeric antigen receptor-engineered T cells for the treatment of metastatic prostate cancer. *BioDrugs*. 2015;29(2):75-89.
6. Yano H, Thakur A, Tomaszewski EN, Choi M, Deol A, Lum LG. Ipilimumab augments antitumor activity of bispecific antibody-armed T cells. *Journal of translational medicine*. 2014;12(1):191.
7. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer. *New England Journal of Medicine*. 2010;363(5):411-422.
8. Ricciardelli C, Jackson MW, Choong CS, et al. Elevated levels of HER-2/neu and androgen receptor in clinically localized prostate cancer identifies metastatic potential. *Prostate*. 2008;68(8):830-838.
9. Okegawa T, Kinjo M, Nutahara K, Higashihara E. Pretreatment serum level of HER2/neu as a prognostic factor in metastatic prostate cancer patients about to undergo endocrine therapy. *IntJUrol*. 2006;13(9):1197-1201.
10. Signoretti S, Montironi R, Manola J, et al. Her-2-neu expression and progression toward androgen independence in human prostate cancer. *JNatlCancer Inst*. 2000;92(23):1918-1925.
11. Osman IA, Scher H, Drobnjak M, Fazzari M, Cordon-Cardo C. HER-2/neu membrane overexpression in prostate cancer. *ProcAmAssocCancer Res*. 2000;41:4572a.
12. Craft N, Shostak Y, Carey M, Sawyers CL. A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. *NatMed*. 1999;5(3):280-285.
13. Lara PN, Jr., Chee KG, Longmate J, et al. Trastuzumab plus docetaxel in HER-2/neu-positive prostate carcinoma: final results from the California Cancer Consortium Screening and Phase II Trial. *Cancer*. 2004;100(10):2125-2131.
14. Morris MJ, Reuter VE, Kelly WK, et al. HER-2 profiling and targeting in prostate carcinoma. *Cancer*. 2002;94(4):980-986.
15. James ND, Atherton PJ, Jones AJ, Howie AJ, Tchekmedyian S, Curnow RT. A phase II study of the bispecific antibody MDX-H210 (anti-HER2 x CD 64) with GM-CSF in HER2+ advanced prostrate cancer. *British Journal of Cancer*. 2001;85(2):152-156.
16. Lum LG, Sen M. Activated T-cell and bispecific antibody immunotherapy for high-risk breast cancer. Bench to bedside. *Acta Haematol*. 2001;105(3):130-136.
17. Reusch U, Sundaram M, Davol PA, et al. Anti-CD3 x anti-epidermal growth factor receptor (EGFR) bispecific antibody redirects T-cell cytolytic activity to EGFR-positive cancers in vitro and in an animal model. *Clin Cancer Res*. 2006;12(1):183-190.
18. Davol PA, Smith JA, Kouttab N, Elfenbein GJ, Lum LG. Anti-CD3 x anti-HER2 bispecific antibody effectively redirects armed T cells to inhibit tumor development and growth in hormone-refractory prostate cancer-bearing severe combined immunodeficient beige

mice. *Clin Prostate Cancer*. 2004;3(2):112-121.
19. Lum HE, Miller M, Davol PA, Grabert RC, Davis JB, Lum LG. Preclinical studies comparing different bispecific antibodies for redirecting T cell cytotoxicity to extracellular antigens on prostate carcinomas. *Anticancer Res*. 2005;25(1A):43-52.
20. Chan JK, Hamilton CA, Cheung MK, et al. Enhanced killing of primary ovarian cancer by retargeting autologous cytokine-induced killer cells with bispecific antibodies: a preclinical study. *ClinCancer Res*. 2006;12(6):1859-1867.
21. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med*. 2002;8(8):793-800.
22. Sen M, Wankowski DM, Garlie NK, et al. Use of anti-CD3 x anti-HER2/neu bispecific antibody for redirecting cytotoxicity of activated T cells toward HER2/neu+ tumors. *J Hematother Stem Cell Res*. 2001;10(2):247-260.
23. Irvine DJ, Purbhoo MA, Krogsgaard M, Davis MM. Direct observation of ligand recognition by T cells. *Nature*. 2002;419(6909):845-849.
24. Lum L, Thakur A, Rathore R, Al-Kadhimi Z, Uberti J, Rathanatharathorn V. Phase I clinical trial involving infusions of activated T cells armed with anti-CD3 x anti-HER2ne*u* bispecific antibody in women with metastatic breast cancer: Clinical, immune, and trafficking results.Oct. 1-3, 2010, Washington, DC. *ASCO Breast Cancer Symposium*. 2010.
25. Lum HE, Miller M, Van Epps D, Lum LG. Targeting prostate cancer cells with T Cells armed with two novel bispecific antibodies (OKT3 x 9187 and OKT3 x 9189). *Experimental Hematology*. 2001;29(Suppl 1):63.
26. Lum LG, Rathore R, Lum HE, et al. Targeting of Prostate Cancer With T Cells Armed With OKT3 x Anti-HER2/New Bispecific Monoclonal Antibodies(Biabs). *Experimental Hematology*. 2001;29(Suppl 1):75.
27. Van Wauwe JP, De Mey JR, Gooseens JG. OKT3: a monoclonal anti-human T lymphocyte antibody with potent mitogenic properties. *JImmunol*. 1980;124(6):2708-2713.
28. Meuer SC, Hodgdon JC, Hussey RE, Protentis JP, Schlossman SF, Reinherz EL. Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *JExpMed*. 1983;158:988-993.
29. Meuer SC, Hussey RE, Cantrell DA, et al. Triggering of the T3-Ti anti-receptor complex results in clonal T cell proliferation through an interleukin 2 dependent autocrine pathway. *ProcNatlAcadSciUSA*. 1984;81:1509-1513.
30. Weiss A, Imboden JB. Cell surface molecules and early events involved in human T lymphocyte activation. *AdvImmunol*. 1987;41:1-38.
31. Ochoa AC, Gromo G, Alter BJ, Sondel PM, Bach FH. Long-term growth of lymphokine-activated killer (LAK) cell: role of anti-CD3, beta-IL 1, interferon-gamma and -beta. *JImmunol*. 1987;138(8):2728-2733.
32. Anderson PM, Bach FH, Ochoa AC. Augmentation of cell number and LAK activity in peripheral blood mononuclear cells activated with anti-CD3 and interleukin-2. Preliminary results in children with acute lymphocytic leukemia and neuroblastoma. *Cancer ImmunolImmunother*. 1988;27:82-88.
33. Chen BP, Malkovsky M, Hank JA, Sondel PM. Nonrestricted cytotoxicity mediated by interleukin 2-expanded leukocytes is inhibited by anti-LFA-1 monoclonal antibodies (MoAb) but potentiated by anti-CD3 MoAB. *Cell Immunol*. 1987;110:282-293.
34. Lotzova E, Savary CA, Herberman RB, McCredie KB, Keating MJ, Freireich EJ. Augmentation of antileukemia lytic activity by OKT3 monoclonal antibody: synergism of OKT3 and interleukin-2. *NatImmunCell Growth Regul*. 1987;6:219-223.
35. Yang SC, Fry KD, Grimm EA, Roth JA. Successful combination immunotherapy for the generation *in vivo* of antitumor activity with anti-CD3, interleukin 2, and tumor necrosis

factor alpha. *ArchSurg*. 1990;125:220-225.
36. Ueda M, Joshi ID, Dan M, et al. Preclinical studies for adoptive immunotherapy in bone marrow transplantation: II. Generation of anti-CD3 activated cytotoxic T cells from normal donors and autologous bone marrow transplant candidates. *Transplantation*. 1993;56:351-356.
37. Uberti JP, Joshi I, Ueda M, Martilotti F, Sensenbrenner LL, Lum LG. Preclinical studies using immobilized OKT3 to activate human T cells for adoptive immunotherapy: optimal conditions for the proliferation and induction of non-MHC-restricted cytotoxicity. *ClinImmunolImmunopathol*. 1994;70(3):234-240.
38. Anderson PM, Blazar BR, Bach FH, Ochoa AC. Anti-CD3 + IL-2-stimulated murine killer cells: i*n vitro* generation and *in vivo* antitumor activity. *JImmunol*. 1989;142(4):1383-1394.
39. Anderson PM, Ochoa AC, Ramsay NKC, Hasz D, Weisdorf D. Anti-CD3 + interleukin-2 stimulation of marrow and blood: comparison of proliferation and cytotoxicity. *Blood*. 1992;80:1846-8153.
40. Ting C-C, Hargrove ME, Yun YS. Augmentation by anti-T3 antibody of the lymphokine-activated killer cell-mediated cytotoxicity. *JImmunol*. 1988;141:741-748.
41. Ochoa AC, Hasz DE, Rezonzew R, Anderson PM, Bach FH. Lymphokine-activated killer activity in long-term cultures with anti-CD3 plus interleukin 2: identification and isolation of effector subsets. *Cancer Res*. 1989;49:963-968.
42. Scher HI, Halabi S, Tannock I, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. *JClin Oncol*. 2008;26(7):1148-1159.
43. Higuchi CM, Thompson JA, Petersen FB, Buckner CD, Fefer A. Toxicity and immunomodulatory effects of interleukin-2 after autologous bone marrow transplantation for hematologic malignancies. *Blood*. 1991;77:2561-2568.
44. Postow MA, Callahan MK, Wolchok JD. Immune Checkpoint Blockade in Cancer Therapy. *J Clin Oncol*. 2015.
45. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *EurJCancer*. 2009;45(2):228-247.
46. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clinical Cancer Research*. 2009;15(23):7412-7420.
47. Grabert RC, Cousens LP, Smith JA, et al. Human T cells armed with HER2/neu bispecific antibodies divide, are cytotoxic, and secrete cytokines with repeated stimulation. *Clinical Cancer Research*. 2006;12(2):569-576.
48. Lum LG, Thakur A, Pray C, et al. Multiple infusions of CD20-targeted T cells and low-dose IL-2 after SCT for high-risk non-Hodgkin's lymphoma: a pilot study. *Bone Marrow Transplant*. 2014;49(1):73-79.
49. Lum LG, Thakur A, Al-Khadimi Z, et al. Phase I Dose Esclation of Activated T Cells (ATC) Armed with Anti-CD3 x Anti-CD20 Bispecific Antibody (CD20Bi) after Stem Cell Transplant (SCT) In Non-Hodgkin's Lymphoma (NHL). *The American Society of Hematology (ASH)*. 2010;December 4-7, 2010, Orlando, FL:Abstract # 488.
50. Adams S, Gray RJ, Demaria S, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. *J Clin Oncol*. 2014;32(27):2959-2966.
51. Fukunaga A, Miyamoto M, Cho Y, et al. CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. *Pancreas*. 2004;28(1):e26-31.
52. Pages F, Kirilovsky A, Mlecnik B, et al. In situ cytotoxic and memory T cells predict

outcome in patients with early-stage colorectal cancer. *J Clin Oncol*. 2009;27(35):5944-5951.
53. Hintze J. PASS 12 (ed www.ncss.com). Kaysville, Utah, USA: NCSS, LLC.; 2013.
54. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *Annals of Statistics*. 2001;29(4):1165-1188.

16.0 APPENDICES

16.1 ECOG/Zubrod Performance Status:
For this study only ECOG/Zubrod score is used to evaluate patients.

ECOG PERFORMANCE STATUS	KARNOFSKY PERFORMANCE STATUS
0—Fully active, able to carry on all pre-disease performance without restriction	100—Normal, no complaints; no evidence of disease
	90—Able to carry on normal activity; minor signs or symptoms of disease
1—Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	80—Normal activity with effort, some signs or symptoms of disease
	70—Cares for self but unable to carry on normal activity or to do active work
2—Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours	60—Requires occasional assistance but is able to care for most of personal needs
	50—Requires considerable assistance and frequent medical care
3—Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours	40—Disabled; requires special care and assistance
	30—Severely disabled; hospitalization is indicated although death not imminent
4—Completely disabled; cannot carry on any selfcare; totally confined to bed or chair	20—Very ill; hospitalization and active supportive care necessary
	10—Moribund
5—Dead	0—Dead

*Karnofsky D, Burchenal J, The clinical evaluation of chemotherapeutic agents in cancer. In: MacLeod C, ed. Evaluation of Chemotherapeutic Agents. New York, NY: Columbia University Press; 1949:191–205.

**Zubrod C, et al. Appraisal of methods for the study of chemotherapy in man: Comparative therapeutic trial of nitrogen mustard and thiophosphoramide. *Journal of Chronic Diseases*; 1960:11:7-33.

16.2 Common Terminology Criteria for Adverse Events V4.0 (CTCAE):

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

16.3 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.

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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.