Study Title	A Phase I/II Study of Anti-CD3 x Anti-HER2/*neu* (Her2Bi) Armed Activated T Cells (ATC) and Pembrolizumab combination therapy in Women with metastatic breast cancer.
Study Intervention	MK-3475 (Pembrolizumab) with Her2Bi Armed Activated T Cells (ATC)
Indication Studied	Metastatic Breast Cancer
Sponsor	University of Virginia
Principal Investigator	Patrick Dillon, MD
Biostatistician	Qin Liu, PhD Wistar Institute, Philadelphia, PA
Protocol Number	Breast-47
IND Number	IND 9985
Development Phase of Study	Phase I/II
GCP Statement	This study is to be performed in full compliance with acceptable Current Good Clinical Practices (cGCP) and regulations. All required study documentation will be archived as required by regulatory authorities.
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1.0 SUMMARY

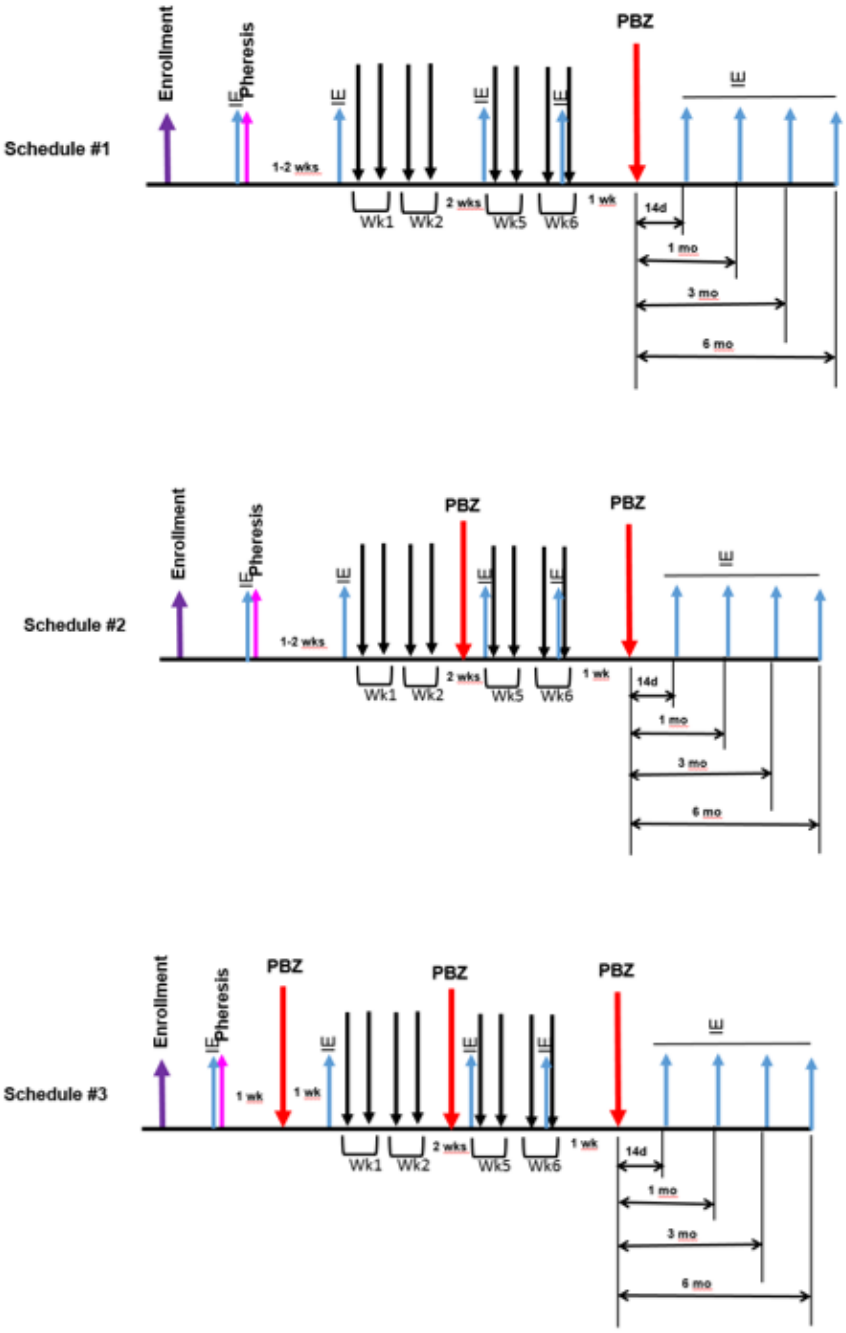
Non-toxic approaches are needed to improve progression free survival (PFS) and overall survival (OS) in all patients (pts) who have metastatic breast cancer (MBC). In our previous clinical trials, anti-CD3 activated T cells (ATC) armed with anti-CD3 x anti-HER2 bispecific antibody (HER2Bi) traffic to tumors, exhibit anti-HER2 cytotoxicity, proliferate, and secrete immunokines upon tumor engagement (1).

This proposal uses HER2**B**i **a**rmed A**T**Cs (HER2 **BATs**) to target breast cancer in combination with pembrolizumab (PBZ) in women with MBC. Women with MBC who have failed two or more lines of therapy are eligible regardless of their HER2 or hormonal status. The strategy of this protocol is to increase functional T cell activity at the tumor site and increase local and systemic anti-tumor responses. Combining BATs with a checkpoint inhibitor by blocking PD-1 signaling is likely to enhance anti-breast cancer immune responses more than BATs or PBZ alone. We hypothesize that multiple IV infusions of HER2 BATs in combination with PBZ will be safe without producing intolerable toxicity, infiltrate metastatic lesions, lyse tumor, and induce a "vaccine" response and that immunotherapy (IT) mediated anti-tumor “immunization” will induce both functional and phenotypic immune changes detectable in the blood that translate into improved total toxicity profile (TTP) and overall survival (OS) for subjects with advanced MBC.

The primary objective is to conduct a phase I/II clinical trial in women with MBC to estimate the safety and maximum tolerated dose (MTD) of BATs in combination with PBZ. The secondary objectives are to estimate the safety of the MTD, preliminarily estimate objective response rate (ORR), duration of response (DoR), and disease control rate (DCR), to assess OS and progression-free survival (PFS), to establish a dose-expansion cohort (DEC), and to evaluate immune responses. Immune responses will be tested to determine if phenotype and function of peripheral blood mononuclear cells (PBMC) and cytokine profiles are altered from baseline in various schedules for the combination of BATs and PBZ.

The study design assesses safety and immune responses to PBZ administered in combination with BATS infusions (~10^{10}/infusion ± 20%), as follows:

Figure 1


Schedule #1
Enrollment
IE
Pheresis
IE
1-2 wks
IE
IE
IE
PBZ
IE
Wk1
Wk2
2 wks
Wk5
Wk6
1 wk
14d
1 mo
3 mo
6 mo
Schedule #2
Enrollment
IE
Pheresis
IE
1-2 wks
PBZ
IE
IE
PBZ
IE
Wk1
Wk2
2 wks
Wk5
Wk6
1 wk
14d
1 mo
3 mo
6 mo
Schedule #3
Enrollment
IE
Pheresis
PBZ
1 wk
IE
1 wk
PBZ
IE
IE
PBZ
IE
Wk1
Wk2
2 wks
Wk5
Wk6
1 wk
14d
1 mo
3 mo
6 mo

2.0 OBJECTIVES

2.1 Primary objective

[1] To determine the MTD for BATs combined with PBZ in subjects with MBC.

2.2 Secondary objectives

[1] To estimate the safety of the MTD for BATs combined with PBZ in subjects with MBC.

[2] To evaluate immune responses in MBC subjects by sequential monitoring of phenotype, IFN-γ EliSpots, anti-breast cancer cytotoxicity of peripheral blood mononuclear cells (PBMC) directed at breast cancer cell lines, Th_1/Th_2 serum cytokine patterns, and anti-breast cancer antibodies in the serum during the "vaccinate and consolidate" process.

[3] To obtain a preliminary estimate of the objective response rate (ORR), duration of response (DoR), and disease control rate (DCR) in subjects treated at the MTD for BATs combined with PBZ.

[4] To establish a dose-expansion cohort (DEC) of an additional 12 subjects to further evaluate the safety and preliminary efficacy (PFS and OS).

3.0 BACKGROUND & RATIONALE

3.1 Background

Unmet Need for Treatment of Breast Cancer Metastases. Metastatic Breast Cancer represents a significant clinical challenge. About 20-85% of the patients who present with localized breast cancers will progress to MBC in 5 years and approximately 6-10% of the patients present with metastatic disease *de novo*. Depending on the stage at which metastasis is detected the median survival is in the range of 18-24 months(2). Both chemotherapy and hormonal therapy have been used to treat MBC with different levels of success. Most patients experience objective responses associated with palliation of symptoms (3-5), but complete responses (CR) are uncommon and short-lived. Initial responses may last between 8 and 14 months (6-8), however, progression of disease is inevitable, and durable responses to subsequent therapies are progressively fewer. For the 20-25% of women with MBC who are HER2/*neu* (HER2) overexpressed, Herceptin®, pertuzumab, lapatinib and ado-trastuzumab have become mainstays of treatment in combination with chemotherapy. New treatment strategies, however, are needed to prolong progression-free and overall survival in the 75-80% of the MBC patients who are HER2 normal (not over-expressed) and thus, not eligible for the FDA approved HER2 targeted therapies. In our phase I clinical trial, we have administered anti-CD3 activated T cells (ATC) armed with anti-CD3 x anti-HER2/neu bispecific antibody (HER2Bi) to women with

both HER2-positive and HER2-negative MBC to determine their safety and the MTD. Arming ATC with HER2Bi makes every T cell into a HER2-specific cytotoxic T lymphocyte (CTL) with the potential to induce high levels of specific cytotoxicity that is independent of HER2 receptor-mediated mechanisms and thus, HER2 expression levels (9). Indeed, evaluation of immune responses in our phase I clinical trial subjects suggests that infusions of HER2 BATs induce robust immune responses regardless of the subject's HER2 status. Consistent with this observation, log rank analyses comparing Kaplan-Meier estimates of PFS and OS between HER2(3+) subjects (n =7) and HER2(0-2+) subjects (n = 7) enrolled in the phase I trial found no significant difference between the stratified groups. In the HER2(3+) group, median overall survival was 21 months, but did not differ significantly (p = 0.98) from the OS curve of the HER2(0-2+) group for which median OS remained undefined (> 50% of subjects still survive) strongly suggesting that HER2 BATs may prolong survival independent of HER2 status. From a mechanistic standpoint, this is not surprising. For example, unlike monoclonal antibody therapy with Herceptin®, which induces cellular death through interference with receptor-mediated pathways and is thus, more effective in cells heavily reliant on those signaling pathways, low levels of receptors may be sufficient to redirect enough BATs to induce T cell mediated mechanisms of cellular death. A subset of clinical situations thus exists where tumors of MBC subjects with low level HER2 expression/amplification—a characteristic that would preclude therapy with Herceptin®—may still possess enhanced susceptibility to the directed cytotoxicity of HER2 BATs.

Additional clinical data is available from larger studies to further support the use of HER2 targeted therapies in breast cancer patients deemed HER2 normal by ASCO CAP guidelines (10, 11). In summary, Chumsri et al report mutations in breast cancer subjects responsive to trastuzumab in the absence of HER2 amplification. Additionally, Koumarianou et al describe a study of 130 subjects deemed HER2 normal who achieve benefit from HER2 targeting with Herceptin®. Recently, Brunello et al showed that subsets of triple negative breast cancer subjects derive benefit from HER2 targeted therapies(12). Intriguingly, even some subjects deemed HER2 negative by radiolabeled HER2 PET imaging, achieved responses to ado-trastuzumab in the Zephir study by Gephir et al (13). Older reports of trastuzumab responses in IHC intermediate subjects are well known in the breast cancer community (14).

Role of Immune Checkpoint Inhibitors: Experimental and clinical evidence strongly suggests that cancer cannot develop or progress without escaping immune surveillance(15); the PD-1 immune checkpoint pathway is one mechanism tumors use to escape detection. PD-1 (programmed cell death protein-1) is a receptor expressed by T cells upon activation. PD-1 induction on activated T cells occurs in response to PD-L1 or L2 engagement and limits effector T-cell activity in peripheral tissues. Such interaction limits normal tissue damage (16), and can be hijacked by cancers to limit anti-tumor immune response (17). The relevance of the PD-1 signaling pathway in limiting anti-tumor immune responses is suggested by the observation of higher levels of PD-1 on tumor infiltrating lymphocytes (TILs) compared to circulating cells(18). In addition, animal studies show that tumors

expressing PD-L1 have increased resistance to T cell immune responses and are more invasive. Inhibiting the PD-1/PD-L1 interaction can reverse resistance to anti-tumor T cell response and shrink tumors (19). Both nivolumab and PBZ have been approved by the FDA for malignant melanoma. Recently, the data in previously untreated advanced NSCLC has also been encouraging(20). All enrolled subjects had to have PD-L1 (≥ 1% staining) positive cancers. Of the 73 pts screened for the trial, 78% had PD-L1 positive tumors. The overall response rate in these subjects was 26% and the disease control rate was 64%. The median duration of response was not reached and 100% of the responders continued to have disease response at the time of reporting. Data with PBZ suggests that tumor PD-L1 expression may predict for higher probability of clinical benefit, though clinical benefits were also observed in subjects with PD-L1 negative tumors (20).

MK-3475 (PBZ)] in Breast Cancer. Drugs targeting either PD-1 or PD-L1 have shown promising rates of clinical benefit. PBZ is a humanized high affinity IgG4 PD-1 blocking antibody designed to directly block the interaction between PD-1 and PD-L1 and PD-L2 (21). Blockade enhances the functional activity of the target lymphocytes to facilitate tumor regression and ultimately immune rejection. In a recent phase Ib trial for metastatic triple negative breast cancer (TNBC) reported at the San Antonio Breast Cancer (SABCS) meeting on December 10, 2014 by Dr. Rita Nanda, safety, tolerability, and anti-tumor activity was evaluated in bi-weekly infusions of MK-3475 in 27 subjects (29-79 years old) who had relapsed after treatment of early stage breast cancer or had progressed with advanced disease (22). All subjects had tumors that expressed high levels of PD-L1. There was 1 clinical response, 4 partial responders, 6 with stable disease, and 12 with progressive disease. In those with measurable disease, 5 (18.5%) had encouraging results. Additional clinical evidence of PBZ activity in breast cancer was presented at SABCS 2015 (abstract S5-07) by Hope Rugo from the Keynote-28 trial. That trial used PBZ in pretreated metastatic ER+ subjects with >1% PD-L1 positivity on tumor or stroma. An overall response rate of 12% and a clinical benefit rate of 20% was observed in breast cancer subjects. Further proof of principle comes from positive responses observed with alternative PD-1 and PD-L1 agents. For example, Avelumab monotherapy in metastatic breast cancer yielded an 8.6% partial response rate and a 31% disease control rate (abstract S1-07). Likewise a trial of atezolizumab and nab-paclitaxel observed an overall response rate of 89% in nine first-line metastatic subjects and an overall response rate of 42% in all 24 metastatic subjects (first through third line) (abstract 850477). Toxicities in all four of these highlighted checkpoint inhibitor studies have been tolerable and no treatment-related deaths in breast cancer subjects have been reported.

There is strong interest in combining immune check point inhibitors with active immunotherapy strategies such as vaccines and T cell therapy. Our group has already developed HER2 BATs and conducted clinical trials showing that infusion of HER2 BATs is both safe and feasible. ***We propose to evaluate a novel strategy of combining immune checkpoint PD-1 inhibitor, PBZ with BATs (BATs+PBZ). This study will assess the feasibility and preliminary efficacy of this novel therapy in subjects with MBC.***

MK-3475 (PBZ) in ER+ and HER2+ breast cancer. In regard to the use of Pembrolizumab in ER+ and HER2+ patients, a goal of this project is to uncover a method to render immunologically silent tumors such as ER+ breast cancer, to be immunologically recognizable. Employing armed T cells (BATS) and a checkpoint inhibitor seeks to address this highly valuable goal in breast cancer. We have already shown an example of an ER positive/HER2 negative patient with a partial response to BATS alone (figure 5d). Activity of PD-1 inhibitors in ER positive metastatic breast cancer was reported in the two following abstracts:

a. Rugo HS, Delord J-P, Im S-A, et al. Preliminary efficacy and safety of pembrolizumab (MK-3475) in patients with PD-L1–positive, estrogen receptor-positive (ER+)/HER2-negative advanced breast cancer enrolled in KEYNOTE-028. Presented at: 2015 San Antonio Breast Cancer Symposium; December 8-12, 2015; San Antonio, TX. Abstract S5-07.

b. Dirix LY, Takacs I, Nikolinakos P, et al. Avelumab (MSB0010718C), an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a phase Ib JAVELIN solid tumor trial. Presented at: 2015 San Antonio Breast Cancer Symposium; December 8-12, 2015; San Antonio, TX. Abstract S1-04.

In the Rugo KEYNOTE-28 study, there have been 3 PR's and 4 SD subjects reported out of 25 evaluable subjects to date, We propose and hypothesize that BATS may synergize with PD-1 inhibitors to improve response rates in ER positive and negative populations and in HER2 amplified populations. If preferential response is observed in a subset of subjects, then a future trial will focus on efficacy in that subpopulation.

BATs. HER2 BATs mediate high levels of cytotoxicity directed at both low and high HER2-expressing breast cancer cell lines (23). BATs showed repeated killing, proliferation, and release of Th_1 cytokines, RANTES and MIP-1, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α),and granulocyte-macrophage colony stimulating factor (GM-CSF) that induce DC maturation. Dying tumor cells release tumor associated antigens (TAA), and lead to increased cross-presentation of TAA by antigen presenting cells. This mechanism, illustrated in **Fig 1**, may vaccinate subjects against their own tumor antigens**.** In preclinical studies, we showed that intravenous infusions of HER2 BATs inhibited the growth of established HER2+ PC-3 tumors in SCID/Beige mice significantly more than ATC alone ($p<0.001$) and prevented tumor development in co-injection WINN assays (24, 25). Furthermore, arming ATC with HER2Bi makes every T cell into a non-MHC

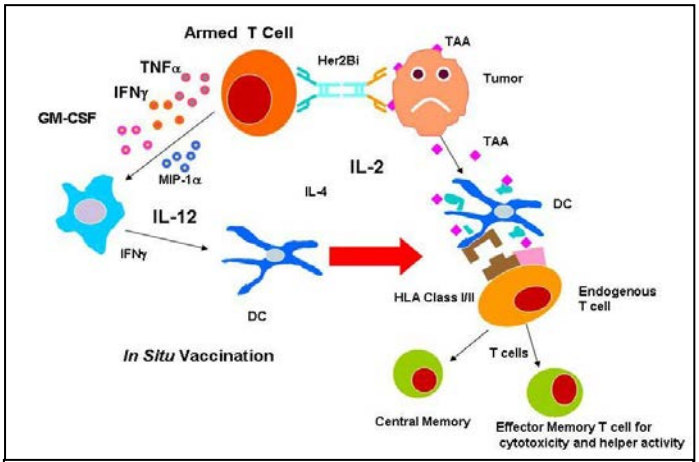


Fig 1: Infusion of targeted T cells induces anti-tumor immune responses. T cells kill tumor cells releasing cytokines and chemokines that induce DC maturation followed by antigen presentation of unknown tumor antigens to "vaccinate" the patient against their own TAA.

restricted HER2-specific cytotoxic T lymphocyte (CTL)(23). Trastuzumab's clinical effect requires high levels of HER2 expression whereas low levels or nil expression of HER2 receptors is sufficient to allow targeting by potent granzyme/perforin non-MHC restricted killing mediated by BATs. This ability to target low level HER2 or negative expressing tumors provides a unique opportunity to target MBC in subjects with HER2 0-2+ disease. More recently, the expression of HER2 receptors on breast cancer stem cells provides another explanation of why targeting HER2 negative tumors could lead to extended median OS in HER2 negative subjects (26, 27).

Phase I and II clinical trials(28-31); a total of 320 x 10^9 cells were infused in 4 to 8 divided doses without finding a maximum tolerated dose (MTD). There were no dose limiting toxicities (DLTs). No one needed ICU care or blood pressure or ventilator support in contrast to those who received BiAb infusions alone for solid tumors. Furthermore, we showed that ATC armed with CD20Bi are not only highly effective at lysing CD20 resistant targets but also secrete Th_1 cytokines such as IFN-γ upon contact with $CD20^+$ malignant B cells (32). In our phase I clinical trials in 12 non-Hodgkin's lymphoma subjects with high risk or resistant NHL, multiple infusions of CD20 BATs following high dose chemotherapy (HDC) and autologous stem cell transplant (SCT) induce significantly higher levels of anti-lymphoma activity in the peripheral blood after multiple infusions of CD20 BATs following HDC and autologous SCT than subjects undergoing SCT alone (33). Multiple infusions of HER2 BATs in phase I clinical trials have been shown to be safe in subjects with breast cancer with some promising anti-tumor activity and OS (34). In addition, targeted T cells were detected in the blood of these subjects for at least 1 week after infusions, serum cytokine profile shifted to Th_1, and immune monitoring studies demonstrated evidence of highly cytotoxic fresh endogenous peripheral blood mononuclear cells (PBMC) that killed breast cancer cells (34, 35). Chemical heteroconjugation of BiAbs was quick, inexpensive and straightforward, enabling rapid large scale current good manufacturing practice (cGMP) production of clinical grade BiAbs. Anti-HER2, anti-EGFR, anti-GD2, and anti-CD20 strategies have been translated into clinical phase I studies (28, 30).

ATC as mobile serial killers and immunokine factories. Cross-linking the T cell receptor with anti-CD3 triggers activation, proliferation, cytokine synthesis, and non-MHC restricted cytotoxicity directed at tumor targets(36-39).The tumoricidal cytokines produced include interferon-γ(IFNγ), tumor necrosis factor-α(TNFα),and GM-CSF(40-42). In animals, infusions of ATC provide anti-tumor effects (43-46) and improve engraftment when limiting doses of stem cells were given after lethal total body irradiation (47). In humans, a phase I trial treating non-Hodgkin's lymphoma subjects with cyclophosphamide before infusing activated $CD4^+$ cells resulted in one complete, 2 partial and 8 minor responses in 31 subjects with advanced cancers (48, 49). Our earlier clinical trials showed that even unarmed ATC with low dose IL-2, and GM-CSF in subjects with MBC following HDC and SCT were well tolerated and clinically effective even without retargeting them to cancer cells (31). If nonspecific T cells have enhanced cancer-directed activity after chemotherapy, BATs may have even greater anti-tumor activity.

HER2 BATs target high (SK-BR-3) and low (SUM 1315) HER2 cell lines. In our preclinical studies, we showed that HER2 BATs were able to lyse breast cancer cell lines that expressed not only high amounts of HER2 (SK-BR-3, high 3+ expression) but more importantly target and lyse lines that express little (MCF-7, a negative control for immunohistochemistry)(50) or essentially no HER2 expression (SUM1315). SUM 1315 is known to be negative for HER2 by Western blotting (**Fig. 2a**) and flow cytometry (**Fig. 2b**). **Fig. 2c** shows specific cytotoxicity of HER2 BATs directed at SUM 1315 and SK-BR-3. Flow cytometry showed that SUM 1315 cells had a very low mean fluorescent intensity (MFI) and only 6.7% were positive. These results are consistent with the Western blot (**Fig. 2a**). Goat anti-HER2/neu was used to detect HER2/*neu* receptors on SUM 1315 and SK-BR-3 cells. **Fig. 2c** shows that BATs exhibit high levels of cytotoxicity directed at SUM 1315 cells. It is clear that only a few molecules of HER2/*neu* on their surface are sufficient to allow binding and triggering of specific cytotoxicity. This is consistent with the observation that as few as 10-30 TCR-ligand interactions are sufficient to mediate T cell killing (51). *These data and our clinical and immunologic data on subjects treated with HER2 BATs who were HER2-negative in our phase I/II clinical trials provide the rationale to target tumors that may have low or variable antigen expression.*

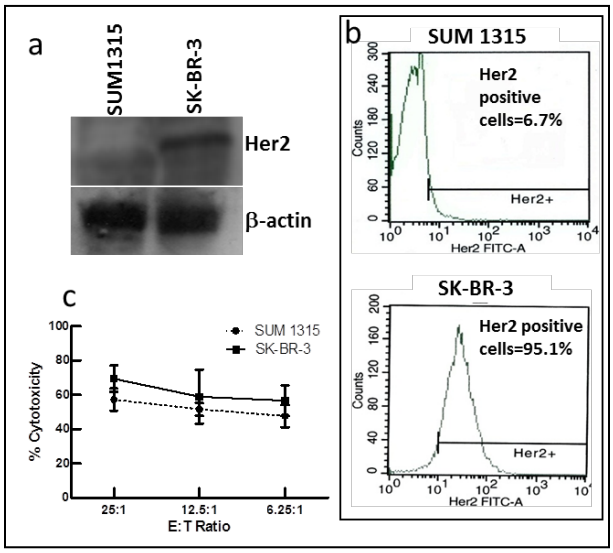


Fig. 2: Cytotoxicity remains high in very low HER2/neu expressing SUM 1315 cells. a) Western blot shows very low (SUM 1315) and high (SK-BR-3) HER2/neu expression; **b)** Flow cytometry shows surface expression of HER2/neu on 6.7% of SUM 1315 with very low mean fluorescent intensity (MFI) and 95.1% of SK-BR-3 with a high MFI; and **c)** Cytotoxicity remained high in the very low expressing HER2/neu SUM 1315 cell line.

BATs inhibit differentiation of myeloid derived suppressor cells (MDSC) and T_{regs}. In a 3D matrigel model, co-culture of PBMC with tumor spheres showed increased proportions of tumor induced MDSC ($CD33^+/CD11b^+/HLA\text{-}DR^-$) and T_{reg} ($CD4^+/CD25^{hi}/CD127^{lo}$) populations. Comparison of tumor sphere killing and MDSC and T_{reg} populations in culture conditions with or without BATs showed a significantly higher killing of tumor spheres ($p<0.0001$) and reduced percentage of MDSC ($p<0.0005$) and T_{reg} ($p<0.028$) populations in BATs containing co-cultures compared to control culture conditions without BATs (**Fig. 3**). In addition to decreased proportions of MDSC, we have shown that BATs induced a shift in the STAT3/STAT1 ratio. In the presence of BATs, tumor derived immune suppressive STAT3 activation decreased significantly while immune activating STAT1 expression and activation increased significantly. *These data suggest that targeted immunotherapy with BATs directed at tumor targets not only kills tumor spheres but also reduces MDSC and T_{reg} populations as part of the targeting response.*

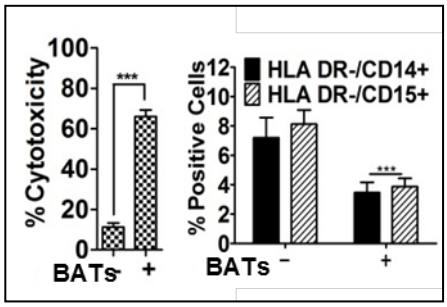


Fig 3. Right panel shows the BATs mediated cytotoxicity against MB-231 tumor spheres in 3D culture. Left panel shows significantly reduced granulocytic and monocytic MDSC populations in the presence of BATs.

BATs induce cytokines and chemokines that inhibit MDSC differentiation and activation. We recently reported that in Th_1 cytokine enriched microenvironment, IFN-γ induced chemokines, MIG/CXCL9 and IP-10/CXCL10, were upregulated while IL-1β and IL-6 were down regulated with concomitant reduction in the percentage of MDSC (**Fig. 4**) in a 3D co-culture model. Similarly, levels of IFN- γ, IL-2, IL-2R and IL-12p41/71 were significantly higher in culture supernatants from BATs containing co-cultures compared to control condition without BATs. High levels of IFN- γ, IL-2, IL-12p41/71, CXCL9, CXCL10, upregulation of STAT1 and downregulation of STAT3 are likely to activate anergic immune cells in the tumor microenvironment and corroborate with reduced number of MDSC. *These data suggest that BATs inhibited differentiation of MDSC and Tregs through cytokines/chemokines.*

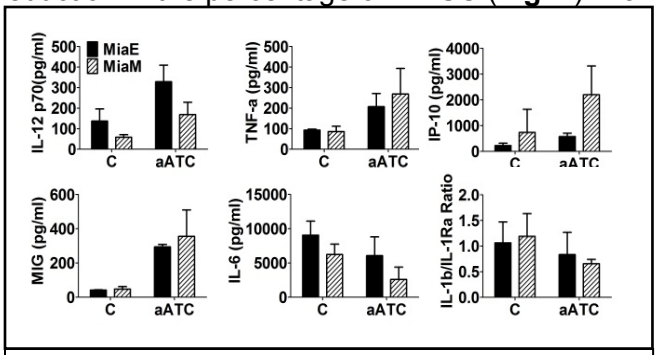


Fig 4. Cytokine profile of culture supernatants detected by multiplex luminex system show the increased levels of cytokines IL-12, TNF-α and IFN-γ inducible chemokines MIG/CXCL9 and IP-10/CXCL10 in the presence of BATs (aATC) compared to control condition (without BATs). While levels of immune suppressive cytokines IL-6 and IL-1β were reduced in the presence of BATs vs. control.

<u>Phase I study of HER2 BATs in subjects with metastatic breast cancer (MBC)</u>

Targeting of MBC with HER2 BATs. In a phase I trial, 8 infusions of HER2 BATs were given twice per week for 4 weeks with low-dose IL-2 (300,000 IU/m^2/day) and GM-CSF (250 µg/m^2/dose given twice weekly) in a standard 3+3 dose escalation schema with 5, 10, 20, and 40 × 10^9 BATs per dose for total doses of 40, 80, 160, and 320 × 10^9 BATs. Up to 320 × 10^9 HER2 BATs were frozen and infused into women with MBC without DLTs(16). However, the dose of 320 × 10^9 was not consistently achievable due to technical feasibility. These women were heavily pretreated and all but one of the HER2 3+ subjects had received prior Herceptin®. PBMC exhibited high levels of cytotoxicity directed at a HER2+ MBC cell line (SK-BR-3) that persisted up to 4 months (35) and there were significant increases in serum Th$_1$ cytokines (**Fig. 5a**) and IL-12 (**Fig. 5b**)(31, 34) during and after infusions. *The marked increases in serum IL-12 (not produced by T cells) show that endogenous monocytes activated by BATs infusions secreted high levels of IL-12. The Kaplan-Meier (K-M) showed that OS was 36.2 months for the entire group, 57.4 months for the HER2 3+ group, and 27.4 months for the HER2-negative group (**Fig. 5c**).* Of 22 evaluable subjects, one had very good partial response (**Fig. 5d**) with nearly all of the liver metastases responding, and 12 subjects had stable disease at 15 weeks after initiation of immunotherapy. There was a reduction in CEA (3 of 5), CA 27.29 (2 of 4), and HER2 serum receptor (2 of 5) levels.

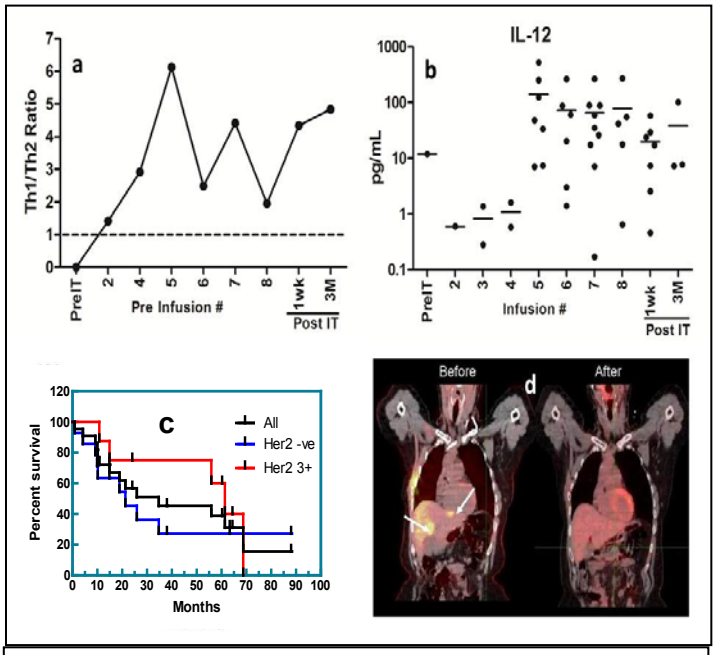


Fig. 5: **a**) Mean ratio of Th$_1$/Th$_2$ cytokine as a function of infusion #, where Th$_1$/Th$_2$ = [IL-2+IFN-γ]/[IL-4+IL-5] ; **b**) IL-12 levels as a function of infusion # with means denoted as horizontal bars; **c**) the median OS is 57.4 months for the HER2 3+ group (**red**), 27.4 months for the HER2 0-2+ (**blue**) group, and is 36.2 months for the entire group (**black**); **d**) HER2-negative partial responder showing 2 liver mets pre-immunotherapy (left) PET/CT which disappeared post-immunotherapy (right).

Very good partial response in HER2-negative subject. A partial responder in the HER2-negative group had two well-defined liver metastases on a PET/CT (2.5 × 1.7 cm and 2.5 × 1.3 cm) as shown in **Fig. 5d** (**left panel**). This subject was ER positive. Re-imaging after immunotherapy showed regression of the multiple lesions after 6 months (**Fig. 5d, right panel**). The sum of longest diameters was decreased by 30% at 4 weeks after immunotherapy and was decreased by >70% at 6 months. The subject was progressing on Femara and was restarted on Femara after immunotherapy.

Clinical toxicities of BATs. The most frequent manageable clinical toxicities were fever, chills, transient hypotension, headaches, and fatigue. Fever, chills, and hypotension were preempted with pre-hydration and pre-medication with diphenhydramine and acetaminophen. No MBC subject required ICU admission for cardiorespiratory support. There was one death due to a toxic level of digoxin and one subject developed a subdural hematoma due to hypertension associated with GM-CSF and T cells. The subdural hematoma was successfully evacuated without any complications.

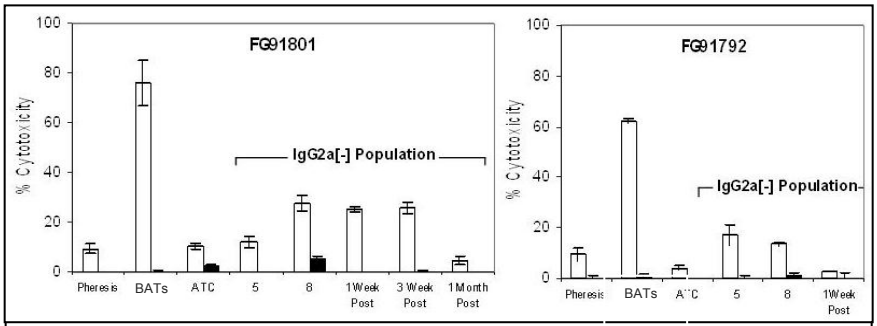


Fig 6: Pheresis, BATs, unarmed ATC and IgG2a- populations were tested for specific cytotoxicity directed at SK-BR-3. Specific cytotoxicity (E/T = 10:1) was performed in ^{51}Cr assays. IgG2a+ were depleted using Miltenyl beads. The clear bars show cytotoxicity directed at SK-BR-3 targets and the solid bars show cytotoxicity directed at Raji targets.

Evidence for activation and immunization of endogenous immune cells in MBC subjects. PBMC exhibited high levels of cytotoxicity directed at SK-BR-3 that persisted up to 4 months. **Fig 6** shows specific cytotoxicity mediated by endogenous lymphocytes (IgG2a-negative population) directed against SK-BR-3 in two representative subjects. PBMC from these subjects were depleted of IgG2a+ bearing cells (BATs) using Miltenyi columns and the IgG2a negative population was used in the killing assay. The results strongly suggest that endogenous lymphocytes had developed robust immune responses to breast cancer antigens.

No Human Anti-Mouse Antibody (HAMA) responses. Because anti-CD3 mAb moiety comprising the BiAb is a murine IgG2a mAb, we evaluated subjects' sera before and after HER2 BATs infusions for the development of HAMA to IgG2a in an ELISA. Of the 10 subjects evaluated, none developed clinically significant HAMA levels (>10 ng/ml) during or immediately after the treatment regimen.

Pilot study: Vaccination with HER2 BATs infusions and boost after SCT with "immune" ATC. In 5 of 7 evaluable subjects who had undergone the phase I clinical trial consisting of 8 infusions of HER2 BATs in combination with low-dose IL-2 and GM-CSF (**see below**), a second leukapheresis was performed to obtain "anti-breast cancer immune" PBMC. ATC were produced and cryopreserved for multiple infusions (4-15 infusions) after HDC and SCT to create immunologic space. A third leukapheresis was performed after G-

CSF stimulation to obtain the stem cells for SCT. Two of 7 subjects had rapidly progressing disease and were not evaluable. Three subjects received 3 infusions of 5 × 10^9 twice per week for 3 weeks and 1 infusion of 5 × 10^9 per week for 6 weeks post-SCT. After moving the protocol to KCI, 2 subjects received 1 infusion up to 40 × 10^9 cells per week for 4 weeks. **Figure 7a** shows that fresh PBMC mediated high levels of anti-breast cancer cytotoxicity in 5 women pre-, during (mid-immunotherapy), post-immunotherapy , pre-SCT (post-immunotherapy), and at various time points after SCT (1 month, 2 months, 3 months, 6 months, 1-2 years). When fresh PBMC from 2 pts were stimulated with SK-BR-3 targeted T cells and using a new assay for inducing anti-breast cancer antibodies *in vitro(52)*, the 14-day B cell cultures secreted anti-SK-BR-3 antibodies. **Figure 7b** shows amounts of anti-SK-BR-3 antibody produced *in vitro* detected by whole cell ELISA in four subjects at the same time points. The ability of PBMC to produce antibodies directed at SK-BR-3 *in vitro* is confirmed by the presence of IgG anti-SK-BR-3 antibodies (levels ranging from 10 ng to 200 ng/ml) in all 5 subjects more than 1 year post-SCT (data not shown).These results show that the PBMC contained memory B cells that produce anti-breast cancer antibodies *in vitro*. This proof-of-concept pilot study shows that both cellular and humoral anti-tumor immune responses can be easily detected during immunotherapy and these immune responses can be transferred via the "immune" ATC and detected after SCT. Two out of the 5 women remain alive at 2,664 and 1,302 days from their original enrollment data prior to receiving BATs in the phase I trial. These results strongly suggest that BATs provided a survival benefit and may have induced a vaccination response.

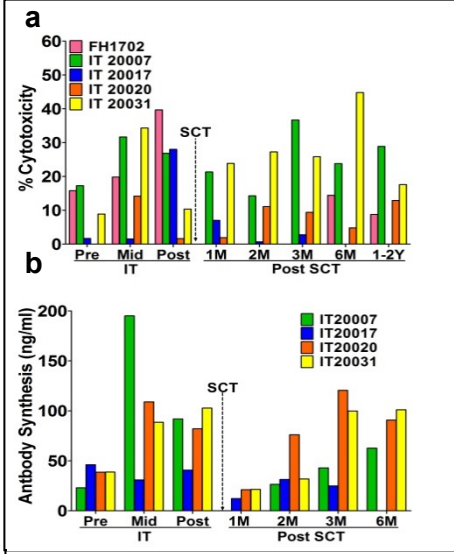


Fig 7: a) Multiple infusions of ATC after SCT promoted the development of cytotoxicity that could be detected during immunotherapy (Mid IT) and at 2-4 weeks post immunotherapy (Post IT), and 1 mo (1M), 2 mos (2M), 3 mos (3M), 6 mos (6M) and 12-24 mos (1-2Y) after SCT. **b)** Shows the anti-SK-BR-3 antibody levels in culture supernatants tested after 14 days of culture. These data suggest that subjects had been immunized against her own tumor antigens by multiple infusions of BATs that can be transferred in ATC product after SCT.

<u>Phase I Clinical Trial in Stage II/III, HER2 0-3+ High Risk Breast Cancer Subjects (> 4+ Nodes):</u> In our phase I clinical trial, women with locally advanced breast cancer were infused with HER2 BATs in combination with IL-2 and GM-CSF after Texel, Adriamycin, and Cytoxan. Seven of 9 subjects were 0-2+ HER2 and 2 of 9 had 3+ HER2 expression. The median OS remains undefined with survival ranging from 14.2 to 136.3 months (**Fig 8**). Five of 9 subjects are alive, 4 out of the 5 subjects have no evidence of disease and 1 subject relapsed at 91.8 months. There were no DLTs. These clinical responses suggest that BATs infusions in the adjuvant setting may have been exceptionally efficacious.

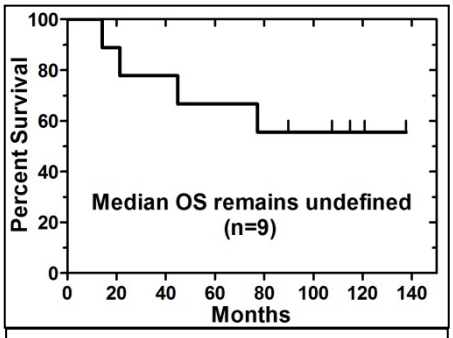
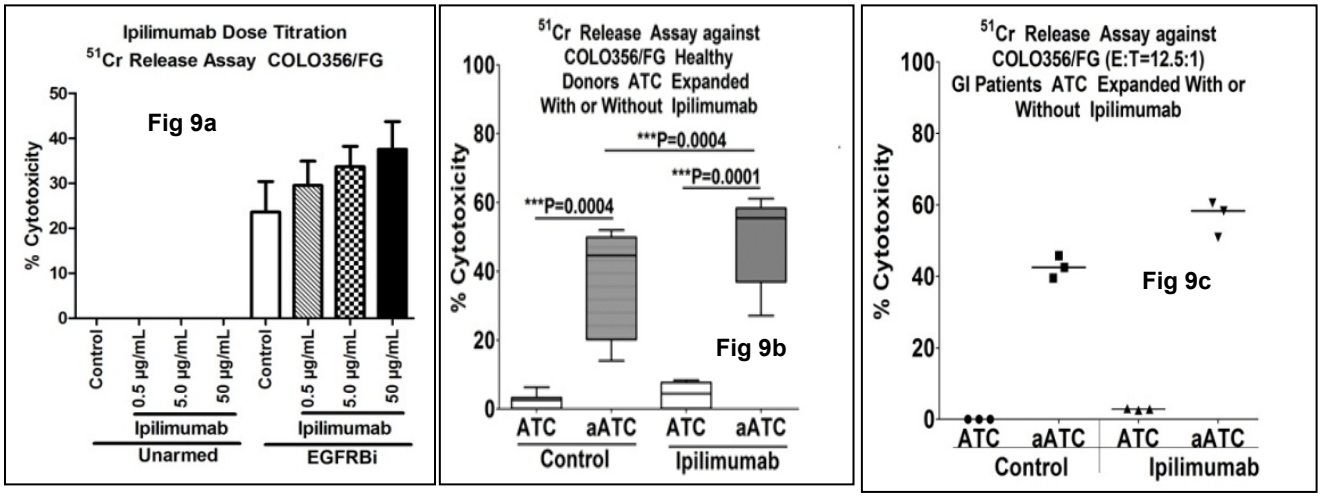


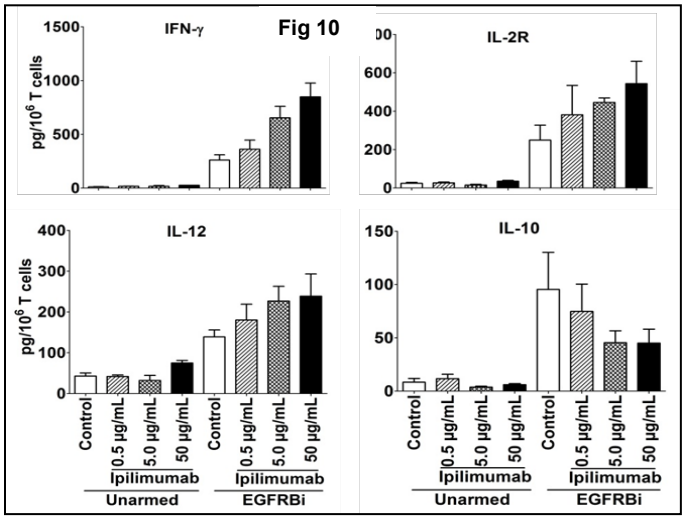
Fig 8 The K-M curve for phase I clinical trial in high risk subjects treated with adjuvant chemotherapy and HER2Bi-BATs infusions.

Summary of clinical trials using BiAb BATs (BATs). Our clinical trials show that: 1) large numbers of ATC can be expanded *in vitro,* armed with HER2Bi for breast cancer or CD20Bi for NHL and infused safely without DLTs(16, 33); 2) BATs can be detected by simple flow cytometry *in vivo* and are found to circulate up to 5 weeks (1-2%) after the last infusion without IL-2 infusions (data not shown)(31, 33); 3) most of the time, the targeted total infusion doses have been achieved without difficulty; no HAMA responses inhibited T cell infusions; 4) BATs can target and kill even low antigen-expressing tumors; and 5) BATs infusions induced the development of cytotoxic endogenous immune cells that could be detected in MBC subjects (34) as well as in subjects who received CD20 BATs after SCT(33); and 6) multiple infusions of BATs can induce robust cellular and humoral responses that can be collected, expanded, and used to boost anti-tumor responses after



HDC and SCT.

Immune Check Point Inhibitors enhance EGFR BATs function. Our group has demonstrated that combining ipilimumab, an immune checkpoint inhibitor, with 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 9a** shows the dose titration effect on cytotoxicity in the presence or absence of EGFR BATs (Armed ATC-aATC in the figure) and **Fig 9b** shows increased killing by normal EGFR BATs in the presence or absence of ipilmumab at an E:T of 25:1 for COLO356/FG. **Fig 9c** shows the same experiments for 3 colorectal subjects. BATs infusions increased the secretion of chemokines CCL2, CCL3, CCL4, CCL5, CXCL9, and GM-CSF [data not shown]. **Fig 10**



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.(53)

Induction of Serum IL-2 and GM-CSF Levels after 2 Weeks or 4 Infusions of BATs.

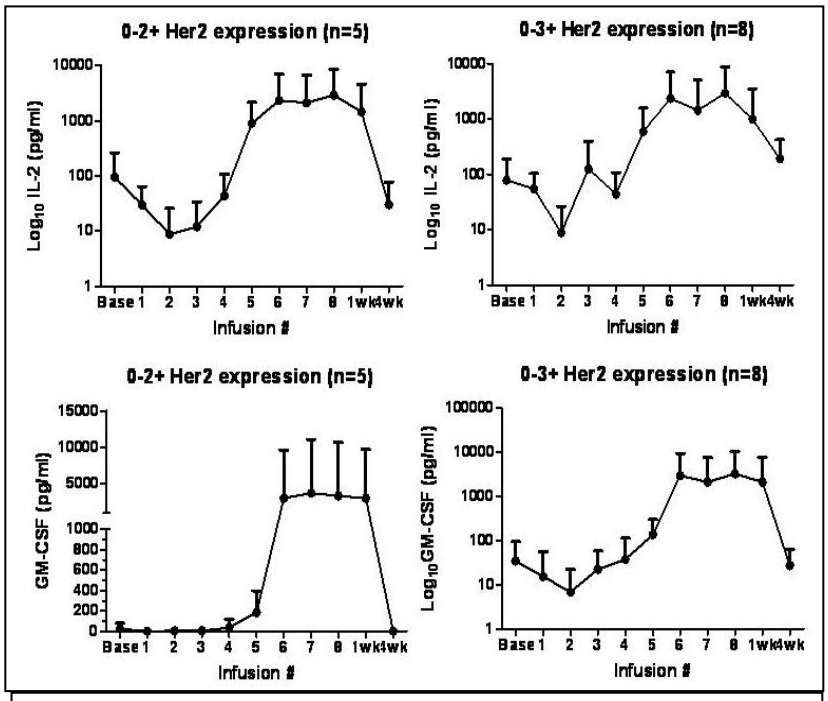


Fig 11: Serum IL-2 and GM-CSF Levels. The mean is indicated ± S.D. Serial serum samples collected at the indicated time points were quantitated with the BioPlex.

The serum concentration of IL-2 and GM-CSF were determined for several reasons. First, we asked whether we could detect the IL-2 and GM-CSF that was being given to subjects. Second, we wanted to assess the levels of IL-2 and GM-CSF that would be induced by multiple infusions of BATs in subjects with MBC. The mean levels of IL-2 (upper panels) and GM-CSF (lower panels) markedly increased by the 4th or 5th infusion (**Fig 11**). Low levels of IL-2 (<100 pg/ml) were detected until the 5th infusion and low levels of GM-CSF were detected up until the 4th infusion. The left panels show that a subset of Her2/*neu* 0-2+ subjects have similar response patterns to the entire group of subjects (0-3+ group in the right panels. The IL-2 data show that there is no increase or accumulation of the injected IL-2 up until the 3rd infusion (nearly 2 weeks of IL-2 injections). Similarly, there is no increase in serum GM-CSF until after the 3rd infusion (2 weeks after the twice weekly GM-CSF shots began). These data suggest that infusions of BATs in combination with low dose IL-2 and GM-CSF induce systemic immune responses. But it remains unclear whether IL-2 and GM-CSF were required to induce the observed responses. Our recent studies using anti-CD3 x anti-EGFR BiAb armed T cells (EGFR BATs) in 5 subjects with unresectable or metastatic pancreatic cancer showed that once weekly infusions of EGFR BATs was enough to induce a minor response that lasted 6.5 months with the median overall survival of 29.4 months. Furthermore, two subjects developed complete responses of their disease after they received subsequent chemotherapy after they were treated for progressive disease following their immunotherapy (SITC Abstract, Lum et al, 2016). Based on this observation, we elected to not add low dose IL-2 and GM-CSF to the combination of BATS and Pembrolizumab (PBZ) (MK-3475).

3.1.1 Pharmaceutical and Therapeutic Background

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 ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

3.1.2 Preclinical and Clinical Trial Data

Refer to the most recent Investigator's Brochure for PBZ for a review of the current preclinical and clinical data. In the phase Ib KEYNOTE-012 Study, MK-3475 was used to treat triple negative breast cancer (TNBC), gastric cancer, urothelial cancer, and head and neck cancer subjects IV at 10 mg/kg every 2 weeks in subjects with PD-L1+ tumors by immunohistochemistry. The report focused on the TNBC subjects. There were 27 evaluable subjects for anti-tumor activity with an overall response rate of 18.5%. The time to response was 17.9 weeks (range 7.3 -32.4 weeks) with a median duration of response ≥47.3 weeks (R. Nanda, JCO 34;2460). Rugo et al reported the preliminary efficacy and safety of MK-3475 for PKD-L1-positive estrogen receptor-positive (ER+)/HER2-negative advanced breast cancer enrolled in KEYNOTE-028 (SABCS S5-07, 2015).

3.1.3 Rationale for Dose Selection/Regimen/Modification

The planned dose of pembrolizumab for this study is 200 mg every 3-4 weeks for Arm 3 (less frequent for other arms). As pembrolizumab is being combined with an investigational treatment (HER2 BATs) in this study, pembrolizumab will not be given at a fixed q3 week frequency. Initially (to the first group of patients), one dose of pembrolizumab will be given only after HER2 BATs is completed. Based on safety, an additional dose will be added about halfway through BATs treatment and, if that is deemed safe, a dose will also be given prior to starting BATs infusions. Based on the totality of data generated in the Keytruda development program, 200 mg Q3W is the appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies in melanoma and NSCLC indications demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg Q2W, representing an approximate 5- to 7.5-fold exposure range (refer to IB, Section 5.2.2)
- Population PK analysis showing that both fixed dosing and weight-based dosing provides similar control of PK variability with considerable overlap in the distributions of exposures, supporting suitability of 200 mg Q3W
- Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3W across multiple indications, and

Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W.

3.1.4 Phase II toxicity data for the combination of PBZ and HER2 BATs

In a phase II study in metastatic castrate-resistant prostate cancer (mCRPC) patients, 8 infusions of Her2 BATs (up to 10^{10}/infusion) are being given twice per week for 4 weeks in combination with anti-PD-1 therapy with pembrolizumab given once every 3 weeks starting 3 weeks before the 1st BATs infusion. The primary objective is to assess the percentage of patients free of clinical progression at 6 months after registration. We hypothesized 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. The objective is to estimate the magnitude of change in anti-PC immune functions and markers, after infusions of Her2 BATs in mCRPC and to 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. The clinical toxicities in the first 9 patients is summarized in the table below. The toxicities observed were not different than reported in the phase I for hormone-refractory prostate cancer (HRPC) by Vaishampayan et al (54)and Lum et al (16).

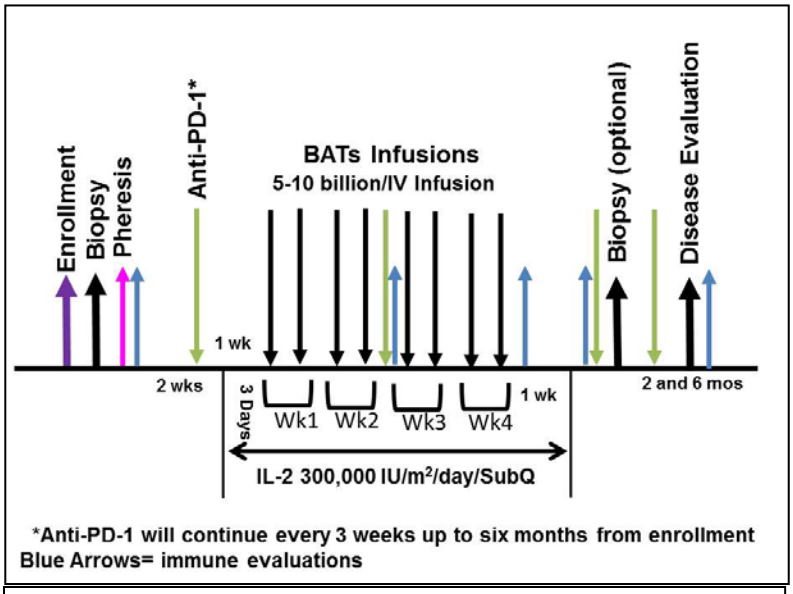


Fig 12. Schema for Treatment of mCRPC, phase II.

Number of Subjects	(%) of Subjects	Total Adverse Events	Grade 1	Grade 2	Grade 3
Subjects with AEs	55.6	16			
Chills	44.4	2	1	1	
Fatigue	22.2	2	2		
Fever	22.2	2	2		
Infusion reaction	22.2	1	1		
Headache	44.4	4	4		
Nausea	22.2	2	2		
Heartburn	11.1	1	1		
Immune system other	11.1	1			1
Hypertension	11.1	1			1

4.0 METHODOLOGY

4.1 Entry Criteria

4.1.1 Subject Inclusion Criteria

[1] Histologically confirmed breast cancer (infiltrating ductal or lobular breast carcinoma) with evidence of measurable metastatic disease. Metastatic disease must be biopsy proven.
   a. Since histologic type, lymphatic permeation, blood vessel invasion, and degree of anaplasia may be prognostic variables, appropriate slides of the primary lesion will be requested for future review. HER2, estrogen, and progesterone receptor positivity will be recorded.

[2] Measurable lesion. Patients are required to have at least one measurable non-bone lesion ≥10 mm that has not been irradiated.
   a. Measurable metastatic disease documented by radiograph, CT scan, PET/CT, MRI, or physical exam is required. Each subject will be required to have at least one measurable lesion that has not been irradiated with a minimum size in at least one diameter of ≥ 10 mm for liver lesions, lung, skin, and ≥ 15 mm lymph node metastases. Biopsy of recurrent site(s) is not required.

[3] Patients must have HER2 status determined by CISH, FISH or IHC. HER2 status of positive or negative are both eligible for the study.

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

[4] Be female ≥ 18 years of age
[5] Be willing and able to provide written informed consent for the trial.
[6] Have a performance status (PS) ECOG 0-1
[7] Have a life expectancy ≥ 3 months
[8] Demonstrate adequate organ function as defined in **Table 1**, all screening labs in the table should be performed within 10 days prior to on-study date.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute lymphocyte count	≥ 500/mm^3
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥ 100,000 / mcL Note: If there is an absence of other medical conditions related to thrombocytopenia that would preclude participation in the protocol as determined by the treating investigator, the platelet requirement is ≥ 75,000 / 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 creatinine clearance[1] (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**
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] Creatinine clearance should be calculated per institutional standard.	

[9] Female patients of childbearing potential should have a negative pregnancy test within 10 days prior to on-study date. If a urine or serum test is positive or cannot be confirmed as negative, the other (urine or serum pregnancy test, whichever was not performed first) will be required.

[10] Female patients of childbearing potential must be willing to use an adequate method of contraception as outlined in Section 4.5.2, for the course of the study through 120 days after the last dose of study medication.

[11] Patients must have had two or more lines of prior therapy (chemo or hormonal) in the metastatic setting

4.1.2 Subject Exclusion Criteria

The patient must be excluded from participating in the trial if the subject:

- Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to on-study date.
- Has a known history of active TB (Bacillus Tuberculosis)
- Hypersensitivity to PBZ or any of its excipients.
- Lack of recovery (i.e., ≤ Grade 1 or baseline prior to last line of cancer therapy) from non-laboratory adverse events except ≤ Grade 2 neuropathy

- Has history of another malignancy within the past 5 years. 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.

- 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 on-study date 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 leukapheresis. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

- 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.

- Has a known history of (non-infectious) pneumonitis/interstitial lung disease that required steroids or has current pneumonitis/interstitial lung disease.

- Has an active infection requiring systemic therapy.

- 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.

- Is pregnant or breastfeeding, or expecting to conceive 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.

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

- Has Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies) or known history of Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (HCV) antibody is detected. Note: Patients may be eligible if HCV antibody is detected as long as HCV viral load is undetectable following an FDA approved treatment regimen.

- Has received a live vaccine or live-attenuated vaccine within 30 days prior to on-study date. Administration of killed vaccines is allowed.

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.

- Has a history of significant cardiac disease, including:
    - History of a recent myocardial infarction (within one year), a past myocardial infarction (more than one year ago) along with current coronary symptoms requiring medications and/or evidence of depressed left ventricular function (LVEF < 45% by MUGA or ECHO).
    - Current history of angina/coronary symptoms requiring medications and/or evidence of depressed left ventricular function (LVEF < 45% by MUGA or ECHO)
    - Clinical evidence of congestive heart failure requiring medical management (irrespective of ECHO results).
- Pt may be excluded if, in the opinion of the PI and investigator team, the pt is not capable of being compliant.

4.2 Treatment Regimen

The administration of BATS and PBZ will be increased in a standard 3 + 3 dose escalation/dose de-escalation approach, starting with schedule #1. This approach combines 8 infusions of BATs (~10^{10}/infusion ± 20%) and one to three infusions of PBZ using a previously established schedule of BATs (1 x 10^{10} per infusion) and PBZ (200 mg). PBZ will be added to 8 infusions of BATs in 3 schedules: #1) after the 8th BATs infusion; #2) after the 4th and 8th BATs infusions; and then, #3) before the 1st and after the 4th and 8th BATs infusions. Once the maximum tolerated dose is reached, there will be a dose expansion cohort (DEC) of 12 evaluable subjects using the MTD to confirm the toxicity profile and estimate TTP, PFS, and OS.

According to standard 3 + 3 dose escalation/dose de-escalation rule, if there are 2 or more DLTs at Schedule #1, the study will be closed. If there are no dose limiting toxicities (DLTs) in the first 3 subjects (0/3) or at maximum 1 subject with DLT out of 6 subjects (1/6) with treatment of Schedule #1, we will advance to Schedule #2. If there are 0/3 or 1/6 DLTs at Schedule #2, we will advance to Schedule #3. If there are no more than 1/6 DLTs, Schedule #3 will be used as the MTD in this study. Otherwise, Schedule #2 will be used as the MTD if no more than 1 out of 6 patients at Schedule #2 have DLTs. This design will require a minimum of 3 and a maximum of 18 evaluable patients to identify the MTD. To take the eligible but not evaluable patients into account (estimated at 10%), the following plan is considered. Among 18 eligible patients, if the true unevaluable rate is 10%, 2 unevaluable

patients are expected. Since the probability of 4 unevaluable patients out of 18 eligible patients is 7% and the null hypothesis that the unevaluable rate is 10% can't be rejected at a one-sided type I error rate of 2.5%, a maximum of 4 unevaluable patients will be accepted. Thus, a maximum of 22 eligible patients may be recruited and among them 4 unevaluable patients will be allowed. Note that patients that are enrolled but do not receive any BATs infusions will not be included in this maximum. Similar consideration is taken for a DEC of 12 patients at the MTD. Among the 12 eligible patients in the DEC, a maximum of 3 unevaluable patients will be accepted considering that the probability of 3 unevaluable patients out of 12 eligible patients is 8.5%, which cannot reject the null hypothesis that the unevaluable rate is 10% at a one-sided type I error rate of 2.5%. Thus, a total of 15 eligible patients may be recruited in the DEC. As in the dose escalation phase, these maximums do not include patients that enroll but do not receive any BATs infusions.

This treatment regimen is illustrated in Figure 1.

4.2.1 Rationale for Staggered Enrollment Plan

In the original version of this protocol, a staggered enrollment was based on the historical information from the phase I studies using HER2 BATs wherein the acute toxicities are mostly resolved in 2-3 days and the side effects of PBZ are usually resolved before the next infusion 3 weeks after the first PBZ dose. In an ongoing phase II clinical trial in patients with hormone refractory prostate cancer (HRPC) [Personal communication Vaishampayan and Lum], PBZ and HER2 BATs with the same doses and schedule have been given to 9 patients on schedule #3 planned for this protocol without any dose limiting toxicities. Therefore, staggered enrollment is no longer required for this protocol.

4.2.2 BATS

4.2.2.1 BATS Preparation

Apheresis will be performed according to UVA standards. The apheresis procedure may be repeated (once) if adequate and viable cells are not available or cannot be used for other reasons from the first apheresis procedure.

Exclusions for apheresis related to past treatment prior to apheresis. Participants must not undergo apheresis if any of the following apply:

- Current or recent (within 4 weeks prior to apheresis) use of an investigational agent or investigational device.
- Current or recent (within 14 days prior to apheresis) use of an anti-cancer monoclonal antibody (mAb) except denosumab (Xgeva)
- Current or recent (within 14 days prior to apheresis) chemotherapy, targeted small molecule therapy, hormonal therapy, radiation therapy to the axial skeleton, monoclonal antibody therapy, or radiation therapy

- Note: If the participant was receiving hormonal therapy prior to study therapy, it may be restarted after the restaging scan 1 month after the 8th BATs infusion.

Leukapheresis Procedure. Reported toxicity from leukapheresis is minimal. Subjects have a small potential for infection, bleeding, and bruising from the placement of peripheral or central catheters used for leukapheresis. Use of the anticoagulant citrate dextrose may cause symptoms of mild hypocalcemia, which are controlled with calcium replacement. Peripheral IV catheters will be used for leukapheresis. If the apheresis nurses cannot obtain adequate venous access by pre-assess, the subject will be scheduled to have a central venous catheter placed by interventional radiology. The risks for central venous catheter placement include pain, blood loss, infection, pneumo/hemothorax (rare), cardiac arrest (extremely rare), and death (extremely rare). A large bore groin catheter may be placed for apheresis instead of a central catheter. Unless AEs lead to failure of lymphocyte collection or meet the criteria for a serious adverse event, they will not be reported.

Production of BATs. BATs are prepared in the UVa cGMP facility. Immediately after leukapheresis, the lymphocytes are activated with soluble monoclonal anti-CD3 antibody (OKT3), which cross-links the CD3 receptors on T cells and activates them. The ATC are expanded in the presence of IL-2 for up to 14 days. After culture, ATC are harvested, armed with OKT3 x Herceptin® (HER2Bi), washed to remove unbound HER2Bi, and cryopreserved in 10% dimethyl sulfoxide (DMSO) and 20% protein (human albumin or serum) using rate controlled freezing and storage in liquid nitrogen. No exogenous IL-2, OKT3, or other culture reagents (e.g. medium components) are present in the final cryopreserved product. Armed product is released for clinical use after Quality Control testing for sterility (bacterial and fungal culture, endotoxin and mycoplasma), phenotype (% of CD3 cells), and activity (*i.e*. cytolytic activity against the SKBR-3 cell line or equivalent).

OKT3. This is a murine IgG2a monoclonal antibody directed at human CD3 commercially available from Miltenyi Biotec, Auburn, CA. It is purchased in vials containing 1 mg/1 ml of reconstituted bacteriostatic water. OKT3 is used to activate T cells for growth and for heteroconjugation with Herceptin® to produce the HER2Bi bispecific antibody for arming subject ATC.

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. For this study, Herceptin® is heteroconjugated to OKT3 to produce the HER2Bi bispecific antibody for arming subject ATC.

Anti-CD3 (OKT3) x anti-HER2/*neu* heteroconjugated bispecific monoclonal antibody (HER2Bi). Anti-CD3 x anti-HER2 is produced under GMP conditions. IND #9985 was cleared for clinical trials by the FDA that specifies the production of bispecific antibody, sterility testing, and the standard operation procedures for arming of activated T cells. The

HER2Bi has been retested for targeting and cytotoxicity and shown to be stable for over 3 years stored at 4°C.

Facility. Qualified personnel who are familiar with procedures which minimize undue exposure to themselves and to the environment will undertake the preparation, handling, and safe disposal of immunotherapeutic agents in a self-contained protective environment. Cells will be generated in the UVA cGMP Facility under FDA #BB-IND 9985 with standard operating procedures (SOPs) for producing HER2Bi and growing, splitting, harvesting, arming of ATC, and cryopreservation of BATs.

Activation, Culture, and Freezing of BATs. Lymphocytes are obtained by leukapheresis and cultured at a density of 1-3 × 10^6 cells/ml in RPMI 1640 media (Lonza) containing 100 IU/ml of IL-2 (Novartis), 10-20 ng/ml of OKT3, and 2% human serum (Valley Biomedical or Hyclone). Cells will be cultured for a maximum of 14 days in medium to which no additional OKT3 will be added during the expansion period.

Preparation of anti-CD3 x anti-HER2 (HER2Bi) Bispecific Antibody. The specific details for the production, purification, and quality control testing are part of IND #9985.

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 hr 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.

Cytotoxicity Assay and IFNγ EliSpots. Cytotoxicity is measured in a 20 hr ^{51}Cr - release assay to ensure activity (minimum ≥ 10% cytotoxicity) of BATs over their unarmed counterparts. Tumor target 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. These wells containing tumor cells will be washed and armed or unarmed ATC will be added at different E:T ratios for 20 hr incubation at 37°C. The next day, the supernatants harvested from the microtiter wells will be counted and the percent specific lysis will be calculated. IFNγ EliSpots will be performed by plating SK-BR-3 targets.

Quality Assurance of BATs 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 sterility, mycoplasma, and endotoxin level. Records of all quality control measures will be maintained by the laboratory. Several separate cryovials of HER2Bi armed ATC will be frozen at the time of harvesting and cryopreservation of the armed cells for the future QC testing. Release criteria for armed products for clinical use include: negative results for anaerobic and aerobic bacterial cultures, fungal cultures, negative mycoplasma and endotoxin assays (endotoxin < 2.5 EU/mL, otherwise a calculated infusion rate will be used to ensure the subject received no more than 5 EU/kg/hr), and ≥10% cytotoxicity directed at HER2 target cells (25:1

E/T). Phenotyping of the product will be recorded. The preferred criteria for ATC are >50% CD3+ cells, <10% for CD19, CD20, CD16, and CD56+ cells. The product can still be used at the discretion of the investigator if some of the phenotyping criteria are not met.

4.2.2.2 BATS Infusions

Toxicity associated with BATs has been minimal. The reported symptoms have been reversible with cessation of therapy. Most subjects treated with BATs experienced no major side effects during infusions. Fever and chills were the most common reported reactions Other rarely reported effects included mild gastrointestinal and neurologic symptoms. Toxicity associated with reinfusion of cryopreserved cells may be partially attributable to the DMSO preservative. The BATs have been documented to cause fever, chills, nausea, vomiting, headache, hypotension, hypertension, and fatigue. See section 4.2.4 for dose limiting toxicities and Appendix for Supportive Care Guidelines for BATs infusions

4.2.2.2.1 Bispecific Antibody Armed Activated T-Cells (BATs) Infusion Instructions

BATs infusions will be done in the outpatient clinic infusion center (infusions may be performed within the UVA hospital or within the clinical research unit as long as appropriate code cart is available). All appropriate assurances for identification of product, subject, sterility, etc., will be performed prior to infusion according to the SOPs for the BATs IND. Frozen BATs will be thawed at the bedside of the subject just prior to infusion. If there is evidence of infusion-related toxicities, subsequent BATs will be thawed, washed, and resuspended in medium prior to infusion. BATs will be infused intravenously (IV) at a rate based on the endotoxin content of the product through a central venous access catheter (to minimize the risk of clotting). All subjects will be observed for at least 6 hours after each infusion. If stable, subjects will be discharged home with detailed instructions regarding when and how to contact the study team (see Appendix for an example) under the care of his/her caregiver. If the subject's home is more than 40 minutes away from UVA hospital, the subject will be instructed to stay locally with his/her caregiver for at least two nights following each BATs infusion. If the subject is asymptomatic after staying two nights within 40 minutes' drive of UVA, the subject may resume travel as usual.

Via connection to central venous access catheter, (CVC) infuse 500 ml of 0.9% NaCl over 1 hour prior to BATs infusion. Infusion rate for BATs will be subject specific. Flush CVC line with 100ml 0.9% NaCl following BATs infusion then run 0.9% NaCl at 200 ml/hr x 4 hrs.

Have emergency medications available for every BATs infusion (use institutional order set).

Notify treating MD if systolic BP is ≥ 160 or if diastolic is ≥ 90 prior to starting infusion. Hold infusion and confirm with Dr. Lum's staff that the infusion will be delayed.

4.2.3 Pembrolizumab

PBZ 200 mg will be administered as a 30 minute IV infusion at the times indicated by their assigned schedule. 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).

4.2.3.1 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 4.

Table 4 Product Descriptions

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

4.2.3.2 Packaging and Labeling Information

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

4.2.3.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor-Investigator 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.

4.2.3.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. The PBZ obtained from Merck will be stored in the investigational pharmacy at the University of Virginia Emily Couric Clinical Cancer Center.

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.

4.2.3.5 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.

4.2.4 Dose Modifications

If there are delays for the administration of BATs or PBZ, the schedules for administration of both BATs and PBZ will be delayed.

4.2.4.1 Dose Modification and Toxicity Management for Immune-Related AEs

There is no dose reduction allowed on the protocol. Adverse events (both non-serious and serious) associated with PBZ or BATs 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. Study treatment must be withheld for drug-related toxicities and severe or life-threatening AEs as per the table below. See *section 4.4.1* for supportive care guidelines, including use of corticosteroids and stopping guidelines, monitoring and follow-up for infusion reactions. In addition, the drugs can be held for any grade of toxicity by the treating physician if it is felt that it is the best interest of the subject.

Attribution of Toxicity: When study interventions are administered in combination, attribution of an adverse event to a single component is likely to be difficult. Therefore, while the investigator may attribute a toxicity event to pembrolizumab alone, to BATs alone, or to the combination, for adverse events listed in the table below, both interventions must be held according to the criteria in the table.

Holding Study Interventions: When study interventions are administered in combination, if the AE is considered immune-related, both interventions should be held according to recommended dose modifications.

Restarting Study Interventions:

Participants may or may not have any dose modifications (no change in dose or schedule) of pembrolizumab or BATs in this study, as described in the table.

If the toxicity does not resolve or the criteria for resuming treatment are not met, the participant must be discontinued from all study interventions.

If the toxicities do resolve and conditions are aligned with what is defined in the table, the combination of BATs and pembrolizumab may be restarted at the discretion of the investigator. In these cases where the toxicity is attributed to the combination or to BATs alone, re-initiation of pembrolizumab as a monotherapy may be considered at the principal investigator's discretion.

General instructions:

1. The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks. This criterion may also be met if the toxicity resolves to pre-study baseline grade (if toxicity was present at baseline).

2. For situations where study intervention has been withheld, study intervention may resume after the irAE decreased to ≤ Grade 1 (or baseline, as described above) after corticosteroid taper.
3. Study intervention must be permanently discontinued if the irAE does not resolve within 12 weeks of last dose or corticosteroid dose is not ≤10 mg prednisone or equivalent per day within 12 weeks of the last study treatment.
4. Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids.

Table 5: Dose Modification Guidelines for Immune Related Adverse Events

Immune-related AEs	Toxicity grade or conditions (CTCAE v4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
	Recurrent Grade 3 or Grade 4	Permanently discontinue		
AST / ALT elevation or Increased bilirubin	Grade 2[a]	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3[b] or 4[c]	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	

Type 1 diabetes mellitus (T1DM) or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold[d]	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[d]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[d]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological toxicities	Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3 or 4	Permanently discontinue		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN or DRESS	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes •
	Confirmed SJS, TEN or DRESS	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event[e].		

	Grade 4 or recurrent Grade 3	Permanently discontinue		

AE=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; GI=gastrointestinal; IO=immuno-oncology; ir=immune related; IV=intravenous; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

a. AST/ALT >3.0 to 5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin >1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal
b. AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 to 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 to 10.0 x ULN if baseline normal; >3.0 to 10.0 x baseline if baseline abnormal
c. AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal
d. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.
e. Events that require discontinuation include, but are not limited to: encephalitis and other clinically important irAEs. (eg. vasculitis and sclerosing cholangitis).

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, subject vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor-Investigator. The reason for interruption should be documented in the subject's study record.

With investigator and Principal Investigator agreement, subjects with a laboratory adverse event still at Grade 2 after 12 weeks may continue treatment in the trial only if asymptomatic and controlled. Subjects who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of PBZ should be discontinued from trial treatment.

4.2.4.2 Other Dose Modification

If there is grade 3 toxicity at any time, HER2BATs treatment will be held until toxicity improves to grade 0 or baseline. The treating physician may decide not to proceed with the next infusion for any grade of toxicity if it is deemed appropriate for the subject’s overall clinical care.

4.2.5 Trial Blinding/Masking

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

4.3 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in section 4.3.2 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 final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician. However, the decision to

continue the subject on trial therapy requires the mutual agreement of the Investigator and the subject.

4.3.1 Acceptable Concomitant Medications

Subjects will 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 will be recorded. Subjects will receive calcium replacement during the pheresis procedure, if needed for symptoms of hypocalcemia.

Subjects will be routinely pre-medicated before BATs infusion with diphenhydramine 50 mg IV or PO or per MD discretion and acetaminophen, 650 mg PO 20-60 minutes prior to infusion of cryopreserved BATS, to prevent fever, chills, and pain. The subject will alternate ibuprofen 600 mg every 8 hours with acetaminophen 650 mg every 8 hours for 24 hours after the BATs infusion.

If the participant was receiving hormonal therapy prior to study therapy, it may be restarted after the restaging scan 1 month after the 8th BATs infusion.

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. This includes antineoplastic systemic chemotherapy or biological therapy (except immunotherapy) after apheresis through 1 week prior to first study treatment (BATs infusion or pembrolizumab). All concomitant medication including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids should be noted in the medical record.

Concomitant medications should be monitored regularly during the study according to standard clinical care and to confirm that no prohibited concomitant medications are used. Concomitant medications do not need to be recorded or reported for research unless they relate to reporting for an SAE or an ECI.

4.3.2 Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy may not be administered prior to apheresis and from 1 week prior to first study treatment (BATs infusion or pembrolizumab) through 1 week following last BATs infusion
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents not specified in this protocol
- Radiation therapy

  - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.

- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.
- 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 (e.g. equivalent to or less than oral prednisone 10 mg daily) is allowed.
- Subjects CAN continue on bone modifying agents such as zolendronic acid or densumomab if deemed necessary by the treating physician.
- Subjects with HER2+ breast cancer should not be receiving an anti-HER2 drug while receiving combination treatment with BATS and PBZ. Anti-HER2 treatment should be discontinued at least 14 days prior to the first BATS infusion.

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.

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

4.4 Rescue Medications & Supportive Care

4.4.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Guidelines for supportive care for BATs infusions are included in the Appendix.

- **Ancillary therapy:**

  Subjects will 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 will be recorded.

- **Steroids/other therapy:**
  With the exception of steroids for anti-emetic regimen, adrenal failure, septic shock, pulmonary toxicity or hormones administered for non-disease-related conditions (e.g. insulin for diabetes), steroids will not be administered. The subjects will stop hormonal

therapy 2 weeks prior to on-study date. Hydrocortisone (50-100 mg IV every 6 hours +/- 30 minutes) for severe adverse reactions related to BATs infusions is allowed.

Suggested supportive care measures for the management of immune-related adverse events at least possibly related to study treatment 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 PBZ. Supportive care guidelines for events determined to be related to HER2 BATs may be found in the Appendix.

Note: if after the evaluation the event is determined not to be immune-related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to section 4.2.4 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 (1-2 mg/kg/day of prednisone or equivalent). Higher doses of steroids may be used if deemed clinically indicated.
    - 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.
    - Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment.
    - 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:** In subjects with moderate enterocolitis (Grade 2), BATs infusion or PBZ should be withheld and anti-diarrheal treatment should be started. If symptoms are persistent for more than one week, systemic corticosteroids should be initiated (0.5 mg/kg/day of prednisone or equivalent).
- For **Grade 3 or 4 diarrhea/colitis:** In subjects with severe enterocolitis (Grade 3 or greater), BATS infusion or PBZ will be permanently discontinued and treatment with systemic corticosteroids should be initiated at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. If the treating physician believes that the subject is deriving clinical benefit from the study drug, then the treating physician after consultation with the Principal Investigator may discuss with the subject regarding continuing protocol related therapy.
- 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) or evidence of β-cell failure**
  - 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.
    - Administer anti-hyperglycemic in participants with hyperglycemia
    - Evaluate subjects 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 (or baseline if existing prior to study entry), 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. Consider monitoring subjects for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

- **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
  - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
  - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
- **Grade 3-4** hyperthyroidism
  - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less (or baseline if existing prior to study entry), 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 or more frequently) or baseline, if existing prior to study entry.
  - Treat with IV or oral corticosteroids (0.5-1 mg/kg/day of prednisone or equivalent).
  - For **Grade 3-4** events, treat with intravenous corticosteroids (1-2 mg/kg for 24 to 48 hours.
  - When symptoms improve to Grade 1 or less (or baseline, if existing prior to study entry), a steroid taper should be started and continued over no less than 4 weeks.
- **Renal Failure or Nephritis**:
  - For **Grade 2-4** events, treat with corticosteroids (1-2 mg/kg/day of prednisone or equivalent).
  - When symptoms improve to Grade 1 or less (or baseline, if existing prior to study entry), steroid taper should be started and continued over no less than 4 weeks.
  - Monitor changes in renal function
- **Nausea/vomiting:**

  Nausea and vomiting should be treated aggressively, and consideration should be given in subsequent cycles to the administration of prophylactic antiemetic therapy

according to standard institutional practice. Subjects should be strongly encouraged to maintain liberal oral fluid intake.

- **Infection:**

  Subjects with a documented infectious complication should receive oral or IV antibiotics or other anti-infective agents as considered appropriate by the treating investigator for a given infectious condition, according to standard institutional practice.

- **Management of Infusion Reactions**:

  Acute infusion reactions (which can include cytokine release syndrome, angioedema, or anaphylaxis) are different from allergic/hypersensitive reactions, although some of the manifestations are common to both AEs. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Signs/symptoms may include: Allergic reaction/hypersensitivity (including drug fever); Arthralgia (joint pain); Bronchospasm; Cough; Dizziness; Dyspnea (shortness of breath); Fatigue (asthenia, lethargy, malaise); Headache; Hypertension; Hypotension; Myalgia (muscle pain); Nausea; Pruritis/itching; Rash/desquamation; Rigors/chills; Sweating (diaphoresis); Tachycardia; Tumor pain (onset or exacerbation of tumor pain due to treatment); Urticaria (hives, welts, wheals); Vomiting.

  Table 6 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of PBZ (MK-3475).

Table 6: Infusion Reaction Treatment Guidelines for PBZ

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	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of PBZ (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

Table 6: Infusion Reaction Treatment Guidelines for PBZ		
NCI CTCAE Grade	**Treatment**	**Premedication at subsequent dosing**
	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.**	
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 that led to this toxicity.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

4.5 Diet/Activity/Other Considerations

4.5.1 Diet

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

4.5.2 Contraception

PBZ and/or BATs may have adverse effects on a fetus in utero. Furthermore, it is not known if PBZ and/or BATs have transient adverse effects on the composition of sperm.

Female subjects will be considered of non-reproductive potential if meets one of the below criteria:

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

replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);
(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;
(3) has a congenital or acquired condition that prevents childbearing.

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

(1) practice abstinence† from heterosexual activity;
(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are**:

Single method (one of the following is acceptable):

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

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

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

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

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

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

days after the last dose of PBZ. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

4.5.3 Use in Pregnancy

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

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to Merck.

4.5.4 Use in Nursing Women

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

4.5.5 Duration of Treatment

Subjects will continue to receive study treatment (defined as BATS in combination with PBZ) until:

- Subject has completed all protocol specific treatments
- Confirmed disease progression (per irRECIST)**
- Unacceptable adverse event(s)
- Subject withdraws consent for further treatment
- Female subject has a confirmed positive serum pregnancy test
- Subject is determined to not be eligible for apheresis as required by the study, as determined by the Stem Cell Transplant Team
- Subject's cells do not grow adequately (to the assigned dose level) during activation and arming process or cannot be used for other reasons (e.g., viability, contamination or bag breakage in the freezer). As indicated in section 4.2.2.1, apheresis may be repeated (once) if adequate and viable cells are not available from the first apheresis procedure.
- Subject receives a non-study treatment (not protocol-specified) for their cancer
- Subject non-compliance
- General or specific changes in the subject's condition render the subject unacceptable for further treatment in the judgment of the investigator

** Subjects do not have to be discontinued from treatment for pseudoprogression (flare). Progression occurring within the first 12 weeks of treatment must be confirmed by repeat cross sectional imaging within 4 weeks as per irRECIST.

If any of the above events occurs, the subject will be considered off treatment and in follow-up. Subjects that do not receive any doses of BATs will not be in follow-up, but will only be followed for safety as described in section 6.3. Subjects that discontinue prior to receiving all scheduled doses of BATs and/or PBZ may continue to participate in the remaining aspects of the study (tissue samples, immune studies, follow-up, etc.) and will be included in the intent to treat analysis. A subject who is withdrawn or discontinues from the study after receiving at least 2 BATs infusions is considered a discontinuation and will not be replaced.

4.5.6 Discontinuation/Withdrawal from Study

Subjects may withdraw or be discontinued from the study at any time for any of the following reasons:

- Subject decides to withdraw from the study
- Subject is unable or unwilling to complete the follow-up procedures
- The subject is lost to follow-up
- Death

All subjects that discontinue or withdraw from the study will continue to receive standard of care treatment. Subjects who have withdrawn consent for the study will not be followed for any reason.

4.5.7 Subject Status Definitions

Enrolled: All subjects who sign an informed consent will be considered enrolled on the study. All subjects consented to the study must be entered into OnCore.

Screen Failure: A subject who is withdrawn or discontinues from study screening prior to being determined eligible is considered a screen failure. Screen failures are not considered a study accrual and will be replaced. Note: The UVa IRB-HSR defines any individual that has signed an informed consent as an enrollment in this study and so screen failures should be reported to the IRB with enrollment numbers.

On-Study: A subject is considered on-study on the date when the study team has confirmed the subject has met all of the inclusion and none of the exclusion criteria, and the treating physician/surgeon or study PI has signed off on the confirmation.

On-Treatment: A subject is considered on-treatment on the date that they receive the first treatment of either BATS or PBZ.

Off-Treatment: A subject is considered off-treatment on the date that they have met any of the criteria listed in Section 4.5.5.

On Follow-up: A subject is considered on follow-up on the date that they have met any of the criteria listed in Section 4.5.5.

Off-Study: A subject is considered off-study if they are removed from the study for any of the reasons listed in Section 4.5.6, or if they have completed the follow-up interval.

4.6 Clinical Criteria for Early Trial Termination

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

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

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

5.0 TRIAL FLOW CHART

5.1 Study Flow Chart

Trial Period:	Pre-Treatment		Study Treatments											Post-Treatment Follow-Up[1]			
Visit:	Prior to On--Study[10]	Pheresis	Pembro #1	BATS #1	BATS #2	BATS #3	BATS #4	Pembro #2	BATS #5	BATS #6	BATS #7	BATS #8	Pembro #3	Visit 1	Visit 2	Visit 3	Visit 4
Timing:		-30d--21d	-7d	Week 1		Week 2		Week 3-4	Week 5		Week 6		Week 7	14 d	30 d	90 d	6m
Scheduling Window (Days):			± 2d	± 3d	± 3d	± 3d	± 3d		± 3d	± 3d	± 3d	± 3d	± 3d	± 5d	+ 10d	+ 20d	± 10d
Administrative Procedures																	
Informed Consent	X																
Inclusion/Exclusion Criteria	X																
Demographics and Medical History	X																
Study Treatments/Procedures																	
Pheresis		X															
BATS infusion[2]				X	X	X	X		X	X	X	X					
PBZ			X[3]					X[4]					X				

[1] Follow-up visit timing listed is based on the date of the last study treatment. See *section 6.2.5.3*.
[2] Subject should receive BAT #1-8 infusions twice per week (+/- 3 days).

[3] Initiate PBZ infusion 7 ± 2 days prior to BATS infusion for Schedule 3 only.
[4] Schedules 2 and 3 only.

Trial Period:	Pre-Treatment		Study Treatments											Post-Treatment Follow-Up[1]			
Visit:	Prior to On--Study[10]	Pheresis	Pembro #1	BATS #1	BATS #2	BATS #3	BATS #4	Pembro #2	BATS #5	BATS #6	BATS #7	BATS #8	Pembro #3	Visit 1	Visit 2	Visit 3	Visit 4
Timing:		-30d--21d	-7d	Week 1		Week 2		Week 3-4	Week 5		Week 6		Week 7	14 d	30 d	90 d	6m
Scheduling Window (Days):			± 2d	± 3d	± 3d	± 3d	± 3d		± 3d	± 3d	± 3d	± 3d	± 3d	± 5d	+ 10d	+ 20d	± 10d
Clinical Procedures/Assessments																	
Review Adverse Events			X	X	X	X	X	X	X	X	X	X	X	X	X	X (SAEs only)	
Physical Examination/Performance status	X		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		
ECG	X																
MUGA or ECHO	X													X			
Disease Status, Additional Treatment[1]															X	X	X
Laboratory Procedures[6]																	
β-HCG (for WOCBP)	X	X[11]															

5 If clinically indicated, additional evaluation studies (one 10 mL red top tube) may be drawn at any or all of the following time points: pre-infusion, 1, 2, and 4 hours (+/- 15 minutes), 8 and 16 hours (+/- 1 hour), 24, 48, and/or 72 hours (+/- 2 hours) after each infusion, to study cytokine release. "Clinically indicated" is based on the clinical judgment of the investigator.
6 Labs required prior to treatments do not need to be repeated if labs have been performed within 72 hours prior to dose
7 A total of 60 mL of blood will be drawn at each of the designated time-points. One 10 mL red top tube and five 10 mL green or lavender top tubes containing anti-coagulant. Immune evaluation blood drawn on the same day as BATs #1 and #5 should be drawn prior to BATs infusion. Blood for immune evaluation does not need to be collected after first disease progression following participation.
8 Tumor imaging should be performed at intervals consistent with routine clinical care as close to recommended times as possible. Alterations to the usual schedule are not considered protocol deviations as long as the reason for the alteration is documented.
9 Please refer to Table 8 for full list of lab requirements
10 As described in the eligibility criteria, hematological, renal, hepatic, and coagulation labs should be completed within 10 days of on-study. The screening MUGA scan or ECHO may be done within 60 days of on study date. All other screening procedures must be done within 4 weeks of on-study date.
11 β-HCG (for WOCBP) prior to apheresis may be completed within 7 days prior to apheresis. It does not need to be repeated if the screening test was completed within 7 days prior to apheresis. This may be completed at a non-UVA laboratory at the convenience of the participant.
12 TSH, T3 and T4 testing at the time of PBZ treatment following all BATs infusions is only required for participants on Schedule #3

Trial Period:	Pre-Treatment		Study Treatments											Post-Treatment Follow-Up[1]			
Visit:	Prior to On--Study[10]	Pheresis	Pembro #1	BATS #1	BATS #2	BATS #3	BATS #4	Pembro #2	BATS #5	BATS #6	BATS #7	BATS #8	Pembro #3	Visit 1	Visit 2	Visit 3	Visit 4
Timing:		-30d--21d	-7d	Week 1		Week 2		Week 3-4	Week 5		Week 6		Week 7	14 d	30 d	90 d	6m
Scheduling Window (Days):			± 2d	± 3d	± 3d	± 3d	± 3d		± 3d	± 3d	± 3d	± 3d	± 3d	± 5d	+ 10d	+ 20d	± 10d
HBsAg B & C and HIV	X																
PT/INR and aPTT	X																
CBC with Differential	X	X	X	X				X	X				X	X	X	X	
Serum Chemistry Panel[9]	X	X	X	X				X	X				X	X	X	X	
Urinalysis	X																
TSH, T3, T4	X												X[12]	X			
Immune Evaluations[7]		X		X					X					X	X	X	X
Cytokine assays[5]				X	X	X	X		X	X	X	X					
Efficacy Measurements																	
Tumor Imaging[8]	X														X	X	X

6.0 TRIAL PROCEDURES

6.1 Trial Procedures

The Trial Flow Chart summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

6.1.1 Administrative Procedures

6.1.1.1 Informed Consent

An investigator must obtain documented consent from each potential subject prior to participating in a clinical trial. Consent must be documented by the subject'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.

6.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

6.2 Registration

Subject must meet all of the eligibility requirements listed in section 4.1 prior to registration.

Subjects who are consented to the study must be registered in OnCore in accordance with the University of Virginia Cancer Center OnCore SOP, which can be found on the OnCore Resources page (see the Oncore Help tab). General guidelines are provided below for reference, but the procedure should follow the OnCore SOP in case of any discrepancy.

All subjects who have signed an informed consent should have the following information entered into OnCore:

- demographics
- date of signed informed consent

6.2.1.1 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, past conditions that affect eligibility and history of all past cancers and cancer treatment. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

6.2.2 Clinical Procedures/Assessments

6.2.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Please refer to section 6.3 for detailed information regarding the assessment and recording of AEs.

6.2.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period and prior to the first PBZ (for schedule #3) or HER2 BATs infusion (schedules #1 and #2) (for baseline conditions) only. Clinically significant abnormal findings should be recorded as medical history.

At subsequent visits specified in the Trial Flow Chart, the investigator or qualified designee will perform a directed physical exam as clinically indicated.

6.2.2.3 Performance Scale

The investigator or qualified designee will assess ECOG performance status at time points specified in the Trial Flow Chart.

6.2.2.4 Tumor Imaging and Assessment of Disease

Tumor measurements should be done clinically by physical exam and by CT, MRI or PET/CT prior to initiation of treatment.

Imaging should be repeated throughout the study at intervals consistent with routine clinical care. The images should be evaluated using immune related RECIST criteria (irRECIST) (55, 56) (see Section 6.2.2.4.1) and RECIST. If progression is noted on radiographic imaging, confirmation by a second scan in the absence of rapid clinical deterioration is required. Data will be collected for post-hoc analysis by RECIST criteria, but RECIST will not be used for any decisions related to study treatment.

6.2.2.4.1 Response Definitions Immune-Related RECIST Criteria (irRECIST)

Measurable lesions must be accurately measured in at least one dimension with a minimum 10 mm in the longest diameter for non-nodal lesions, and a minimum 15 mm in short axis for lymph nodes. At baseline, the sum of the longest diameter (SumD) of all target lesions will be measured. At each subsequent tumor assessment, the longest diameters of non-nodal target and new non-nodal measurable lesions, and short axes of nodal target and new nodal measurable lesions should be added together to provide the total measurable tumor burden (TMTB):

Total Measurable Tumor Burden = SumD index lesions + SumD new, measurable lesions

Response categories are defined as immune-related complete response (irCR), immune-related partial response (irPR), immune-related stable disease (irSD), immune-related progressive disease (irPD) and confirmed progressive disease. Decreases in total measurable tumor burden are compared to the baseline tumor burden. New measurable lesions (≤ 2 lesions per organ, ≤ 5 lesions total, per time point) must meet the same criteria as defined for baseline target lesion selection (i.e., at least 10 mm in the longest diameter). These new patterns are considered clinically meaningful because they appear to be associated with favorable survival. Importantly, early increases in the size of lesions may be due to infiltration of lymphocytes and does not preclude an irCR, irPR, or irSD at the next consecutive time point. If a subject is classified as having irPD, confirmation by a second scan in the absence of rapid clinical deterioration is required. Thus, the definition of confirmation of progression represents an increase in tumor burden of at least 20% (minimum of 5 mm) compared with baseline or nadir at two consecutive time points at least 4 weeks apart. It is recommended that this confirmation be done at the discretion of the investigator in the context of the subject's tumor type, disease stage, and clinical status because awaiting a response after tumor burden increase may not be appropriate for subjects with rapid symptomatic progression accompanied by a decline in performance status.

Table 7. Derivation of irRECIST overall responses

Complete Response (irCR)	Complete disappearance of all measurable and non-measurable lesions. Lymph nodes must decrease to < 10 mm in short axis. No new lesions Confirmation of response as indicated.
Partial Response (irPR)	Decrease of ≥ 30% in TMTB relative to baseline No unequivocal progression of non-target lesions No new lesions. Confirmation of response as indicated.
Stable Disease (irSD)	Failure to meet criteria for irCR or irPR in the absence of irPD.
Progressive Disease (irPD)	Increase of ≥20% (minimum of 5 mm) in TMTB compared to baseline or nadir OR Progression of non-target lesions OR New lesion

	Confirmation of progression is recommended minimum 4 weeks after the first irPD assessment.
Confirmed Progressive Disease	New unequivocal progression or worsened progression from initial PD visit OR Appearance of another new lesion
Other	**irNE**, used in exceptional cases where insufficient data exists. **irND**, in adjuvant setting when no disease is detected.

6.2.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below in **Table 8**.

Table 8. Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	(β-hCG)†
Hemoglobin	Alkaline phosphatase	Glucose	PT (INR)
Platelet count	Alanine aminotransferase (ALT)	Protein	aPTT
WBC	Aspartate aminotransferase (AST)	Specific gravity	Total triiodothyronine (T3)
Red Blood Cell Count	Carbon Dioxide ‡	Microscopic exam *(If abnormal)*	Free thyroxine (T4)
Absolute Neutrophil Count	*(CO_2 or biocarbonate)*	β-hCG †	Thyroid stimulating hormone (TSH)
Absolute Lymphocyte Count	Uric Acid		
	Calcium		
	Chloride		
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Total protein		
	Blood Urea Nitrogen		

† WOCBP only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.

6.2.4 Immune Evaluations

To measure functional changes in immune cell populations as a consequence of immunotherapy, anti-tumor cytotoxicity will be examined. Samples of serum will be stored for future evaluations such as cytokine and anti-tumor antibody immune responses. The specific procedures are well-described in the literature. Blood drawing studies will be performed as outlined in the Trial Flow Chart. The subsequent paragraphs summarize the tests and procedures.

Serum cytokine/chemokine levels: Serum will be stored for subsequent cytokine analysis at the indicated time points, if clinically indicated.

PBMC subsets and phenotype analysis: Peripheral blood mononuclear cell (PBMC) and tumor-infiltrating lymphocyte (TIL) samples using immunohistochemistry to determine T cells and T cell subpopulations.

Cytotoxic activity and IFNγ production in PBMC populations: PBMC will be tested in specific cytotoxicity and IFNγ EliSpot assays after exposure to SK-BR-3 (HER2+ breast cancer), or Daudi and/or Raji (Burkitt's lymphoma) at the indicated time points. Specific activity of pre-IT samples will be compared to those obtained during and post-IT.

6.2.5 Visit Requirements

6.2.5.1 Screening

Prior to the performance of any study-specific procedures, the subject will have the nature of the study explained to them, and will be asked to give written informed consent. Informed consent must be obtained prior to any study-specific procedures that do not form a part of the subject's normal care. However, assessments performed according to standard of care prior to receipt of informed consent may be utilized to fulfill the screening requirement, if completed within the required window for screening.

Eligibility criteria will be assessed as stated in section 4.1. Eligible subjects will be leukapheresed after being determined eligible to obtain lymphocytes for expansion or when the lymphocyte population has recovered to ≥ 500 lymphocytes/mm^3.

6.2.5.2 Treatment Period

The treatment period will consist of the duration of intervention with any of the treatments listed in the Treatment Regimen section. Subject should receive study assessments as defined in the Trial Flow Chart.

6.2.5.3 Post-Treatment Visits

Follow-up visits should be scheduled within 14 (±5) days, 1 month (±5 days), 3 months (±10 days) and 6 months (±10 days) following administration of the last study treatment in all subjects to perform the assessments defined in the Trial Flow Chart. As described in the Trial

Flowchart, IEs will be done if there continue to be immune responses at 6 month ((± 2 weeks) intervals until the responses have disappeared (e.g. if there is a positive response at 6 months post study treatment, then an IE will be collected approximately 6 months later. If that test is positive, then she would be tested again 6 months later).

Following the 6 month post-treatment visit, study staff will contact subjects (by phone or email, or passively via medical record) every 3 months for survival, disease status, and information on cancer treatment until death, withdrawal of consent, or the end of the study, whichever occurs first. If this information is in the medical record, no contact is necessary. Subjects who discontinue trial treatment for a reason other than disease progression should continue to have imaging performed and tumor measurements by irRECIST until first progression following study treatment.

6.3 Assessing and Recording Adverse Events

After informed consent has been obtained, and prior to initiation of investigational treatment, only serious adverse events caused by a protocol-mandated intervention should be reported (e.g., serious adverse events related to procedures such as leukapheresis). AEs reported between the time the subject signed the informed consent and the administration of the first dose of any study medication will be captured as concurrent medical history unless due to a protocol-related procedure.

After initiation of study treatment, all adverse events will be reported according to the guidelines in the following sections. Throughout the study, investigators should report any deaths, serious adverse events, or other adverse events of concern that are believed to be related to the investigational intervention.

An adverse event is defined as any untoward medical occurrence in a subject 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 an investigational agent, is also an adverse event.

Adverse events may occur during the course of clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events.

Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

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

6.3.1 Assessment of Adverse Events

After initiation of investigational intervention (BATs or PBZ), all adverse events, whether reported by the subject or noted by study personnel, should be reported according to the guidelines in the following sections. After this period, investigators should report any deaths, serious adverse events, or other adverse events of concern that are believed to be related to the investigational intervention.

Adverse events clearly related to prior chemotherapy (e.g. neuropathies that have not changed with treatment or symptoms such as pre-existing nausea, fatigue) will be recorded as baseline.

Adverse events should be assessed for seriousness, severity, attribution and expectedness, in relation to PBZ treatment and BATs treatment, by the Principal Investigator or designee. The following sections provide definitions for adverse event characteristics and reporting requirements.

AEs should be followed to resolution or stabilization, and reported as SAEs if they become serious. This also applies to subjects experiencing AEs that cause interruption or discontinuation of study intervention, or those experiencing AEs that are present at the end of their participation in the study. Such subjects should receive post-treatment follow-up as appropriate.

If an ongoing AE changes in its severity or in its perceived relationship to study drug, a new AE entry for the event should be completed with the following exception. During the time between one infusion and the next, or in the 72 hours following the 4th and 8th BATs infusions, events should only be recorded once with the maximum severity grade that the participant experienced.

AE and SAE recording will continue until 30 days after the last dose of study drug. SAE recording for all events (regardless of relation to study treatment) will continue through at least 90 days following the last dose of study treatment. SAEs considered related to study drug may be recorded and reported at any time, even after the patient's final visit.

6.3.1.1 Adverse Event Definition

An adverse event is any untoward medical occurrence in a subject who has received an investigational intervention, whether or not related to the investigational intervention(s). Medical conditions present before starting the investigational intervention will be considered adverse events only if they worsen after starting study treatment. Adverse events include unfavorable, harmful or pathological changes in the general condition of a subject; subjective

or objective symptoms (spontaneously offered by the subject and/or observed by the Investigator or the study nurse); intercurrent events or exacerbation of pre-existing diseases which occurred after the administration of the study drug; clinically significant changes in laboratory abnormalities; or any undesirable and unintended effect of research occurring in human subjects as a result of the collection of identifiable private information under the research. Progression of the cancer under study is not considered an adverse event.

6.3.1.2 Secondary Adverse Events

In general, events occurring secondary to other events (e.g., cascade events or clinical sequelae) should be considered a single event identified by the primary cause. However, a secondary event should be listed as an independent event if it meets one of the following criteria:

- severe
- serious
- separated in time from the primary event

If it is not clear as to whether events are dependent, then record as separate adverse events.

6.3.1.3 Preexisting Medical Conditions

A preexisting medical condition is one that is present during the pre-study screening assessments. These conditions should be noted on the Medical History form. Preexisting medical conditions are not considered adverse events unless any of the following characteristics worsen following initiation of any study-related procedure:

- frequency
- severity
- character

If any of the above conditions apply, then this should be recorded as an adverse event. Remember to convey that this is a change in a preexisting condition when describing the event (e.g., “increased frequency of kidney stones”).

6.3.1.4 Expectedness

The expectedness of the adverse event will be determined by the Investigator based on current literature, the Investigator’s Brochure or package insert and the Investigator’s experience. See Appendix for more details on expected risks.

6.3.1.5 Severity

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.03. A copy of the CTCAE version 4.03 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

To assess severity of adverse events not included in the CTCAE version 4.03, use the table below.

Grade	Severity
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL[a]
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]
4	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE

a. Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
b. Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

6.3.1.6 Attribution

The Principal Investigator will evaluate all AEs and assess their toxicity and attribution, if any, to PBZ or BATs treatment. See **Table** for further guidance on evaluation of attribution. The following criteria will define the attribution:

Definite: The AE is clearly related to the investigational intervention.
Probable: The AE is likely related to the investigational intervention.
Possible: The AE may be related to the investigational intervention.
Unlikely*:* The AE is doubtfully related to the investigational intervention.
Unrelated*:* The AE is NOT related to the investigational intervention.

6.3.1.7 Seriousness

A serious adverse event (SAE) or serious adverse drug reaction (ADR) is any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

- Results in death;
- Is life threatening (places the subject at immediate risk of death from the event as it occurred);
- Results in a persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Other important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when,

based upon appropriate medical judgment, they may jeopardize the subject or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. For reporting purposes, also consider the occurrences of pregnancy as an event which must be reported as an important medical event.

*Hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event, except if the hospitalization meets at least one of the following criteria:

- The hospitalization is less than 24 hours without an admission
- Hospitalization for respite care
- Planned hospitalization required by the protocol (e.g., for anticipated or protocol specified procedures such as administration of Intravenous fluids, central line insertion, biopsy procedures)
- Hospitalization for a preexisting condition, provided that all of the following criteria are met:
    - The hospitalization was planned prior to the study or was scheduled during the study when elective surgery became necessary because of the expected normal progression of the disease.
    - The subject has not suffered an adverse event.

If the hospitalization meets any of these criteria, then it is not considered a serious adverse event. In addition, hospitalization related to convenience (e.g. transportation issues etc.) will not be considered a SAE.

Refer to **Table** 10 for additional details regarding each of the above criteria.

6.3.1.8 Abnormal Laboratory Values / Clinical Assessments

It is the responsibility of the investigator, or designee, to review and document all laboratory findings and clinical assessments, which may include vital signs, physical exams and ECGs. Medical and scientific judgment should be exercised in deciding whether a laboratory abnormality or clinical finding should be classified as an adverse event. In general, an abnormal laboratory test result or clinical finding should be considered as an adverse event if it meets at least one of the following criteria:

- Is associated with clinical symptoms
- Results in a change in study treatment (e.g., treatment modification, interruption or discontinuation)
- Requires a medical intervention or change in concomitant therapy
- Clinically significant in the investigator's judgment

Note that if a clinically significant laboratory abnormality or clinical finding is a sign of a disease or syndrome (e.g. elevated ALT) then this should be recorded as a single event identified by the diagnosis (hepatic failure).

If a clinically significant laboratory abnormality is not a sign/symptom of another primary event, the abnormality itself should be recorded as an adverse event. The event should be described using a specific clinical term (“hyperkalemia”), if possible, or as test result above or below the normal range (e.g., "elevated Vitamin D," as opposed to "abnormal Vitamin D").

6.3.1.9 Death

Death should be considered an outcome of an adverse event and not an independent adverse event. The event or condition that caused the death should be recorded as the adverse event with the outcome of death. If the cause of death is unknown and cannot be ascertained at the time of reporting, then the event should be reported as an “unexplained death”. If the cause of death later becomes available (e.g., after autopsy), “unexplained death” should be updated by the established cause of death.

6.3.2 Reporting of Adverse Events to Merck

6.3.2.1 Reporting of Serious Adverse Events to Merck

SAE reports and any other relevant safety information related to PBZ are to be forwarded to the Merck Global Safety facsimile number: +1-215 664-6229. 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 215 664-6229) at the time of submission to FDA.

For the time period beginning when the consent form is signed until treatment assignment, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (reference section 6.3.1.9 for additional details) that occurs to any subject must be reported within 24 hours to the Sponsor-Investigator 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 study treatment through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (reference section 6.3.1.9 for additional details) whether or not related to the Merck product, must be reported within 24 hours to the Sponsor-Investigator and within 2 working days to Merck Global Safety. Note that SAE reporting to UVA DSMC, UVA IRB, and FDA differs and is described in other sections.

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-Investigator and to Merck Global Safety.

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

6.3.2.2 Reporting of Overdose to Merck

For the purposes of this trial, an overdose of PBZ 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 PBZ. 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-Investigator and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 661-6229)

6.3.2.3 Reporting of Pregnancy and Lactation to Merck

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

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

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of 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-Investigator and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 661-6229).

6.3.2.4 Reporting of Events of Clinical Interest to Merck

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-Investigator and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 661-6229).

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-Investigator 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-Investigator and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in section 6.3.2.2, that is not associated with clinical symptoms or abnormal laboratory results.

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

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

6.3.2.5 Protocol-Specific Exceptions to Serious Adverse Event Reporting to Merck

Efficacy endpoints as outlined in this section will not be reported to Merck as described in Section 6.3.2.3, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor-Investigator 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-Investigator 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.

6.3.3 UVA Cancer Center DSMC Reporting Requirements

All adverse events must be reported into the University of Virginia Cancer Center OnCore database within the time frames specified below:

Table A: High Risk Studies
Reporting requirements for AEs that occur within 30 days of the last dose of protocol specified treatment

	Grade 1	Grade 2		Grade 3				Grade 4 & 5
	Expected and unexpected	Expected	Unexpected	Expected Without hospitalization	Expected With hospitalization	Unexpected Without hospitalization	Unexpected With hospitalization	Expected and Unexpected
Unrelated Unlikely	OnCore 30 days[a]	OnCore 30 days	OnCore 30 days	OnCore 30 days	OnCore 15 days	OnCore 30 days	OnCore 15 days	OnCore 7 days
Possible Probable Definite	OnCore 30 days[a]	OnCore 30 days	OnCore 15 days	OnCore 30 days	OnCore 15 days	OnCore 7 days	OnCore 7 days	OnCore (24-hrs)* 7 days

*Enter into Cancer Center database within 24 hours if unexpected and definitely related to protocol specified treatment
Hospitalization defined as an inpatient hospital stay or prolongation of a hospital stay equal to or greater than 24 hours
a Grade 1 unexpected or expected hematologic/metabolic events will be recorded in the Cancer Center Database; however, regardless of attribution, these events do not have to be reported as adverse events.

6.3.4 IRB Reporting Requirements

Serious, unexpected adverse events considered at least possibly related to study treatment must be submitted to the site Institutional Review Board according to the participating site institutional policies.

For the University of Virginia clinical site, the Principal Investigator (PI) or designee is responsible for reporting AEs and unanticipated problems to the UVA HSR-IRB according to the following guidelines.

Type of Event	To whom will it be reported:	Time Frame for Reporting	How reported?
Any internal event resulting in death that is deemed DEFINITELY related to (caused by) study participation *An internal event is one that occurs in a subject enrolled in a UVa protocol*	IRB-HSR	Within 24 hours	IRB Online and phone call www.irb.virginia.edu/
Internal, Serious, Unexpected, Related adverse event	IRB-HSR	Within 7 calendar days from the time the study	IRB Online

See Oncore reporting requiremer		team received knowledge of the event. *Timeline includes submission of signed hardcopy of AE form.*	www.irb.virginia.edu/
Unanticipated Problems that are not adverse events or protocol violations This would include a Data Breach.	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Unanticipated Problem report form. *http://www.virginia.edu/vprgs/irb/HSR_docs/Forms/Reporting_Requirements-Unanticipated_Problems.doc)*
Protocol Violations (*The IRB-HSR only requires that MAJOR violation be reported, unless otherwise required by your sponsor, if applicable.)* Or Enrollment Exceptions	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Protocol Violation and Enrollment Exception Reporting Form *http://www.virginia.edu/vprgs/irb/hsr_forms.html* *Go to 3rd bullet from the bottom.*
Data Breach	The UVa Corporate Compliance and Privacy Office, a ITC: if breach involves electronic data- UVa Police if breach includes such things as stolen computers.	As soon as possible and no later than 24 hours from the time the incident is identified. As soon as possible and no later than 24 hours from the time the incident is identified. IMMEDIATELY.	UVa Corporate Compliance and Privacy Office- Phone 924-9741 **ITC:** Information Security Incident Reporting procedure, http://www.itc.virginia.edu/security/reporting.html Phone- (434) 924-7166

6.3.5 Reporting to the FDA

The Sponsor-Investigator for the study (the UVA PI or designee) is responsible for providing safety updates to the FDA per the following guidelines. The reporting times refer to the time the Sponsor-Investigator received knowledge of the AE.

Table 9. FDA Reporting Requirements

UVa PI HELD IND			
Type of Event	**To whom will it be reported:**	**Time Frame for Reporting**	**How reported?**
Life-threatening and/or fatal unexpected events related or possibly related to the use of the investigational agent.	FDA	Within 7 calendar days of the study team learning of the event	Form FDA 3500A (MedWatch) or narrative
Serious, unexpected and related or possibly related adverse events	FDA	Within 15 calendar days after the study team receives knowledge of the event	Form FDA 3500A (MedWatch) or narrative
All adverse events	FDA	Annually	IND annual report

6.3.6 Data Safety Monitoring Plan

The Data and Safety Monitoring Board for this study is the UVA Data Safety Monitoring Committee (DSMC).

6.3.6.1 UVA Cancer Center Data Safety Monitoring Committee

The University of Virginia Cancer Center Data and Safety Monitoring Committee (DSMC) will provide oversight of the conduct of this study. The CC DSMC will report to the UVA Protocol Review Committee (PRC). The DSMC will review the following:

- All adverse events
- Audit results
- Application of study designed stopping/decision rules
- Whether the study accrual pattern warrants continuation/action
- Protocol violations

The CC DSMC will meet every month for aggregate review of AE data. Tracking reports of the meetings are available to the PI for review. Issues of immediate concern by the DSMC are brought to the attention of the PI (and if appropriate to the PRC and IRB) and a formal response from the PI is requested. Per the Cancer Center NIH approved institutional plan this study will be audited approximately every 6 or 12 months.

Table 10. Evaluating Adverse Events

4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during study treatment that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a study treatment and is documented in the subject's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor-Investigator within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor-Investigator and to Merck within 2 working days.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause study treatment to be discontinued?	
Relationship to study treatment	Did study treatment cause the adverse event? The determination of the likelihood that study treatment caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between study treatment and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely study treatment caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to study treatment such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of study treatment? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to study treatment **(continued)**	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was study treatment discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of treatment; or (3) the trial is a single-dose drug trial); or (4) Product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to study treatment in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Product(s) is/are used only one time).
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding study treatment or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of** study treatment **relationship).**	
Yes, there is a reasonable possibility of study treatment **relationship.**	There is evidence of exposure to study treatment. The temporal sequence of the AE onset relative to the administration of study treatment is reasonable. The AE is more likely explained by study treatment than by another cause.	
No, there is not a reasonable possibility of study treatment **relationship**	Subject did not receive study treatment OR temporal sequence of the AE onset relative to administration of study treatment is not reasonable OR the AE is more likely explained by another cause than the study treatment. (Also entered for a subject with overdose without an associated AE.)	

7.0 EVALUATION CRITERIA

7.1 Dose Limiting Toxicity

The incidence of dose limiting toxicity (DLT) in subjects will be monitored. A DLT is defined as any treatment related event (at least possibly related to treatment) following the start of any study mandated treatment which meets one of the below criteria per CTCAE v4.03:

- Grade 3 nausea, vomiting or diarrhea persisting for >48 hours with optimal medical management
- Grade ≥ 3 hypophysitis, or hyperthyroidism lasting > 7 days
- Grade 3 fatigue, flu-like symptoms, BATs infusion reaction, and rash or other skin infections lasting greater than 72 hours
- Grade ≥ 3 dyspnea that persists for more than 6 hours
- Grade ≥ 3 hypotension requiring IV pressors > 72 hours or hospitalization > 72 hours
- Grade ≥ 4 cytokine release syndrome (CRS) EXCEPT that IV pressors may be used for up to 72 hours without being determined as a DLT
- Any other non-hematological toxicity Grade ≥ 3 not resolving within 72 hours (except for those described above)
- Grade 4 BATS infusion related reaction
- Grade 3 or higher PBZ infusion related reaction
- Grade 4 neutropenia lasting more than 5 days
- Grade 4 thrombocytopenia, or Grade 3 thrombocytopenia with bleeding, or any requirement for platelet transfusion
- Grade 4 anemia
- The study chair reserves the right to declare other AEs as DLT's if they are clearly treatment related and are causing substantial morbidity for subjects.

Subjects experiencing autoimmune DLTs will be removed from all study treatment (PBZ and HER2 BATs). Subjects experiencing DLTs other than autoimmune complications may continue on either HER2 BATs or PBZ treatment (e.g. if the patient has a Grade $\underline{\geq}$ 3 BATs infusion related reaction lasting more than 72 hours, she may continue on PBZ as long as the treating physician feels it is in her best interest). Likewise, patients with prolonged high grade hypotension due to meperidine/dehydration/anti-emetics or any other cause not solely due to the BATS infusion may continue on study.

All subjects with DLTs should also participate in the remaining aspects of the study (immune studies, follow-up, etc.)

7.2 Response Criteria

The primary method of evaluation of response to treatment will be irRECIST criteria as described in section 6.2.2.4. Response based on RECIST 1.1 criteria will also be collected for secondary analyses.

7.3 Other Measures of Effect

All irRECIST and RECIST assessments of CR, PR or PD must be independently confirmed by the UVA DSMC prior to publication of study results as per the UVA DSMP charter.

7.3.1 Progression-free survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment to time of progression or death from any cause, whichever occurs first.

7.3.2 Overall survival

Overall survival (OS) is defined as the duration of time from start of treatment to time of death from any cause.

7.3.3 Objective response rate (ORR)

Objective Response Rate (ORR) is defined as the percentage of subjects with a response of irCR or irPR per irRECIST (ORR = irCR + irPR).

7.3.4 Duration of response (DoR)

Duration of response is defined as the time from documentation of tumor response to disease progression.

7.3.5 Disease control rate (DCR)

Disease control rate (DCR) is defined as the percentage of subjects who achieve complete response, partial response and stable disease following treatment.

7.3.6 Immune Evaluations

All subjects receiving any dose of study treatment should continue assessment of immune markers.

An immune response will be defined as a 30 IFNγ Elispots per million cells plated above the background of unstimulated spontaneous IFNγ Elispots per million cells plated.

7.4 Toxicity and Safety

All subjects receiving any dose of study treatment will be evaluated for safety. Safety assessments will include:

i. Incidence and severity of adverse events
ii. Incidence of Dose-Limiting Toxicities (DLTs)

8.0 STATISTICAL ANALYSIS PLAN

8.1 Design

For Aim 1: According to standard 3 + 3 dose escalation/dose de-escalation rule, if there are 2 or more DLTs at Schedule #1, the study will be closed. If there are no dose limiting toxicities (DLTs) in the first 3 subjects (0/3) or at maximum 1 subject with DLT out of 6 subjects (1/6) with treatment of Schedule #1, we will advance to Schedule #2. If there are 0/3 or 1/6 DLTs at Schedule #2, we will advance to Schedule #3. If there are no more than 1/6 DLTs, Schedule #3 will be used as the MTD in this study. Otherwise, Schedule #2 will be used as MTD if no more than 1 out of 6 patients at Schedule #2 have DLTs. This design will require a minimum of 3 and a maximum of 18 evaluable patients to identify the MTD. To take the eligible but not evaluable patients into account (estimated at 10%), the following plan is considered. Among 18 eligible patients, if the true unevaluable rate is 10%, 2 unevaluable patients is expected. Since the probability of 4 unevaluable patients out of 18 eligible patients is 7% and the null hypothesis that the unevaluable rate is 10% can't be rejected at one-sided type I error rate of 2.5%, a maximum of 4 unevaluable patients will be accepted. Thus, a maximum of 22 eligible patients may be recruited and among of them 4 unevaluable patients will be allowed.

Once the MTD is identified, 12 evaluable subjects in the dose expansion cohort (DEC) will be enrolled at the MTD level to further evaluate the safety and explore the preliminary efficacy. Assuming an unevaluable rate of 10%, as above, similar considerations is taken for a DEC of 12 evaluable subjects at the MTD. A maximum of 3 unevaluable patients in the DEC will be allowed (the probability of 3 unevaluable patients out of 12 eligible patients is 8.5%>2.5%) and a total of 15 eligible patients may be recruited in the DEC.

In brief, the table below lists the dose-escalation decision rules for this study.

Number of subjects with DLT/ cohort at a given dose schedule level	Escalation decision rule
0/3	Accrue 3 new subjects for next advanced schedule level
1/3	Accrue additional 3 subjects at current schedule level
1/6	Accrue 3 new subjects for next advanced schedule level
>=2/3 or >=2/6	Stop dose escalation. Enter 3 more subjects to previous schedule/dose level if only 3 subjects were enrolled to that cohort. If 6 subjects had been enrolled to that cohort already, then declare that previous dose / schedule as MTD.

Aim 2 is exploratory, only summary and descriptive statistics will be performed on the immune evaluations.

Immune evaluations (IE) will be done at the time points indicated on the schema to define the pattern of immune responses.

We will calculate the mean, median, and standard deviation for each studied immune variable at each studied time point and explore potential patterns of immune responses. The product-limit method of Kaplan-Meier will be used to obtain preliminary estimates of PFS and OS, and survival curves will be plotted. With all subjects treated at the MTD, the objective response rate (ORR), duration of response (DoR), and disease control rate (DCR) will be calculated and corresponding 90% confidence intervals will be estimated using the Clopper-Pearson method.

Justification for the expansion cohort size of 12 patients. The expansion cohort size of 12 patients is used to get further assessment of the safety at the chosen MTD. This sample size will have the width of 90% confidence intervals (Clopper-Pearson method) of DLT rate within 50%. With an expected maximum DLT rate of 15% at the chosen MTD, a maximum of 4 patients with DLTs will be acceptable (since the probability of 4 DLTs out of 12 patients is 6.8%, the null hypothesis that the maximum DLT rate is 15% can't be rejected at a one-sided type I error rate of 2.5%). In the expansion of this cohort, if one more DLT occurrs after 4 patients have had DLTs, the expansion will be stopped. The table below shows the Clopper-Pearson's 90% confidence intervals for various number of DLTs.

Number of DLTs	Total N in the expansion cohort	DLT rate (90% confidence interval)
0	12	0% (0% - 22.1%)
1	12	8.3% (0.4% -33.9%)
2	12	16.7% (3.0% - 43.8%)
3	12	25% (7.2% - 52.7%)
4	12	33.3% (12.3% - 60.9%)

8.2 Sample Size and Accrual

This study requires a maximum of 37 subjects (MTD identified at Schedule #3 with 6 subjects at each Schedule/Dose level plus the 12 in the DEC and a maximum of 7 unevaluable patients). Accrual is estimated at between 3-10 subjects per year.

8.3 Populations and Analysis Plans

All subjects who are put on-study will be assessed for inclusion in the final report. Eligible subjects who do not complete the scheduled course of treatment due to reasons other than toxicity will not be used to guide decisions about dose/schedule selection.

8.3.1 Outcomes

All eligible subjects who receive any protocol treatment will be evaluated for safety endpoints. Only eligible subjects that receive at least two BATs infusions will be evaluated for efficacy endpoints. Subjects that discontinue or withdraw for a reason other than a DLT prior to receiving two BATs infusions within 20% of the target dose will be replaced.

Immunologic:
All eligible subjects who receive any protocol treatment (BATs infusion or pembrolizumab) will be evaluated for immunologic response if at least one post treatment sample is evaluable for IFN-γ EliSpots analysis.

9.0 ADMINISTRATIVE AND REGULATORY DETAILS

9.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to all ICH E6 principles and Good Clinical Practice (GCP), to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the subject will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the subject and the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a subject's participation in the trial, the written informed consent form should be signed and personally dated by the subject and by the person who conducted the informed consent discussion.

9.2 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study subject requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

9.2.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB-HSR approval/favorable opinion.

For any such emergency modification implemented, a UVA IRB modification form must be completed by study Personnel within five (5) business days of making the change.

9.2.2 Other Protocol Deviations/Violations

Protocol Deviations: A protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

Study personnel will record the deviation, and report to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

Protocol Violations: An unplanned protocol variance is considered a violation if the variance:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

Violations should be reported by study personnel to the IRB within one (1) week of the investigator becoming aware of the event.

9.3 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed subject consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study

documents should be kept on file until six years after the completion and final study report of this investigational study.

9.4 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations, all applicable local regulatory laws and regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study subjects. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion. It is the responsibility of the Principal Investigator to ensure that all study site personnel are aware that the study protocol and all data generated is confidential and should not be disclosed to third parties (with the exception of local and national regulatory bodies which require access for oversight purposes).

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

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11.0 APPENDICES

11.1 Appendix 1: Suggested Supportive Care Guidelines for BATs Infusions and Example Discharge Instruction Document

Suggested Supportive Care Guidelines for BATs Infusions

Note: These are suggested and not required per protocol.

Medical Issue	Grade	Actions
Allergic reactions	Grade 3 of true allergic reaction.	For next infusion: Diphenhydramine hydrochloride 50 mg IV, Hydrocortisone sodium succinate 100 mg IV q 12 hrs (or equivalent) and acetaminophen 650 mg q 8 hrs Appropriate antibiotics, blood products, antiemetics, fluids, electrolytes and general supportive care are to be used as necessary.
Severe cytokine storm		Subjects with severe or life-threatening reactions will be given hydrocortisone sodium succinate 100 mg IV q 12 hrs, diphenhydramine 50 mg IV every 6 hours. **No one out of 130 subjects has developed CRS o**r required high dose steroids. Call treating MD and the study PI immediately for any suspicion of developing CRS. The subject will receive fluid support, pressor support, ventilatory support, dialysis, or plasma exchange support in the intensive care unit as clinically indicated. Workup for macrophage activation syndrome (MAS) and disseminated intravascular coagulation as clinically indicated. Anti-coagulation can be administered based on the clinical situation. MAS and CRS have not been observed in subjects receiving BATs. In the rare occasion that treatment is needed for CRS beyond steroids or pressors, tocilizumab (ACTEMRA®), which is a humanized monoclonal antibody against the interleukin 6 receptor (IL-6R), will be given to target and block IL-6 receptors in treatment of CRS. The dose will be 8 mg/kg IV given as a single 60 min infusion. Serum will be drawn for testing as clinically indicated to assess levels of IL-6 pretreatment and other relevant cytokines post-treatment as clinically indicated. IL-6 cannot be tested due to complexing between IL-6 and the anti-IL-6 antibody. Other cytokines such as IFNγ, TNFα, GM-CSF, IL-2, IL-7, IL-8, IL-10, IL-12, and RANTES will be measured.

Infusional Reactions for IV HER2 BATs (distinct from allergic or hypersensitivity reactions), generally develop during or shortly after infusion	**All grades** **Grade 1** (Transient flushing or rash, fever < 38 ºC (<100.4 ºF)) **Grade 2** (Rash, flushing, urticaria, dyspnea, drug fever ≥ 38 ºC (≥100.4 ºF)) **Grade 3** (Symptomatic bronchospasm with or without urticaria, allergy-related edema/ angioedema, hypotension (except as described in section below) **Grade 4** (Anaphylaxis)	Acetaminophen, diphenhydramine, steroids or other medications may be given for symptom control as needed. Anaphylactic precautions should be observed during HER2 BATs administration. Slow infusion rate by 50% and monitor subject for worsening of condition Stop infusion; symptom control (diphenhydramine hydrochloride) IV, acetaminophen for fever, and oxygen if needed) Cells from this infusion must be discarded but subjects may receive future infusions once symptoms have resolved (cells for this infusion must be discarded). Stop infusion immediately and remove the infusion tube. Cells for this infusion must be discarded. Administer diphenhydramine hydrochloride 50 mg IV, hydrocortisone 100 mg IV every 6 hours. Bronchodilators for bronchospasms, and other medications as medically indicated. Hospital admission should be considered. Stop infusion immediately. Cells for this infusion must be discarded. Administer diphenhydramine hydrochloride 50 mg IV, hydrocortisone 100 mg IV every 6 hours and epinephrine as indicated.
Hypotension: usually delayed several hours after infusion	SBP <90 mm/Hg SBP<70 mm/Hg	Call treating MD and the study PI 500 mL normal saline (NS) bolus. If unresponsive, stop the HER2 BATs infusion and repeat NS 500 mL. Fluid bolus may be repeated as needed or the increased IV rate may be maintained. If unresponsive, call treating MD and the study PI; subject may require IV pressors
Hypertension	SBP >160 mm/Hg or DBP >90	Call treating MD and the study PI: Subject may require anti-hypertensive medication
Capillary Leak Syndrome	Grade 3 (respiratory compromise or fluid	a. Stop HER2 BATs infusion (once stopped, cells should be discarded and infusion may not be restarted)

	support required for hypotension) Grade 4 (pressor support or mechanical ventilation is required)	b. Provide appropriate supportive care depending on manifestations (e.g. oxygen, IV fluids) c. Furosemide 0.5-1 mg/kg (max 40 mg) IV every 6-12 hours as needed for symptomatic pulmonary vascular congestion, tense ascites or significant weight gain (ie, 10% of baseline), if blood pressure allows. If poor response to furosemide alone, add metolazone 0.2-0.4 mg/kg/day (maximum: 10 mg/dose/day) PO prior to IV furosemide. Albumin may be required prior to furosemide if albumin < 3.0. If albumin and furosemide are given together, close attention to volume status to prevent hypotension and prerenal azotemia is important. Stop HER2 BATs infusion
Dyspnea	Oxygen saturation is less than 90%	Brief oxygen supplementation If the subject is fluid overloaded, hypoxic and hypotensive, treatment should be stopped (cells for this infusion should be discarded) and vasopressor support initiated, as needed. If hypoxia continues to worsen, does not respond to the above measures, and is considered to be life threatening, mechanical ventilation should be initiated.
Arrythmia	Any evidence of arrhythmia not present at baseline Asymptomatic atrial irregularities related to an elevated temperature, but without evidence of ischemia or clinically significant hypotension Ischemia or Grade 3 or worse cardiac toxicity	Immediate ECG evaluation Monitor but continue therapy Stop HER2 BATs infusion
Neurotoxicity	Grade 3 or 4, except confusion	If there are persistent neurologic signs and symptoms of possible CNS infection and/or cytokine storm, the attending team will proceed with appropriate diagnostic measures (e.g. spinal tap, MRI, or CT evaluations).

	Grade 3 or 4 confusion (not fever related) Headaches	Persistent confusion beginning after the start of an HER2 BATs infusion which is clearly not temperature related or related to supportive care medicines (diphenhydramine, morphine, etc.) will require immediate treatment stoppage. Imitrex 50 mg dose or ibuprofen, and/or acetaminophen. If the patient experienced headaches at a previous infusion, pre-medicate with 50 mg Imitrex
Fever	Persistent temperature elevations (≥6 hours) of 40°C or greater not responding to antipyretics Grade 2 chills, which may accompany temperature changes Grade 3 Confusion	Hold HER2 BATs Continue acetaminophen (650 mg/dose), maximum dose of 3000 mg/day) every 8 hours alternating with ibuprofen 600 mg every 8 hours as needed Subjects developing fever following their first HER2 BATs treatment through 30 days following their last HER2 BATs treatment should have blood cultures obtained if infection is suspected. If there is a high level of suspicion, the subject should be placed on a broad-spectrum antibiotic, such as cefepime until afebrile and blood cultures have remained negative for 48 hours. Can be treated with meperidine 25 to 75 mg IV (maximum dose of 75 mg). Confusion related to fever will be managed by aggressive use of antipyretics and will not require dose reduction or drug stoppage, if the confusion normalizes with initiation of aggressive antipyretic measures

Example Discharge Instruction Document

Discharge Instructions Following T-cell Infusions

IRB-HSR # _____

Name: _____ Subject ID#: _____

In the 24 - 48 hours following infusion of armed activated T Cells, you may experience any of the following side effects. Although these are usually minimal and will go away, it is important to your well-being that you report any of the following:

- Difficulty breathing
- Shaking chills
- Fever – take your temperature every 4 hours while awake. Notify Dr. _____ if you experience fever higher than 100.5^{o}F.
- Nausea and vomiting
- Headache (unrelieved by acetaminophen (Tylenol) and ibuprofen (Motrin) or caffeinated beverages such as Coke)

Please stay locally (within about 40 minutes' drive) to UVA with a caregiver for two nights. If after 2 nights you feel well and do not have any symptoms, you may travel outside of this area.

Please continue to take acetaminophen (Tylenol) and ibuprofen (Motrin) as directed for 24 hours following your discharge. If you experience shaking chills, you may take 25-50 mg of diphenhydramine (Benadryl). It is also **very important to drink plenty of fluids** during this time. If you develop fever over 100.5 after stopping the medications, restart the combination for another 12 hours (3 doses). Notify the Doctor if your fever persists beyond 36 hours.

Please also complete the table on the following page with the time you take each medication, your temperature and blood pressure and any comments you may have. Please continue filling out the table for as long as you are taking medications for side effects of the infusion. Add rows for any additional medications you are asked to take for these side effects (for example, diphenhydramine (Benadryl)).

If you have any of the above side effects or have anything else that concerns you within the week following your T cell infusion, please notify us at one of the following numbers:

During office hours (Monday – Friday 8am-4:30pm): _____

Outside of office hours: call 434 924-0000 and ask to page the on call Heme-Onc team doctor

Subject signature _____ Date _____

Nurse signature _____ Date _____

Name _________________________ Subject ID# _______________

Date/Time	Medication – protocol prescribed	Other medications	Temperature	Blood Pressure	Heart rate	Symptoms/Comments* *Please include start and stop times for symptoms
	Acetaminophen 650 mg every 8 hrs					
	Ibuprofen 600 mg every 8 hrs					
	Acetaminophen 650 mg every 8 hrs					
	Ibuprofen 600 mg every 8 hrs					
	Acetaminophen 650 mg every 8 hrs					
	Ibuprofen 600 mg every 8 hrs					
	Acetaminophen 650 mg every 8 hrs					
	Ibuprofen 600 mg every 8 hrs					
	Acetaminophen 650 mg every 8 hrs					
	Ibuprofen 600 mg every 8 hrs					
	Acetaminophen 650 mg every 8 hrs					
	Ibuprofen 600 mg every 8 hrs					

Date/Time	Medication – protocol prescribed	Other medications	Temperature	Blood Pressure	Heart rate	Symptoms/Comments* *Please include start and stop times for symptoms
	Acetaminophen 650 mg every 8 hrs					
	Ibuprofen 600 mg every 8 hrs					
	Acetaminophen 650 mg every 8 hrs					
	Ibuprofen 600 mg every 8 hrs					
	Acetaminophen 650 mg every 8 hrs					
	Ibuprofen 600 mg every 8 hrs					
	Acetaminophen 650 mg every 8 hrs					
	Ibuprofen 600 mg every 8 hrs					
	Acetaminophen 650 mg every 8 hrs					

	Ibuprofen 600 mg every 8 hrs					
	Acetaminophen 650 mg every 8 hrs					
	Ibuprofen 600 mg every 8 hrs					

Subject signature _____ Date _____