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Breast Cancer Program
BMT and Immunotherapy Program
Barbara Ann Karmanos Cancer Institute

2010-056: A Phase II Study of Anti-CD3 x Anti-HER2/*neu* (Her2Bi) Armed Activated T Cells (ATC) after Neoadjuvant Chemotherapy in Patients with HER2/*neu* (0-2+)-Negative Stage II-III Breast Cancers

IND 9985

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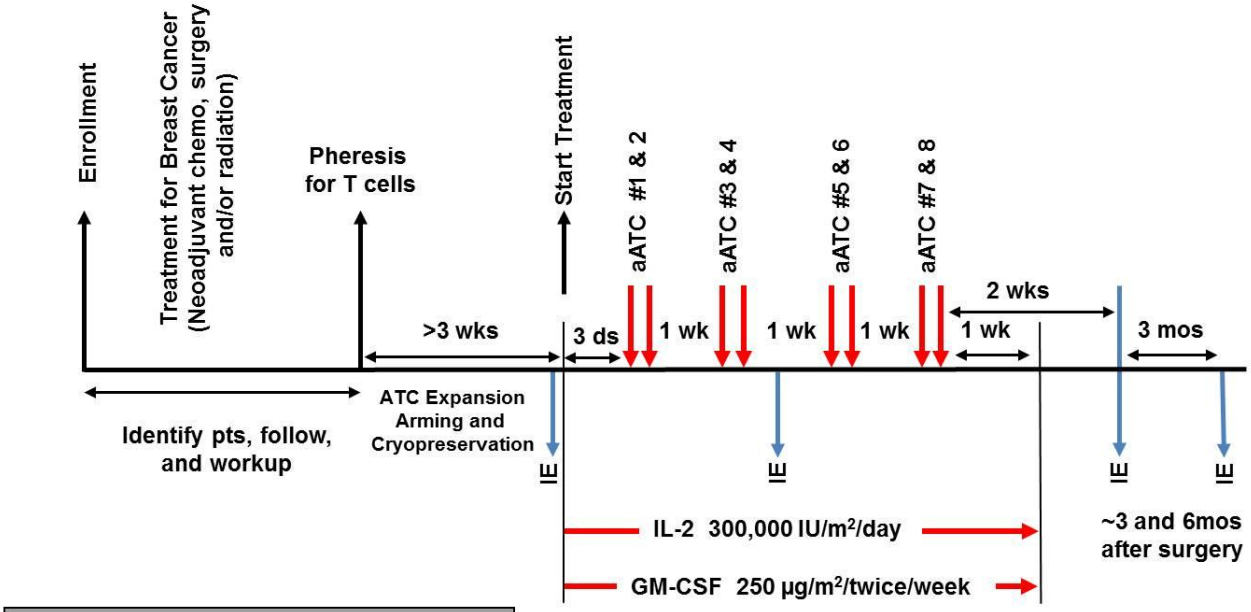
SUMMARY

Women with breast cancer who achieve a complete pathologic response (pCR) after preoperative (neoadjuvant) chemotherapy (chemoT) have a better prognosis than to those with residual disease; pCR is a good surrogate of long-term survival and cure from breast cancer. Women with stage II-III operable triple negative breast cancer with residual disease at the time of surgery are the focus population of this protocol.

Many studies have addressed how to improve the efficacy of chemoT and increase the pCR rate by including different chemotherapeutic agents, biologic agents, regimens and schedules. Up to now, apart from anti-Her2 targeting antibodies which are highly effective for HER2-positive breast cancer, no targeted, non-hormonal agent can be considered a standard therapeutic option for primary systemic treatment. We are proposing a novel combination of Immunotherapy (IT) in addition to neoadjuvant chemoT for women with stage II-III operable triple-negative breast cancer who have residual disease at surgery. We will conduct a phase II trial to evaluate whether infusions of anti-CD3 x anti-Her2/*neu* bispecific antibody (HER2Bi)-armed activated T cells (ATC) after neoadjuvant chemoT, surgery, and/or radiation will improve time to progression (PFS) beyond previously published clinical trials.

Low or moderate expression of HER2 may be targeted by receptor-specific therapies such as targeted ATC. This, indeed, is the case in our preclinical and phase I studies in which we used an immunotherapeutic strategy to target low to high level expression Her2/*neu* breast cancer. **Evidence of clinical and immunologic responses in women with HER2 0/1+ status in our phase I trials would suggest a therapeutic benefit even in the absence of HER2 over-expression and provides a rationale for studying IT with armed ATC in HER2-negative patients**. More importantly, IT after treatment (neoadjuvant chemotherapy, surgery, and/or radiotherapy) may have several clinical advantages including: (1) removal of tumor with only microscopic minimal residual disease remaining prior to IT; (2) the reversal of tumor-induced suppression of immune-based regulatory factors which limits the effectiveness of vaccine-induced, tumor-specific T cells in the tumor microenvironment. Following Leukapheresis the T-cells will - be activated with anti-CD3 and expanded in 100 IU/ml of IL-2 to generate ATC. After culture, ATC will be harvested, armed with HER2Bi, washed, and cryopreserved in 8 aliquots for two infusions per week for 4 weeks in combination with IL-2 and GM-CSF. Patients will be allowed to have received anthracycline and/or taxane based standard regimens. Following chemoT, surgery, and/or radiation, patients will be evaluated for chemoT, surgical, and radiation-related toxicities, and then given ~10-15 billion Her2Bi-armed ATC twice per week for 4 weeks beginning no sooner than 3 weeks (± 1 week) after the breast cancer treatment. In the phase I trial: (1) the maximum tolerated dose (MTD) of HER2Bi-armed ATC was not reached with no dose-limiting toxicities using ~20 billion HER2Bi-armed ATC/infusion; and (2) there was development of cytotoxic T cell responses to breast cancer cell lines with increases in IL-2, IFNγ, TNFα, GM-CSF, and IL-12. Therefore, we selected a dose of ~10-15 billion HER2Bi-armed ATC per infusion. Armed ATC will be thawed and infused twice a week for 4 weeks for a total dose of~80-120 billion armed ATC in combination with low dose IL-2 and GM-CSF.

SCHEMA



Eligibility:
1. Her2 0-2+, ER -, PR -
2. KPS ≥70%
3. Standard LFT, RFT, PFT, cardiac (EF ≥45)
4. No metastatic disease

IE= Immune Evaluation

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1.0 OBJECTIVES

1.1 To estimate PFS in women with stage II-III triple-negative breast cancer without a complete pathologic response (cPR) who receive a regimen of neoadjuvant chemoT, surgery, and/or irradiation followed by 8 infusions of ~10-15 x 10^9 Her2Bi-armed ATC (aATC) given twice per week for 4 weeks in combination with IL-2 (300,000 IU/m^2/day) and GM-CSF (250 µg/m^2/twice per week) beginning 3 days before the 1st infusion and ending 1 week after the last infusion [defined as immunotherapy].

1.2 To estimate the change from baseline (pre IT) to post-IT in specific cytotoxicity and IFN-γ Elispots of lymphocytes in the blood directed at breast cancer cells.

1.3 To investigate if pathologic response and the changes in numbers and proportion of infiltrating cells and cancer stem cells in the tumor at the time of surgery are associated with progressive disease.

2.0 BACKGROUND AND RATIONALE

2.1 Clinical problem of increased relapse in patients who do not achieve a pCR after neoadjuvant chemoT.

In a study by Guarneri *et al*. the prognostic value of pCR was confirmed in 1,731 patients with breast cancer who received neoadjuvant chemoT[1]. pCR was defined as complete disappearance of invasive carcinoma in both breast and axillary lymph nodes. Ninety-one percent of patients received anthracycline-based chemoT and 66% received an additional taxane. After adjustment for other prognostic factors, patients who achieved a pCR had 0.41 times the risk of relapse and 0.36 times the risk of death compared with patients who did not achieve a pCR. Among patients without hormone receptor (HR) expression, pCR was achieved in 24% compared to 8% in HR positive patients.

A study by Carey *et al*. evaluated responses to neoadjuvant chemoT in 107 patients with stage II and III breast cancer[2]. Patients received neoadjuvant doxorubicin (60 mg/m^2) plus cyclophosphamide (600 mg/m^2) chemoT (AC) given i.v. for 4 cycles, either alone or as the first component of a sequential AC-taxane neoadjuvant regimen. Twenty-eight patients (26%) received AC on a dose-dense schedule (every 2 weeks), whereas the rest of the patients received AC on an every 3 weeks schedule. Most patients (80 of 107, 75%) received additional neoadjuvant chemoT following AC, which primarily involved either paclitaxel or docetaxel. pCR to chemoT (defined as post operatively stage 0, no invasive cancer) was significantly better among the basal-like subtype (27%), defined as the immunohistochemical surrogate ER-negative, PR-negative, and HER2-negative (triple-negative), and the HER2-positive/ER-negative subtype (36%) versus the combined luminal subtypes (7%, P = 0.01). However, despite initial chemosensitivity, patients with the basal-like and HER2-positive/ER-negative subtypes had worse DFS (P = .04) and OS (P = .02) than those with luminal subtypes.

Liedtke et al published an analysis of a prospectively collected clinical database including 1,118 patients who received neoadjuvant chemoT at M.D. Anderson Cancer Center for stage I-III breast cancer from 1985 to 2004 and for whom complete receptor information was available[3]. The primary goal was to describe the relation between triple-negative breast cancer (TNBC) and major determinants of clinical outcome, such as response to neoadjuvant chemoT (rate of pCR in breast and lymph nodes [pCR]), progression-free survival (PFS), site-specific distribution of recurrence, post recurrence survival (PRS), and overall survival (OS). Two hundred fifty-five patients (23%) were designated as having TNBC and 863 patients (77%) were designated as non-TNBC. Increased pCR rates were observed for patients with TNBC compared with non-TNBC (22% *v* 11%; odds ratio [OR] = 1.53; 95% CI, 1.03 to 2.26; P = .034). The highest pCR rates (28%) in association with TNBC were observed for patients treated with chemoT regimens containing a taxane (weekly/once every 3 weeks paclitaxel/docetaxel) followed by anthracycline-based regimens (fluorouracil, doxorubicin, and cyclophosphamide or fluorouracil, epirubicin, and cyclophosphamide). **TNBC patients with residual disease after**

neoadjuvant chemoT have significantly worse survival, particularly in the first 3 years compared with patients with non-TNBC and residual disease (HR = 1.5; 95% CI, 1.3 to 1.8; *P* < .0001)[3].

2.2 Underserved population of women with TNBC.

The studies cited in the previous section demonstrate the worse prognosis of patients with TNBC, especially when they do not achieve pCR. Since they are not eligible for anti-estrogen (hormonal) therapy, there are no other standard treatments beyond the neoadjuvant chemoT and local treatment (surgery +/- radiation). Treatments targeting this poor risk group of patients is especially important. Of specific relevance to this purpose is that African American women tend to have more TNBC[4-6], and at our center, 44.32% of the neoadjuvant patients from 1997-2008 (KCI database) were African American women with newly diagnosed localized breast cancer.

2.3 Criteria for pCR.

Patients with breast cancer who achieve a pCR after neoadjuvant chemoT have an improved prognosis compared to those with residual disease; pCR is a reliable surrogate marker for long-term survival and cure from breast cancer[1;7]. In fact, the achievement of a pCR is one of the most reliable surrogate markers predicting long-term outcome and the pathologic staging at surgery following preoperative treatment maintains its prognostic significance[7-10]. The definition and methods of assessment of pCR vary across studies. A more stringent definition includes absence of all invasive and non-invasive tumor cells in both breast and axilla,[1] while others define pCR as absence of invasive tumor in the breast irrespective of the nodal status[11;12]. Therefore, the definition and assessment of pCR can influence the reported rates independently from the activity of different regimens. However, regardless of the applied criteria, patients who achieve a pCR have better outcomes[9;13].

2.4 Chemo-resistant and persistent tumorogenic breast cancer $CD44^{hi}/CD24^{lo}$, aldehyde dehydrogenase activity (ALDH1), and CD133 "cancer stem-like cells" (CSC) after ChemoT.

Persistence of chemoT resistant breast CSC is the most likely the cause of disease recurrence, especially if minimal residual disease is attained with chemoT. Breast CSCs are believed to be $CD44^{hi}/CD24^{lo}$ tumor cells resistant to and persisting after chemoT. The results from Li showed that treatment increased the percentage of $CD44^{hi}/CD24^{lo}$ cells (mean at baseline vs. 12 weeks after neoadjuvant chemoT, 4.7% to 13.6%, P < .001)[14]. The ALDH1 population, as measured by the ALDEFLUOR assay, has been used to identify, characterize, and isolate populations from normal and malignant mammary tissue displaying stem cell properties *in vitro* and in NOD/SCID xenografts[15]. Subsequently, Ginestier *et al* showed in a series of 577 breast carcinomas that expression of ALDH1 correlated with poor prognosis[16]. CD133 positivity has also been implicated as a co-marker for CSC in inflammatory breast cancers that express a stem cell-like phenotype ($CD44^{hi}/CD24^{lo}$, ALDH1, and CD133)[17-19]..

2.5 HER2 as a target.

HER2 belongs to the epidermal growth factor receptor family of tyrosine kinases. The HER2 oncogene encodes a 185-kDa transmembrane receptor with significant sequence homology to the class I receptor of the tyrosine kinase family[20]. HER2 is over-expressed in breast, ovarian, lung, gastric, oral[20], and prostate cancers[21-23]. The expression of HER2 makes it an ideal target for biological-based agents such as Herceptin® (trastuzumab), a humanized mAb that targets HER2, which was approved for the treatment of women with HER2-positive breast cancer. Herceptin® is believed to exert its anti-tumor effects via interference with receptor-mediated signaling pathways,[24] inhibiting cell cycle progression by inhibiting the mitogen-activated protein kinase pathway[25;26], suppressing the anti-apoptotic phosphatidylinositol 3-kinase and Akt pathways[27;28], and antibody-dependent cellular cytotoxicity (ADCC)[29-33]. Although Herceptin® is effective as a single agent[34;35] or in combination with cytotoxic chemoT[36;37], data from efficacy trials suggest that the beneficial treatment effects are limited to patients with the highest levels of HER2 expression. This limits the application of Herceptin® to 20-25% of patients with Her2/*neu* 3+ or fluorescence in situ (FISH) amplified disease, leaving 75-80% of the Her2/*neu* 0-2+, FISH-negative patients without antibody-targeted treatment options.

2.6 Targeting low level Her2/*neu* receptor expression.

In view of the inherent low level expression of Her2/*neu* receptors on most breast cancers and the potential for the currently available HER2-targeted therapies to cause increased dimerization and downregulation of HER2 receptors[38], leaving fewer receptor targets on the cell surface for drugs designed to target these tumors, a different strategy is required for HER2 non-over-expressing tumors. In such instances, very low or nearly absent expression of HER2 may still be targeted by receptor-specific therapies such as targeted ATC. This is the case with our preclinical and phase I studies in which we used Her2Bi armed T cells to target very low to high level expression Her2/*neu* breast cancer. **Evidence of clinical and immunologic responses in women with HER2 0/1+ status in our phase I trials would suggest a therapeutic benefit even in the absence of HER2 over-expression and provides encouraging results in the HER2-negative patients with armed ATC[39;40].**

2.7 Combination neoadjuvant chemoT and immunotherapy (IT) in TNBC patients who fail to achieve pCR. Many studies have addressed how to improve the efficacy of chemoT and increase the pCR rate by evaluating different anthracycline-based regimens, anthracycline–taxane combinations, sequential regimens, and dose-dense schedules[41-75]. Taxane (paclitaxel or docetaxel) and anthracycline–based regimens appear to provide higher pCR rates, but the pCR rate is still lower than is optimal. Up to now, apart from trastuzumab for HER2-positive breast cancer, none of the targeted agents can be considered a standard therapy for curable disease. The combination of chemoT followed by IT may have several advantages including: (1) tumor debulking; and (2) the reversal of tumor-induced suppression of immune-based regulatory factors which limits the effectiveness of vaccine-induced, tumor-specific T cells in the tumor microenviroment[76]. Induction of lymphopenia leads to increased availability of IL-7 and IL-15, which enhance CD8+ T cell activity[77;78], as well as depletion of regulatory T cells from the tumor microenvironment. Other studies have shown enhanced T cell trafficking into tumors and intratumoral proliferation of effector cells after induction of lymphopenia.[77-79] Vaccination during homeostasis-driven proliferation may educate the developing T cell repertoire and lead to enhanced T cell memory against tumor antigens[80;81]. Adoptive transfer of naïve or activated antigen-specific T cells after induction of lymphopenia has been reported to induce tumor regression[82;83;84]. **The most effective anti-tumor immunity is induced when vaccination and reconstitution are performed concomitantly**[82].

In cancer patients, Dudley et al[85] demonstrated that lymphodepletion with the combination of cyclophosphamide and fludarabine, followed by infusion of *ex vivo* expanded tumor-specific T cells, mediated a significant tumor regression in heavily pretreated patients with IL-2 refractory metastatic melanoma. Recently, studies showed that paclitaxel enhances IL-12 production in tumor-bearing hosts[86], enhances cytotoxicity of natural killers cells against breast cancer by increasing perforin production[87], and preferentially impairs regulatory T cells rather than effector T cells[88]. Machiels *et al* [89] demonstrated in an animal model that paclitaxel, when given one day before vaccine, depleted regulatory T cells from the tumor microenvironment and enhanced the vaccine efficacy in Her2/*neu* tolerized mice. **In this protocol, we will target patients with operable, triple-negative breast cancer and we propose a strategy that will combine the debulking and immune modulating effects of neoadjuvant chemoT, surgery, and/or radiation with adoptively transferred armed HER2Bi ATC after treatment.** "Third generation" regimens, which are expected to have similar pCR rates and are the most aggressive neoadjuvant standard chemoT regimens are recommended, allowing patients to have the best available standard treatment in addition to the IT. These regimens all consist of doxorubicin (A), cyclophosphamide (C), and a taxane (T). The specific regimens will be: (1) dose dense AC→T [A 60mg/m^2 with C 600 mg/m^2 every 2 weeks for 4 doses (required with G-CSF support), followed by either paclitaxel 175 mg/ m^2 every 2 weeks for 4 doses (required with G-CSF support) or paclitaxel 80 mg/m^2 weekly for 12 doses] or (2) TAC [docetaxel 75 mg/m^2, A 50 mg/m^2, and C 500 mg/m^2 in combination every 3 weeks for a total of 6 doses (required with G-CSF support)].

2.8 Infusions of BiAb alone in other clinical trials for solid tumors.

In a clinical trial involving 27 patients with breast cancer, infusions of 2B1 BiAb (targeting c-erbB-2 and FcγRIII to arm Fc-receptor bearing cells in vivo) alone led to 2 partial responses (PRs) and 3 minor responses[90]. The BiAb MDX-H210 (anti-CD64 x HER-2), was used to treat women with breast cancer[91]. There was 1 PR and one mixed response in 10 evaluable breast cancer patients. Using a similar approach, several groups have developed second and third generation humanized BiAbs reactive to T cells and HER-2 positive tumors[92-96]. In a recent phase II advanced prostate and renal cancer trial using MDX-H210 in combination with GM-CSF, 5 objective responses (>50% decline in PSA) were documented[97]. It is likely that armed ATC may provide a similar or better clinical result.

2.9 ATC as Cytotoxic Vehicles and Cytokine/Chemokine Factories.

Cross-linking the T cell receptor (TCR) of T cells with anti-CD3 leads to activation, proliferation, cytokine synthesis [interferon-γ(IFNγ), tumor necrosis factor-α(TNFα),and GM-CSF][98-108], and non-MHC restricted cytotoxicity directed at tumor targets[109-112]. Many chemotherapies kill tumor cells via apoptosis and apoptotic cells are a good source of antigens[113]. While tumor cell apoptosis induced by chemoT leads to increased cross-presentation of tumor antigens[114;115], there is a lack of priming effective anti-tumor immune responses, suggesting that the transition from cross-presentation to cross-priming needs help[115]. If ATC can mediate enhanced cytotoxicity directed at cancers after chemoT, armed ATC may have greater anti-tumor activity after chemoT.

2.10 ATC armed with HER2Bi for Metastatic Breast Cancer (MBC). In our phase I clinical trial, we have administered ATC armed with HER2Bi to women with both HER2-positive and negative MBC to determine their safety and the maximum tolerated dose. 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[116].** Evaluation of immune responses in our phase I clinical trial patients suggests that infusions of Her2Bi-armed ATC induce robust immune responses regardless of the patient's HER2 status**.** Median OS is 56 months for the Her2 3+ group, 20 months for the Her2 0-2+ group, and 26 months for the entire **MBC group** with median follow-ups of 28, 17, and 21 months, respectively (**Fig-6**). There was no statistical difference between them (p = 0.51), suggesting that armed ATC may prolong survival independent of HER2/*neu* status. Unlike therapy with Herceptin®, which induces cellular death through interference with receptor-mediated signaling pathways, low levels of HER2 receptors can induce immune responses, even in TNBC patients. Our clinical data shows that immune responses were obtained in women with MBC previously treated with taxanes. Therefore, IT in addition to a standard neoadjuvant taxane containing chemoT regimen may be ideal to provide anti-tumor activity and impair T regulatory cell activity while improving NK cell activity. We also expect it would provide a measure of lymphodepletion for the expansion of vaccine responsive immune elements.

2.11 Pre-Clinical Studies: Targeting Cancer Cells with Her2Bi-armed ATC. In preclinical studies, we showed that Her2Bi, a bispecific antibody created by chemical heteroconjugation of anti-CD3 (OKT3; Centocor Ortho Biotech) with anti-HER2 (Herceptin®; Genentech) can be used to arm ATC which were produced by activating PBMC with 20 ng/ml of anti-CD3 and expanded in 100-500 IU/ml of IL-2 up to 14 days[116]. ATC armed with an optimal dose 50 ng of Her2Bi/10^6 ATC exhibits high levels of specific cytotoxicity against HER2-expressing cell lines derived from breast cancer (SK-BR-3, MCF-7)[116], pancreatic cancer (MIA PaCa-2, COLO 356/FG)[117], and prostate cancer (LNCaP, DU 145, PC-3) lines[118]. At effector/target ratios (E/T) from 3.125:1 to 25:1, Her2Bi-armed normal and armed patient ATC are significantly more cytotoxic against low or high HER2-expressing cells than unarmed ATC, or ATC armed with an irrelevant BiAb such as anti-CD3 x anti-CD20 BiAb.

Pertinent to this proposal, **Fig-1** shows high levels cytotoxicity directed at extremely low-expressing Her2/*neu* cell line MCF-7 which expresses <1.0 x 10^4 Her2 receptors /cell. This number of receptors on the tumor cell surface is not detectable by IHC. Her2Bi-armed ATC secrete high levels of Th_1 cytokines when they bind to tumor. Redirected non-MHC restricted T cell killing via the perforin/granzyme system is the mechanism responsible for enhanced killing. In mice, co-injections of Her2Bi-armed ATC with PC-3 prostate tumor cells in a Winn assay completely prevented development of tumors and intravenous infusions of Her2Bi-armed ATC significantly delayed growth of established PC-3 tumors compared to mice that received ATC or vehicle alone ($p< 0.001$) without any side effects[118].

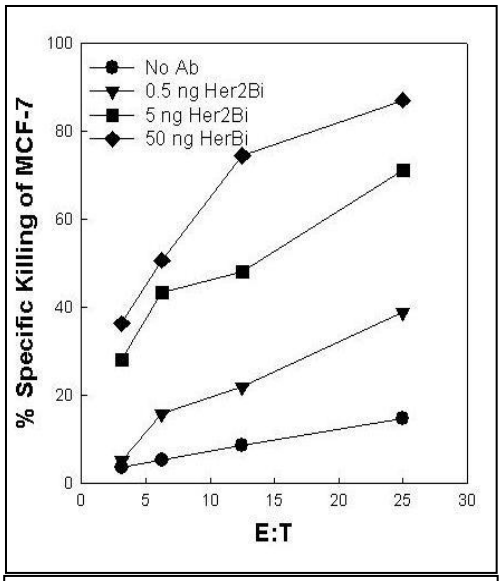


Fig 1: % Cytotoxicity (^{51}Cr release) of MCF-7 Mediated by ATC Armed Normal ATC with Increasing Arming Doses of Her2Bi. ATC were armed with 0.5, 5.0, and 50 ng/10^6 ATC and plated at E/T of 25:1, 12.5:1, 6.25:1 and 3.125:1. Adherent MCF-7 were seeded (40,000 cell/well in flat-bottomed plates, labelled with ^{51}Cr, and effectors were added at the indicated E/T and ^{51}Cr release measured after 18 hrs.

2.12 Her2Bi-Armed ATC Target and Kill SUM 1315 cells (Her2- negative cell line). In order to determine whether armed ATC could target and lyse a Her2-negative cell line, Sum 1315 (a gift from Stephen Ethier) for cytotoxicity was evaluated. Sum 1315 is a breast cancer cell line known to be negative for Her2/*neu* by Western blotting (**Fig-2a)** and flow cytometry(**Fig-2b**). **Fig-2c** shows specific cytotoxicity of Her2Bi armed ATC 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-3c** shows that armed ATC 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[119]. *These data and our clinical and immunologic data on patients treated with Her2Bi armed ATC who were Her2-negative in our phase I clinical trial provide the rationale to target patients with HER2 (0-2+) expression. This approach would potentially benefit up to 80% of patients with BrCA including those with triple negative BrCa.*

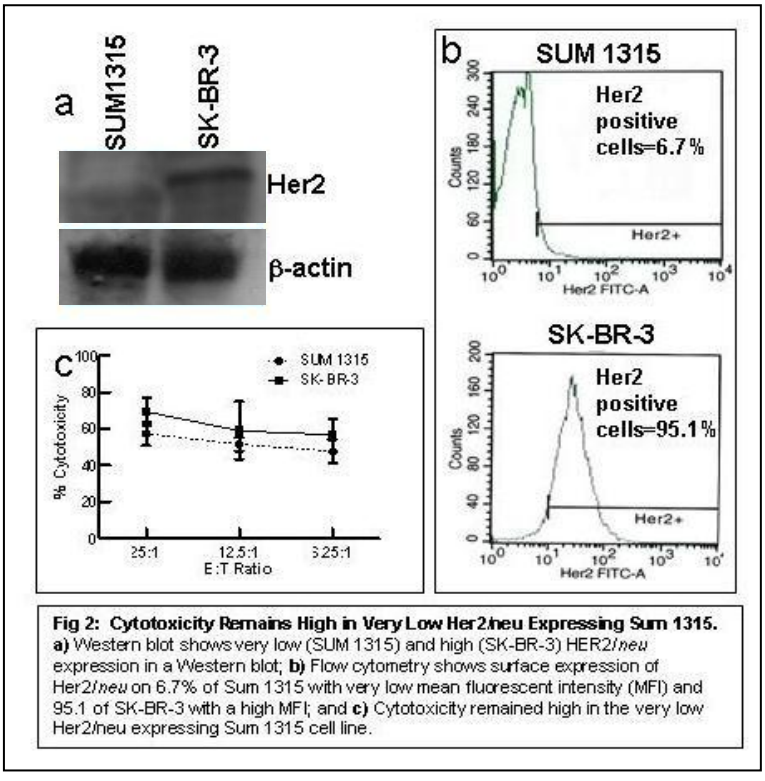


Fig 2: Cytotoxicity Remains High in Very Low Her2/neu Expressing Sum 1315. **a)** Western blot shows very low (SUM 1315) and high (SK-BR-3) HER2/*neu* expression in a Western blot; **b)** Flow cytometry shows surface expression of Her2/*neu* on 6.7% of Sum 1315 with very low mean fluorescent intensity (MFI) and 95.1 of SK-BR-3 with a high MFI; and **c)** Cytotoxicity remained high in the very low Her2/neu expressing Sum 1315 cell line.

2.13 Phase I Clinical Trial Patients and Treatment Schema. We performed a phase I dose escalation clinical trial in women with stable to slowly progressive HER2 0-3+ MBC to determine the maximum tolerated dose (MTD) for Her2Bi-armed ATC. The protocol involved 2 infusions per week for 4 weeks with daily IL-2 and twice weekly GM-CSF beginning 3 days before the first infusion and ending 7 days after the last infusions of armed ATC. **Table 1** shows the characteristics of 19 women enrolled who had a median age of 48 years (range: 31-68 years). All patients were enrolled between September 2002 to January 2008. One patient was treated twice (twice at dose level 2). Patients were leukapheresed for T cells that were expanded with anti-CD3 and IL-2 for 14 days. ATC were harvested, armed with Her2Bi, cryopreserved, thawed, and infused at the bedside. Patients were accrued to each dose level (**Table 2**) with dose escalation based on the criteria that persistent grade 3 non-hematological toxicity did not occur in 3 of 3 patients. If 1 of 3 patients at the dose level developed persistent grade 3 toxicity, then an additional 3 patients would be added to the dose level. The dose would be escalated if 5 of 6 patients at that dose level did not have a dose limiting toxicity (DLT). If 2 of 6 at the dose level had a DLT, then the maximum tolerated dose (MTD) will be the dose below the dose.

Table 1: Patient Characteristics

	No.	%
Age		
< 50	**11**	**58**
≥ 50	**8**	**42**
Cancer Stage		
Stage IV	**19**	**100**
Performance Status (ECOG)		
0	**16**	**84**
1	**3**	**16**
2	**0**	**0**
ER/PR Status		
Positive	**12**	**63.1**
Negative	**6**	**31.5**
Unk	**1**	**5.2**
HER2/neu Status		
0	**8**	**42.1**
1+	**1**	**5.2**
2+	**2**	**10.5**
3+	**7**	**36.8**
Unk	**1**	**5.2**
Prior Treatment w/ Herceptin		
Yes	**7**	**36.8**
No	**12**	**63.2**

Table 2: Dose Escalation Schedule

Dose Level	Her2Bi-armed ATC[a] (# cells/infusion)	IL-2[b] (IU/m²/day)	GM-CSF[c] (µg/m² twice/wk)	Patients	
				No.	%
1	5×10^9	300,000	250	6	31.5
2	10×10^9 *	300,000	250	*4*	21.0
3	20×10^9	300,000	250	*8*	42.1
4	40×10^9	300,000	250	1	5.2

[a] Administered IV twice/week for 4 weeks; [b] Administered SQ beginning 3 days prior to armed ATC and ending 7 days after the last infusion; [c] Administered SQ beginning 3 days prior to armed ATC and ending 7 days after the last infusion
* Dose level selected for Phase II trial

2.14 Phenotype and Viability of the Cryopreserved Cell Products. Phenotyping and viability was performed on cryopreserved products. **Table 3** summarizes the %CD3, CD4, CD8 and viability of the products that were infused into the patients. The mean % CD3, CD4, and CD8 were 86.7%, 52.4%, and 34.6%, respectively. The mean viability was 92.2%.

Table 3: Phenotyping and Viability

Dose Level	%CD3	%CD4	%CD8	Viability
1	97	90	8	95%
1	61	42	18	90%
1	95	50	45	96%
1	93	62	32	94%
1	98	50	48	97%
1	93	61	32	94%
2	96	37	60	91%
2	97	62	37	95%
2	63	46	21	87%
3	95	74	21	95%
3	87	34	55	93%
3	80	55	24	91%
3	57	31	35	92%
3	94	44	37	93%
3	92	40	60	72%
3	91	63	25	98%
4	85	50	*31*	89%
4	*ND*	*ND*	*ND*	98%
Average (%)	86.7 ± 13.5	52.4 ± 15.2	34.6 ± 15.0	92.2 ± 5.9

2.15 Cytotoxicity Mediated by Cryopreserved Armed and Unarmed ATC from Patients. *Specific cytotoxicity directed at SKBR-3 was quantitated on cryopreserved armed ATC and unarmed ATC products using the method previously* described[116]. **Fig-3** summarizes the cytotoxicity mediated by cryopreserved armed ATC (**P aATC**) and unarmed ATC (**P ATC**) from the patients and armed ATC **(P ATC**) and unarmed (**N ATC**) from normal controls. Cytotoxicity was directed at SK-BR-3 targets at an E/T of 12.5:1. Cytotoxicity mediated by P ATC armed with irrelevant anti-CD3 x anti-CD20 BiAb at 12.5:1 was comparable to unarmed ATC (data not shown). These are the product release data for the cell products used for the clinical infusions. M*ean cytotoxicity (±SD) mediated by cryopreserved patients' Her2Bi-armed ATC (49.32% ±*

*21.54) was significantly greater (p < 0.0001) than that mediated by cryopreserved patients' unarmed ATC (3.21 ± 3.20) (**Fig 3**).* The Spearman correlation coefficient comparing % cell type to specific cytotoxicity was r = -0.55 with a p = 0.03 for CD4. There was an inverse correlation between *in vitro* cytotoxicity and the proportion of CD4 cells in the expanded ATC product (Spearman correlation coefficient, comparing % cell type to specific cytotoxicity) with an r = -0.55 with a p = 0.03 for CD4.

2.16 Cytotoxicity Mediated by Her2Bi-Armed ATC Unchanged from Fresh Armed ATC. To determine whether cytotoxicity mediated by armed or unarmed ATC was altered by cryopreservation, cytotoxicity of fresh aATC, cryopreserved aATC, and ATC that were armed after thawed were compared for cytotoxicity (**Fig-4**). **These data show that that cryopreservation did not alter the ability of armed ATC to mediated specific cytotoxicity.** There were no statistical differences in selected samples that were tested fresh versus frozen.

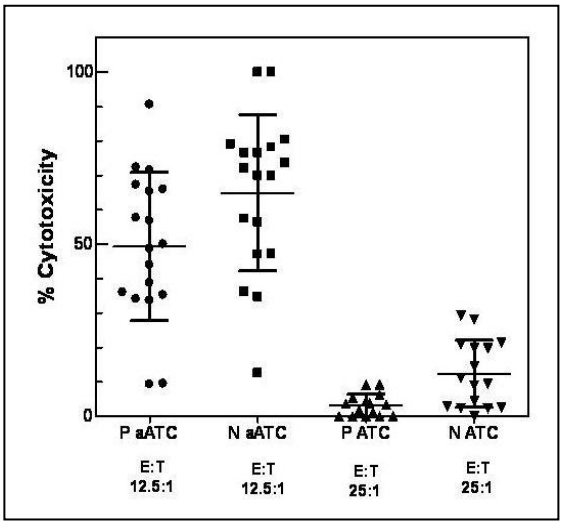


Fig-3: Summarizes cytotoxicity mediated by cryopreserved armed patient ATC **(PaATc**), normal armed ATC **(N aATC**), patient ATC (**PATC**) and normal ATC (**NATC**) The means ± 1 S.D. are indicated by the error bars. All samples were frozen, thawed and then tested as part of quality control product release.

2.17 Methodology to Detect Armed ATC and Correlations with Specific Cytotoxicity Mediated by Known (Spike) Proportions of Armed ATC in the Peripheral Blood Samples. To determine the lower limit of sensitivity for detection of armed ATC-mediated cytotoxicity, we performed a Her2Bi-armed ATC spiking experiment. PBMC were spiked with 5% Her2Bi-armed ATC and Miltenyl selection was performed to separate the IgG2a+ (OKT3-moiety of the BiAB) and IgG2a- populations. Each population was analyzed by flow cytometry to detect cell bearing IgG2a mouse IgG (**Fig-5A**). Staining showed 0.87% IgG2a+ cells in the IgG2a-depleted population whereas the positively selected population contained 82.53 IgG2a+ cells. In a parallel study, known proportions of Her2Bi-armed ATC (0-100%) were mixed with PBMC to create a standard curve for detecting IgG2a+ cells (**Fig-5B**). This standard curve could detect as little as 0.87% IgG2a+ cells (**Fig-5A**) in samples containing known proportions of Her2Bi-armed ATC. These studies established the lower detection limit for flow cytometry for IgG2a+ cells.

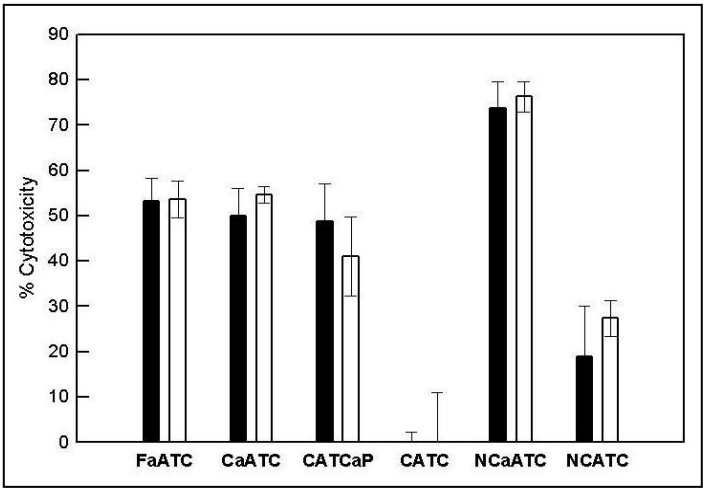


Fig-4: Shows comparable cytotoxicity directed at SK-BR-3 by patient's fresh aATC (**FaATC)**, cryopreserved aATC (**CaATc)**, and cryopreserved ATC that were armed post-thaw (**CATaP)** The also shows cryopreserved ATC (**CATC**), normal cryopreserved ATC (**NCaATC),** and cyropreserved ATC that were not armed with Her2Bi.

2.18 Correlations Between Proportions of Spiked Armed ATC and Specific Cytotoxicity in the Spike Blood Samples. Cytotoxicity assay at an E/T of 25:1 was performed using a range of proportions for armed ATC to PBMC (**Fig-5C**). There was no detectable increase in cytotoxicity against SK-BR-3 cells over PBMC alone in PBMC populations spiked with 0.1% or 1% Her2Bi armed ATC. These spiking levels are above the lower detection limit for flow cytometry. These are levels comparable to circulating armed ATC levels. Flow cytometry is more sensitive than cytotoxicity assay for identification of armed ATC populations. Together, these data show that the cytotoxicity observed in the blood and in the IgG2a- fractions of patient PBMC (**see Fig-17 in section 3.21**) cannot simply be attributed to the few percent of armed ATC detected in the blood. These data show that the enhanced specific cytotoxicity in patients' PBMC

is due to endogenous cytotoxicity.

2.19 The Kinetics and Survival of Armed ATC in a Patient. PBMC were obtained pre and post after each infusion to determine the proportion of IgG2a+ cells within the CD3+ population (**Fig-6**). The post infusion values show transiently elevated numbers of armed ATC (diamonds) in the circulating that return to the CD3+ pre baseline (circles) that range from ~1 to 8%. Up to 50% of the circulating T cells were positive for IgG2a after the second infusion.

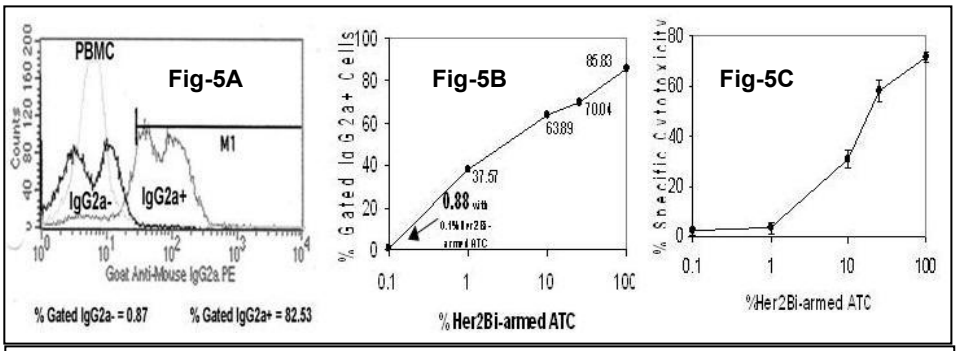


Fig-5: Shows Phenotyping of Miltenyl purified IgG2a+ and IgG2a- populations (Panel A), phenotyping for IgG2a+ cells in spiking experiment wherein various proportion of Her2Bi armed ATC were added to PBMC to determine the lower limit of sensitivity of detection for IgG2a+ cells (Panel B), and cytotoxicity of PBMC spiked with various proportions of Her2Bi-armed ATC. IgG2a+ cells were detected by flow cytometry using goat anti-mouse IgG2a-PE to detect the OKT3 moiety of OKT3 x Herceptin.

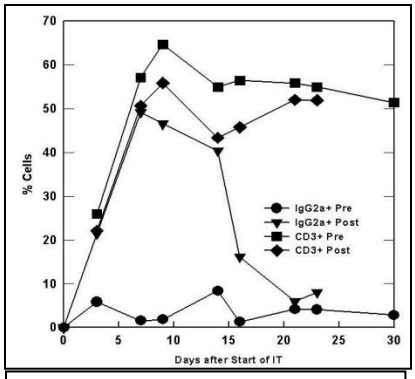


Fig-6: Shows the Proportion of IgG2a+ and IgG2a- CD3+ Cells as a Function of Days after the Start of Immunotherapy. PBMC were double stained with goat PE conjugated anti-IgG2a mouse and anti-CD3 to detect the proportion of CD3+ that were IgG2a+ and IgG2a-. Pre and 2 hour post infusion various were obtained.

2.20 Detection of Circulating murine IgG2a+ (OKT3+) Cells in 8 Patients during Immunotherapy. To confirm the survival of IgG2a+ cells in additional patients, we tested an additional four patients (FG60112, FG60121, FG60056, and FG60191) for circulating armed ATC by phenotyping the peripheral blood for mouse IgG2a+ cells (**Fig-7**). Phenotyping the peripheral blood obtained prior to immunotherapy, before infusions #5 and #8, and 1 wk after the last infusion showed evidence for accumulation and persistence in some patients of armed ATC that last up to 1 weeks after the last infusion. The left panel of **Fig-7** shows Dose Level 1 and the right panel shows Dose Levels 2 and 3.

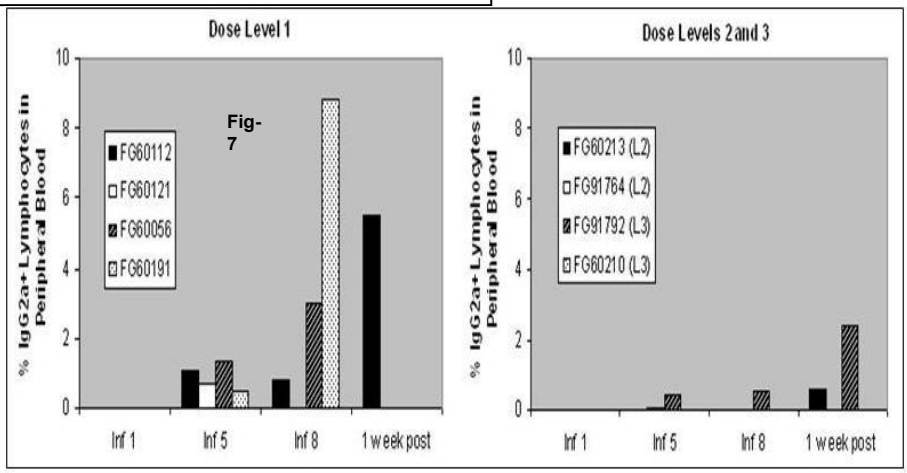


Fig-7: Armed ATC (IgG2a+ cells) Detected Up to 1 Week Post Infusion. Four patients in Dose Level 1 (left panel) and 4 patients in Dose Levels 2 and 3 (right panel). Cells were detected in whole blood using goat anti-mouse IgG2a+ antibodies to phenotyping the circulating cells prior to IT, before infusions 5 and 8 (inf 5 and inf 8) and 1 week after the last infusion (1 week post).

2.21 *In Vivo* Trafficking of Her2Bi-armed ATC. Infusions of ^{111}In labelled Her2Bi-armed ATC localized to the bone marrow, lung, liver, and spleen within 4 hours of injection (**Fig-8 left panel**). By 24 hours, Her2Bi-armed ATC had cleared from the lungs but persisted in the bone marrow, liver and spleen for up to 4 days post-infusion (**Fig-8, right panel**). Serial measurements of ^{111}Indium labeled armed ATC in whole blood (**Fig-9**) showed that about 50% Her2Bi-armed ATC are cleared from the blood within ~30 min. The patient's blood samples continue to be positive for radioactivity (1.1% of the

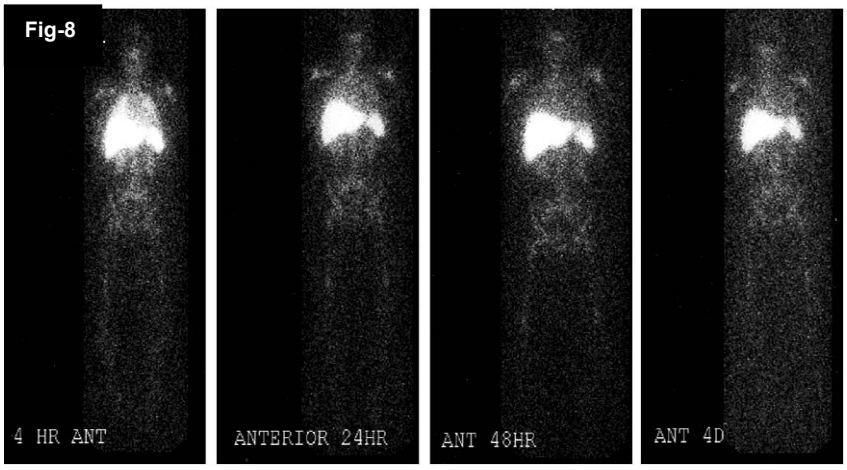


Fig-8: ^{111}Indium Whole Body Scans Show Trafficking of Armed ATC as a Function of Time. Armed ATC (200 x 10^6) were labeled with Indium and infused IV. Serial scans were obtained at 4 hr (left panel), 24 hr (2nd leftmost panel), 48 hr (2nd rightmost panel), and 4 days (rightmost panel).

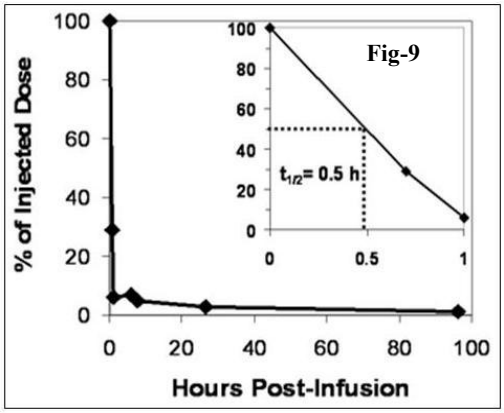
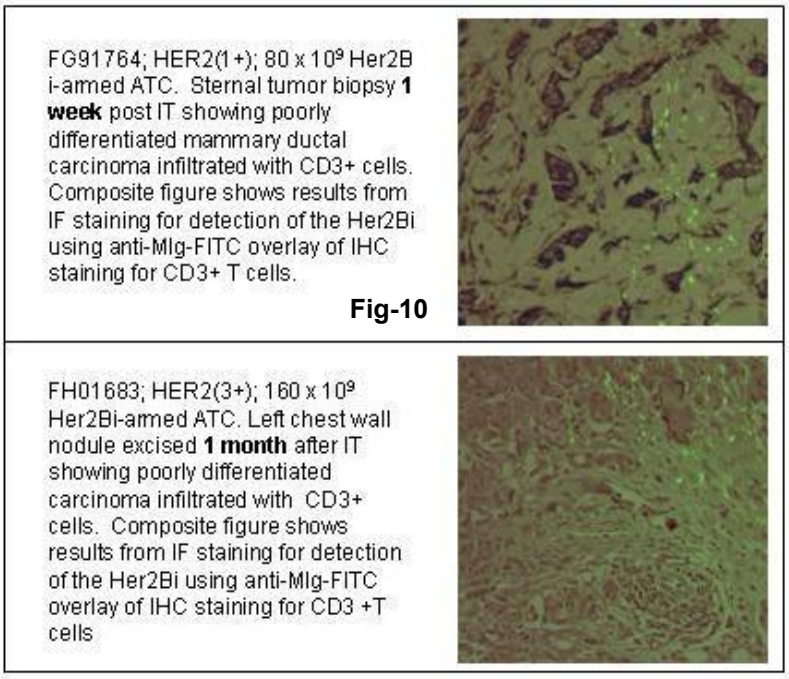


Fig-9: Serial Measurements of ^{111}In Labeled aATC in the Blood after Injection of 200 x 10^6 Decayed.

initial concentration) up to 4 days post infusion. It is clear that armed ATC persist up to 1 week or more post infusion.

2.22 Localization of Her2Bi-armed ATC to Breast Cancer Tumors. Formalin-fixed, paraffin-embedded samples were prepared from a chest wall nodule excision and a sternal tumor biopsy at 1 month and 1 week post treatment, respectively. Anti-CD3 was used to detect human T cells and goat anti-mouse Ig was used to detect mouse IgG2a using an overlay strategy. Tissues samples were sectioned, deparaffinized, stained with H&E and characterized for tumor content by a pathologist. Adjacent sections were stained with anti-CD3 to detect T cells using the CSA Peroxidase System (DAKO, Carpinteria, CA) after target retrieval and endogenous biotin/avidin and peroxidase quenching with the CSA Ancillary System (DAKO). Anti-CD3 antibody (1 μg/ml) was diluted in Background Reducing Components (CSA Ancillary System) and incubated with tissue samples for 30 min at room temperature. Primary antibody was detected by incubating for 15 min with biotinylated goat anti-mouse immunoglobulins, and the signal was amplified and visualized by diaminobenzidine precipitation at the antigen site. In parallel, adjacent sections were stained with biotinylated goat anti-MIg (directed at the mouse IgG2a) followed by streptavidin-FITC to detect the goat anti-MIg. Images acquired using fluorescent filters were overlayed upon images acquired by light microscopy creating composite images (**Fig-10)** to evaluate co-localization of staining.

2.23 Evaluation of MTD and Toxicities. The purpose of this study was to determine the MTD of Her2Bi-armed ATC and to define the toxicity profile. The MTD is defined as the dose below the dose at which dose limiting toxicity is encountered in 2 of 6 patients. Currently, the highest dose level completed is the 40 x 10^9 Her2Bi-armed ATC per infusion (320 x 10^9 total dose of armed ATC); the MTD has not yet been reached. We have accrued one patient at the dose level of 40 x 10^9 Her2Bi-armed ATC per infusion (320 x 10^9 total dose). The most frequent side effect was Grade 3 chills. Grade 3 headaches emerged as the second most common side effect. Fewer than 50% of the total



patients enrolled experienced each of the remaining symptoms. **Table 4** shows the frequency of side effects in the study as a function of dose level based upon the NCI Immunotherapy Protocol Toxicity Table. By episode per infusion, incidence of chills and headache at dose level 1 (8.6% and 3.1%, respectively) increased for dose level 2 (20.8% and 8.3%, respectively) and then again at dose level 3 (43.1% and 19.6%, respectively). All patients with grade 3 chills responded to Demerol. One patient at dose level 3 experienced headache and hypertension that failed to respond to interventional therapy (grade 4); the patient was removed from the study after receiving 3 infusions for a total of 65.7 x 10^9 armed ATC. The patient was suspected of brain metastasis, but surgical follow-up revealed a right frontal subdural hematoma that was evacuated without complications. An adverse event was reported and the patient was removed from the protocol. Accordingly, 3 additional patients were added to dose level 3. None experienced persistent grade 3 toxicities and no fatalities occurred as a result of armed ATC infusions. One patient has received dose level 4 (40 billion/dose) successfully.

Table 4: Side Effects by Dose Level

Dose Level	Reaction	# Patients Affected (% at Dose Level)	Total # of Episodes by Grade			
			1	2	3	4
1	Dyspnea	0 (0%)	0	0	0	0
	Chills	4 (66.7%)	0	2	9	0
	N/V	3 (50%)	4	0	0	0
	Headache	2 (33.3%)	0	0	4	0
	Fever	1(16.7%)	1	0	0	0
	Hypotension	1 (16.7%)	0	1	0	0
	Hypertension	0 (0%)	0	0	0	0
	Back Pain	0 (0%)	0	0	0	0
2	Dyspnea	0 (0%)	0	0	0	0
	Chills	2 (66.7%)	0	0	5	0
	N/V	1 (33.3%)	0	0	1	0
	Headache	2 (66.7%)	0	0	2	0
	Fever	1 (33.3%)	1	0	0	0
	Hypotension	0 (0%)	0	0	0	0
	Hypertension	0 (0%)	0	0	0	0
	Back Pain	0 (0%)	0	0	0	0
3	Dyspnea	1 (14.3%)	0	1	0	0
	Chills	5 (71.4%)	0	0	22	0
	N/V	3 (42.9%)	4	1	0	0
	Headache	6 (85.7%)	0	2	8	1
	Fever	1 (14.3%)	0	1	0	0
	Hypotension	1 (14.3%)	0	2	0	0
	Hypertension	1 (14.3%)	0	0	0	1
4	Dyspnea	0(0%)	0	0	0	0
	Chills	1 (100%)	0	2	0	0
	N/V	1 (100%)	0	0	1	0
	Headache	1 (100%)	0	1	0	0
	Fever	1 (100%)	1	0	0	0
	Hypotension	1 (100%)	1	0	0	0
	Hypertension	0(0%)	0	0	0	0
	Back Pain	0(0%)	0	0	0	0

2.24 Dose Modifications. All patients with grade 4 non-hematologic toxicity were to be removed from protocol. If signs of cardiac toxicity developed, the ejection fraction (EF) by MUGA or echocardiography would have been re-tested and, if the EF fell by >10%, the patient would have been removed. If grade-3 toxicity persisted, treatment would have been held until toxicity resolved to grade 0 or 1 and the same dose of armed-ATC would have been washed to eliminate DMSO. If washing did not reduce toxicity, then the treatment would have been resumed with a 50% reduction in the armed-ATC dose. If the toxicities continued, the IL-2 would have been stopped and the aATC infusions would have been continued at the reduced dose. If grade 3 toxicity persisted, the aATC infusions would have been stopped. One patient at dose level 3 had her infusions stopped due to grade 4 toxicities (hypertension and headache associated with a subdural hematoma). Of the remaining 18 patients, none had their doses reduced due to persistent grade 3 toxicities. One patient had a fatal congestive heart failure that was likely due to Digitoxin toxicity. Three additional patients were added to the dose level without a DLT.

Table 5: Clinical Responses to Her2Bi-armed ATC by Dose Level[a]						
Response (%)	All Pts #	All Pts %	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
PR	1	5.3	0	1(100)[c]	0	0
SD	10	52.6	2(20)	2(20)	6(60)	0
PD	6	31.5	3(50)	2(33.3)	1(16.6)	0
NE	2	10.5	1(50)	0	1(50)	0

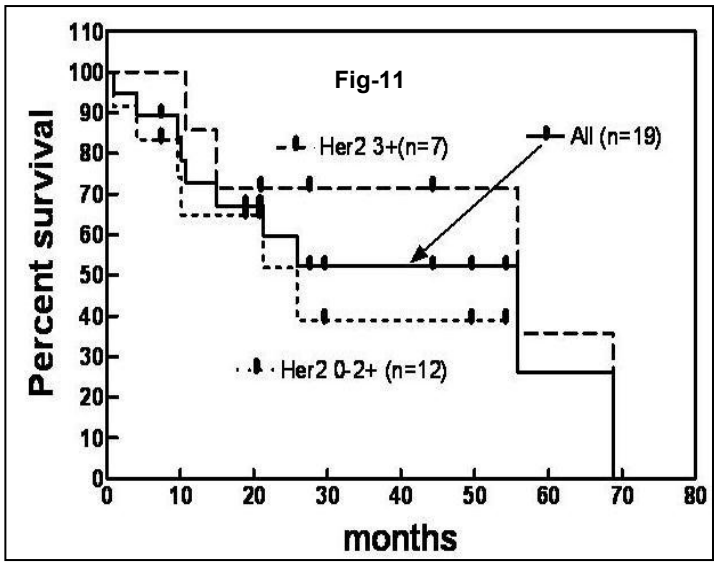
[a] At one month follow-up after the last infusion. [b]Did not complete infusion schedule or died before 1 month followup. [c]Pt received only 80 billion cells due to slow expansion.

Table 6: Clinical Responses to Her2Bi-armed ATC by HER2/*neu* Expression Level [a]			
Response (%)	***All***	HER2(3+)	HER2(0-2+)
PR	**1 (6)**	0 (0)	**1 (8.3)**
SD	**10(59)**	**5 (71.4)**	**5 (50)**
PD	**6(35)**	**2 (28.5)**	**4 (41.6)**

[a] At one month follow-up after the last infusion; excluding 1 unevaluable patient and one patient with unknown HER2/*neu* status who was stable.

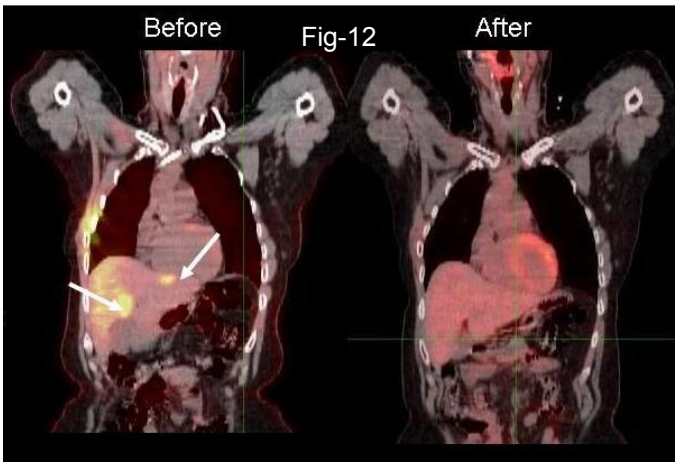
2.25 Clinical Responses to Her2Bi-armed ATC. Although clinical responses are not typically a primary endpoint for phase I clinical studies, we evaluated responses to therapy based upon tumor measurements, in those patients with measurable disease, as well as impact of treatments on levels of tumor markers in those patients who were clinically positive for tumor markers prior to initiation of treatments. One partial remission was obtained and more than half of the patients remain stable with no evidence of increase in tumor size or development of new lesions at their follow-up, one month after completion of the last Her2Bi-armed ATC infusion. Percentages of patients who remained stable during and after treatment with Her2Bi- armed ATC appear to increase as a function of treatment dose level (**Tables 5 and 6**). Serum tumor markers exhibited impressive decreases in Carcinoembryonic Antigen (CEA), CA 27.29, and Her2 receptors in the serum within one month after infusion Her2Bi-armed ATC (**Table 7**).

Table 7: Clinical Tumor Marker Responses			
Tumor Marker	No. Testing Positive Pre-TX	> 50% Reduction (No. HER2[0-2+])	15%-50% Reduction (No. HER2[0-2+])
CA 27.29	5	0	3
CEA	4	2	2
HER2/*neu*	5	2	2

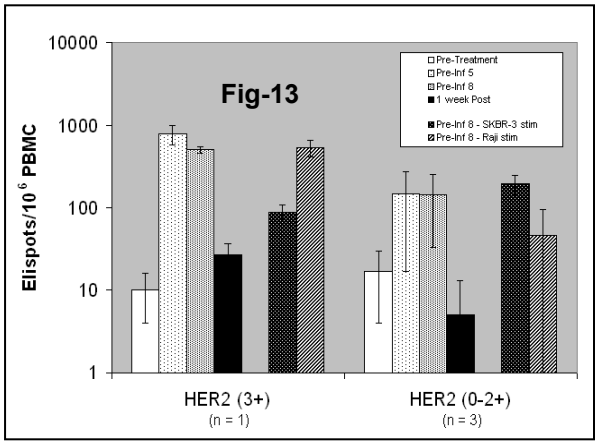


2.26 Overall Survival. For these analyses, we evaluated patients with tumors that had been identified as having HER2(3+) expression levels to patients having HER2(0-2+) expression levels (**Fig-11**). One patient with an unknown HER2 status was analyzed with the Her2 0-2+ group. The median OS is 56 months for the Her2 3+ group, is 20 months for the Her2 0-2+ group, and is 26 months for the entire group with median follow-ups of 27.57 (10.8-68.7), 16.54 (0.93-54.2), and 21.3(0.93-68.7) months, respectively.

2.27 Documentation of Partial Response in Her2 Negative Patient.
A partial responder in the Her-negative group had 2 well-defined liver metastases on a PET/CT (2.5 x 1.7 cm and 2.5 x 1.3 cm as shown in **Before, Fig-12**). Reimaging after IT showed regression of the two lesions after 6 months (**After Fig-12**). The two lesions in the section disappeared. There was one small lesion that remained in a posterior section (not shown). The sum of longest diameters (LDs) was decreased by 30% at 4 weeks after IT and was decreased by >70% at 6 months.



2.28 Immune Responses as a Function of Her2Bi-armed ATC infusions. PBMC were obtained just prior to the indicated infusion number (Inf #) to evaluate steady-state (no ex vivo stimulation) IFNγ EliSpots (3-4 days after the last armed ATC infusion) and stimulated (SK-BR-3) IFNγ EliSpots. Four patients (1 with HER2 [3+] and 3 with HER2[0-2+] were tested. Three of 4 of the patients had significant increases in specific cytotoxicity after Inf #4 (without stimulation) that persisted after treatment (**Fig-13**). Stimulation of the PBMC with SK-BR-3 did not increase the Elispots in the pre-Inf #8 samples. **Fig-14** shows a representative patient in whom the IFNγ EliSpots and serum IFNγ levels represent ongoing pre-existing *in vivo* activation of CTL activity. The IFNγ EliSpots and serum IFNγ levels peaked prior to Inf #5 suggesting that 3-4 infusions was sufficient to maximally activated the endogenous immune system. These data suggest that IFNγ EliSpots will be sufficient to provide a surrogate marker for specific cytotoxicity directed at SK-BR-3 targets. All together, IFNγ EliSpots and specific cytotoxicity appear to correlate even with small numbers of samples. The need for specific monitoring serum IFNγ would not be warranted.



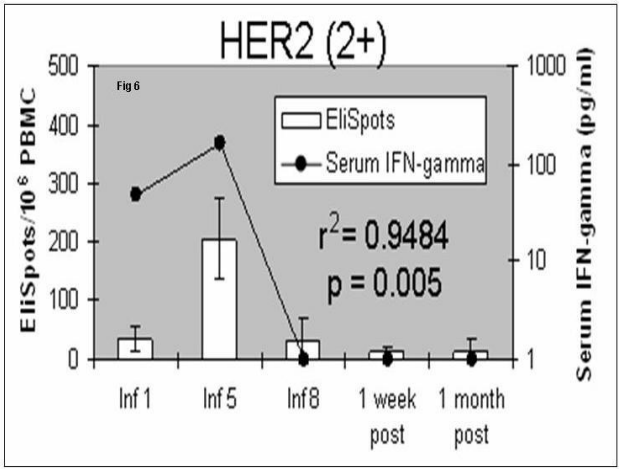


Fig-14: Representative patient had PBMC tested for IFNγ EliSpots (left axis) and serum IFNγ (right axis) tested at the times indicated. Fresh PBMC were plated in Elispot wells and the number of EliSpots were quantitated.

2.29 Enhanced Specific Cytotoxicity in Patient PBMC after Infusions of Her2Bi-armed ATC as Function of Time after Infusions. Five patients undergoing Her2Bi-armed ATC infusions were studied for cytotoxicity directed at SK-BR-3 before, during, and after treatment. **Fig-15** shows 3 of 5 and the **Fig-16** shows 2 of the 5. The PBMC from the 2 patients in **Fig-16** had their PBMC separated into IgG2a+ and IgG2a- subpopulations to determine which populations mediated specific cytotoxicity. A significant increase in specific cytotoxicity directed at SK-BR-3 cells by PBMC obtained from all patients was observed during the course of treatment, with peak levels ranging from 14.15 ± 3.08% to 30.71 ± 0.54%. Specific cytotoxicity tended to increase during treatment with peak cytotoxicity occurring around infusion #8[120]. Cytotoxicity persisted up to 4 months after immunotherapy in patient FG1764. These data suggest that armed ATC not only persist but function *in vivo*.

2.30 Evidence for Activation and Immunization of Endogenous Immune Cells. When IFNγ EliSpots were performed on IgG2a-depleted blood from patients during and after immunotherapy, *there were high numbers of as IFNγ EliSpots in the IgG2a negative population suggesting that endogenous cells were producing the immune response.* To confirm this observation using specific cytotoxicity, we tested IgG2a- populations from 2 patients (FG91801 and FG1792) for specific cytotoxicity directed at SK-BR-3 targets[120]. Total PBMC obtained at times just prior to indicated infusion (Inf#) of armed ATC, original uncultured leukapheresis product, endogenous IgG2a- populations were prepared by depleting IgG2a+ cells from the PBMC sample. The IgG2a- depleted PBMC were tested in specific cytotoxicity against SK-BR-3 or Raji target cells (**Fig-16**). Taken together, these data together with the IFNγ-EliSpot data (**section 3.18, Fig-13**) provide very strong complementary arguments that endogenous immune cell populations are involved in the specific cytotoxicity observed after infusions.

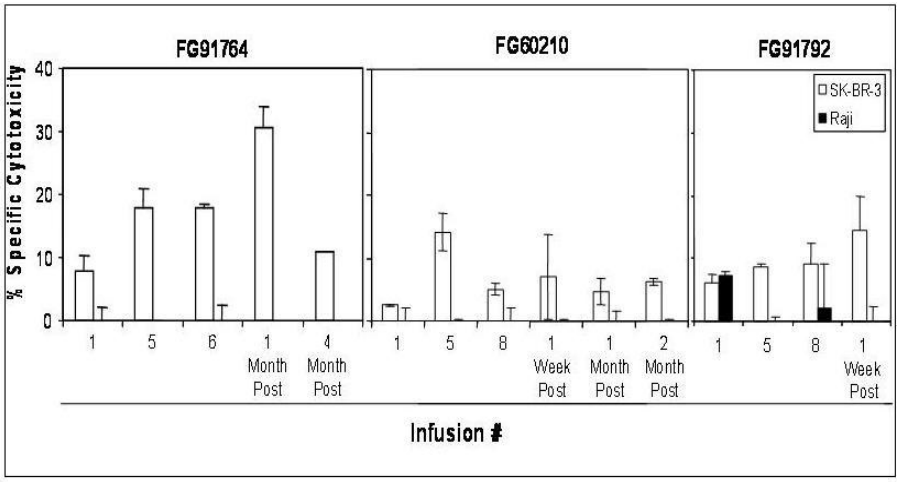


Fig-15: Specific Cytotoxicity Mediated by PBMC obtained at the Designated Time Points from Patients Directed at SK-BR-3 (open bars) and Raji (solid bars). 51Cr was performed at an E/T of 10:1. PBMC were obtained from BrCa patients at the time of leukapheresis to obtain leukocytes for ATC expansion, prior to the 5th, 6th, and 8th infusions. 1 wk. 1 mo. 2 mo. and 4 mo after armed ATC infusions.

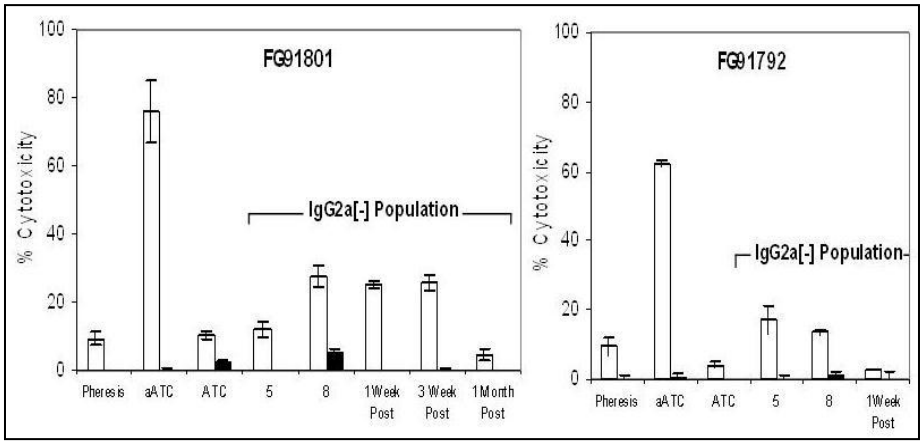


Fig-16: Pheresis, Armed ATC, unarmed ATC and IgG2a- Populations Were Tested for Specific Cytotoxicity Directed at SK-BR-3. Specific cytotoxicity (E/T = 10:1) was performed in 51Cr assays. IgG2a+ were depleted using Miltenyl beads. The clear bars show cytotoxicity directed at SK-BR-3 targets and the solid bars show cytotoxicity directed at Raji targets.

2.31 Cytotoxicity Mediated by PBMC of Women Treated with Her2Bi armed ATC 1 Week after Therapy. Specific cytotoxicity of PBMC from 8 patients directed at SK-BR-3 and Raji (irrelevant control) was tested 1 week after completing immunotherapy using at E/T of 10:1 **(Fig-17).** Her2Bi-armed ATC treatments significantly increased cytotoxicity over cryopreserved PBMC from the leukapheresis product (p = 0.02). The solid black circles are Her2 positive patients and the open circles are Her2 negative patients. These data show that there was a statistically significant increase in the

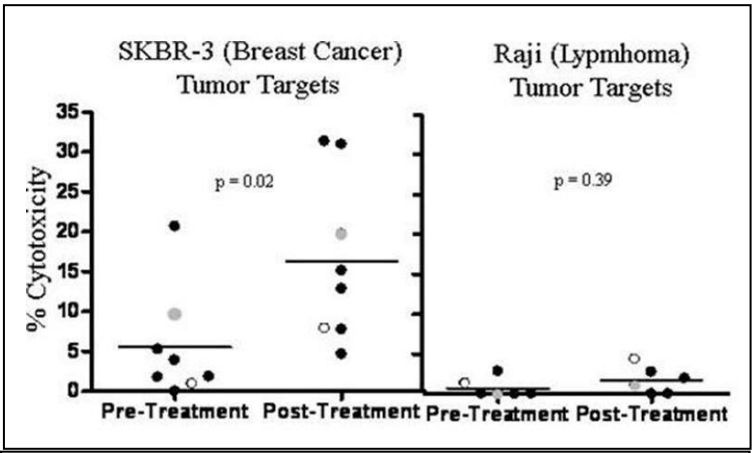


Fig-17: Specific Cytotoxicity by Patient PBMC Directed at SK-BR3 Increases While Nonspecific Killing of Raji Targets Remains Low. ^{51}Cr release was performed with 40,000 adherent targets at a E/T ratio of 10:1. Two Her2 0+ negative patients are indicated by grey and open circles. The solid black circles indicate Her2 3+ positive patients.

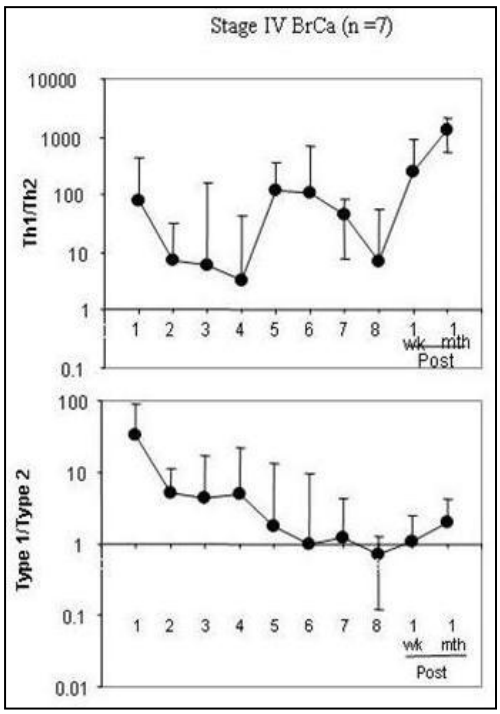


Fig-18: Mean Ratio of Th1/Th2 (upper panel) and Mean Ratio of Type 1/Type 2 (lower panel). Th1/Th2 = [IL-2+IFNγ]/[IL-4+IL-5] and Type 1/Type 2 = [IL-2+IFNγ]/ [IL-4+IL-5+ IL-10+IL-13].

specific cytotoxicity mediated by PBMC from the women who received immunotherapy while non-specific cytotoxicity directed at the Raji lymphoma cell line remained low. Both Her2 negative patients also showed an increase in their specific cytotoxicity directed at SK-BR-3 targets. These data suggest that the Her2 negative patients can also be induce by armed ATC infusions to develop specific cytotoxicity.

2.32 Sequential Serum Cytokine Th1/Th2 and Type 1/Type 2 Ratios in Patients. Serum samples, taken at indicated time points over the course of armed ATC infusions, were tested for cytokines the indicated cytokines using the Bio-Plex System. **Fig-18** shows the mean Th_1/Th_2 and Type 1/Type 2 ratios for 7 patients. Both ratios shifted and remained polarized toward Th1 or Type 1 response throughout treatment. *The overall immune response, calculated as the average ratio of remained polarized towards a Th_1–type response throughout treatment* though more strongly so at the beginning of the infusions (9.46 at Inf #1) than at the end (1.42 at Inf #8). These observations show a shift toward anti-tumor activity as a function of armed ATC infusions.

2.33 Increased Levels of Serum IL-12 in Patients Receiving Her2Bi-armed ATC Infusions. Since IL-12 is produced mainly by activated monocyte/macrophages and IL-12 is the principal cytokine for polarizing T cell responses towards a Th_1 phenotype. We tested the serum of the patients for IL-12. IL-12 is known to enhance the cytotoxic functions of NK and CD8+ T cells. Serum levels of IL-12 increased around 2 weeks or after 4 infusions (**Fig-19**). These data are critical in that they suggest 1) armed ATC infusions establish a systemic Th1-type anti-tumor milieu and 2) endogenous cells of the monocyte/macrophage lineage are activated to produce IL-12 under conditions elicited by armed ATC infusions. *The high serum levels of IL-12 show that the endogenous immunity has been activated in a robust manner, making a major contribution to the Th_1 response observed in **Fig-18**. Armed ATC were not the source of the IL-12 since T cells do not produce IL-12.*

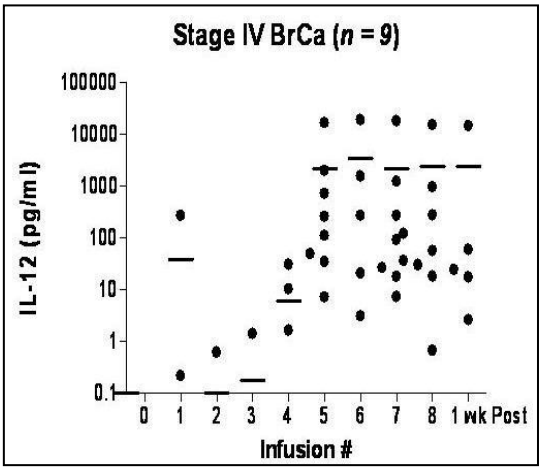


Fig-19: Serum IL-12 Levels in 9 Patients. The mean is indicated by the horizontal bars. IL-12 was quantitated with the BioPlex. Serial samples were obtained prior to each infusion.

2.34 Induction of Serum IL-2 and GM-CSF Levels after 2 Weeks or 4 Infusions of Armed ATC. 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 patients. Second, we wanted to assess the levels of IL-2 and GM-CSF that would be induced by multiple infusions of armed ATC. The mean levels of IL-2 (upper panels) and GM-CSF (lower panels) markedly increased by the 4 for 5th infusion (**Fig-20**). 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 subset of Her2/*neu* 0-2+ patients have similar response pattern to the entire group of patients (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). Such low doses of IL-2 and GM-CSF given on the same

schedule after high dose chemotherapy and stem cell transplant for non-Hodgkin's lymphomas could not be detected during the first 3 weeks after stem cell transplant (Lum, unpublished). Together these data suggest that infusions of IL-2
and GM-CSF may help prime the immune system to induce a brisk Th_1 immune response.

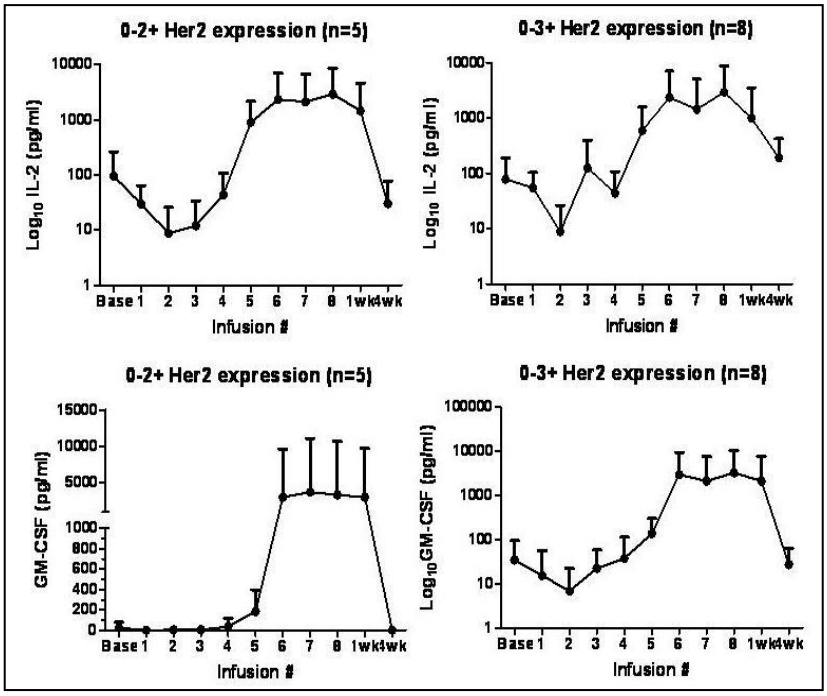


Fig-20: Serum IL-2 and GM-CSF Levels. The mean is indicated ± S.D. Serial serum samples collected at the indicated time points were quantitated with the BioPlex.

2.35 Her2Bi Persists on the ATC and Mediates Multiple Cycles of Specific Cytotoxicity. In studies evaluating the long-term activity and fate of Her2Bi-armed ATC, expansion and division of Her2Bi-armed ATC vs. unarmed ATC have been determined by comparing survival and ability of cells to divide and kill target cells when repeatedly exposed *in vitro* to SK-BR-3 cells over 336 hours of culture. Up to 2 weeks after a single arming, *ex vivo* expanded Her2Bi-armed ATC co-cultured with SK-BR-3 targets increase in number, undergo multiple cell divisions, mediate multiple rounds of specific cytotoxicity, and secrete both cytokines and chemokines without undergoing Fas/FasL-induced apoptosis or activation-induced cell death[120]. *In vitro*, armed ATC divided, maintained surface Her2Bi (**Fig-21**), and expressed a range of activities for extended periods of time. Perforin-mediated cytotoxic activity by armed ATC continued for at least 336 hours, and cytokines and chemokines (IFN-γ, TNFά, GM-CSF, MIPά, and RANTES) were secreted during successive rounds of SK-BR-3 stimulation[120]. Armed ATC would divide, kill, and secrete cytokines/ chemokines multiple times as a result of multiple rounds of targeting SK-BR-3 cells as a function of surface bound Her2Bi was detected by goat anti-IgG2a (OKT3). Cytotoxicity persisted beyond the lower limits of detection for Her2Bi on the cell surface (**Fig-21, right axis**). Specific cytotoxicity by armed ATC and persistence of Her2Bi is shown in **Fig-21**. Mean percent specific cytotoxicity (**Left axis**) directed at SK-BR3 (E:T=10:1) for armed ATC (n=4) and the percent positive IgG2a+ cells at each time point by flow cytometry using polyclonal goat anti-mouse IgG2a PE-conjugated Mab to detect the OKT3 (**Right axis**).

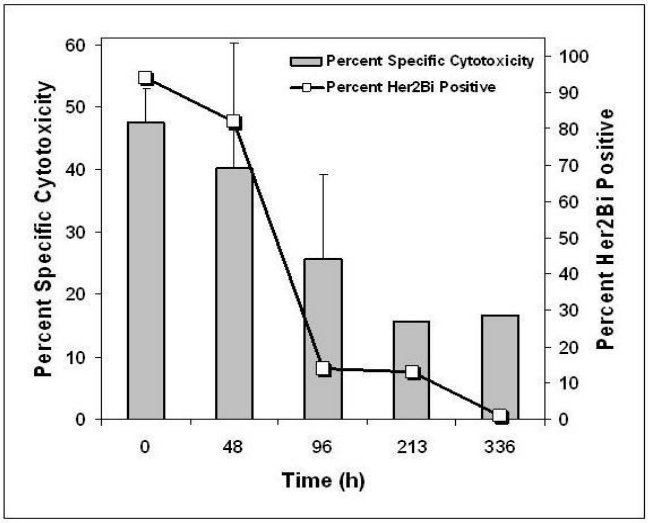


Fig-21: Shows % Specific Cytotoxicity (Left Axis) and % Her2Bi armed ATC (IgG2a+ cells) by Goat anti-mouse IgG2a flow cytometry. ^{51}Cr release was assessed at an E/T of 25:1. Armed ATC were repeated exposed to SK-BR3 targets at 0, 48, 96, 213, and 336 hours after a single arming with Her2Bi. After each exposure, the armed ATC were recounted after the time interval, washed, adjusted, and replated onto a fresh set of SK-BR-3 targets. The means of 4 experiments is presented. Unarmed ATC tested in parallel did not expand or continue to kill at high levels.

2.36 Engagement of Her2Bi-Armed ATC Induces In Vitro Cell Cycling. Her2Bi armed ATC were labeled
with CSFE-dye as a marker for cell proliferation and stimulated with SK-BR-3 to determine whether

engaging the ATC via their Her2Bi receptor would induce proliferation. We found that armed ATC would divide up to 4 times in a 48 hrs (**Fig-22A**) whereas unarmed ATC (**Fig-22C**) underwent far fewer cell divisions after 48 hrs (**Fig-22D**). These data, together with the cytotoxicity data in section 3.25, the persistence of IgG2a on the surface of ATC, and cytokine secretion *in vitro* and *in vivo* suggest that infused armed ATC persist, induce an immune response that "vaccinates" the patient to his own "tumor antigens", may proliferate in vivo with repeated stimulation with tumor antigens, and induce or recruit a systemic immune response to leads to high levels of circulating cytokines in the serum of patients.

2.37 Phase I Clinical Trial in High Risk Breast Cancer (≥ 4 nodes). In order to test whether Her2Bi armed ATC would affect time to progression, nine women received 8 doses of Her2Bi armed ATC twice per week for 4 weeks in combination with daily IL-2 (300,000 IU/m^2/day and twice weekly GM-CSF (250 µg/m^2/twice weekly) beginning 3 days before the first infusion and ending 7 days after the last infusion. There were no dose limiting toxicities. Five of 9 patients are alive free of disease with a median overall survival of 103 months (**Fig 23**).

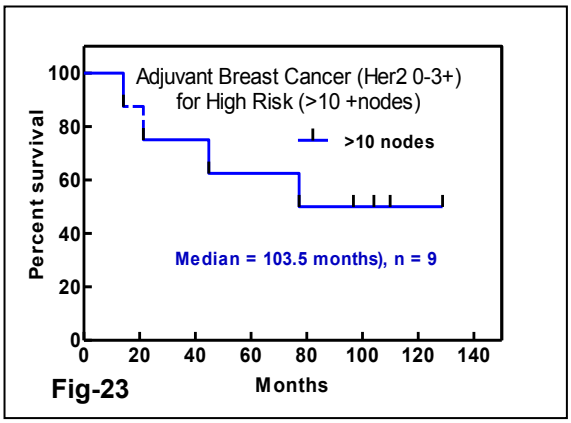


Fig-23

2.38 Human Anti-Mouse Antibody (HAMA) responses. Because one mAb moiety comprising Her2Bi is an unmodified mouse mAb, we evaluated patients' sera for the development of HAMA. Of the 11 patients evaluated, none developed clinically significant HAMA levels (>10 ng/ml) during or following therapy. Average HAMA concentrations in patients did not differ significantly as function of dose level (p =0.55). There was no correlation between levels of IgG2a+ cells in patients' peripheral blood (**Fig-24)** and their respective HAMA levels (r^2 = 0.001; p = 0.86). Therefore, HAMA responses to the IgG2a (OKT3) component of Her2Bi do not appear to play a role in the clearance of armed ATC.

2.39 Summary of Preliminary Data: Our *in vitro* and *in vivo* clinical data show that armed ATC: 1) infusions are feasible and safe; 2) have well demonstrated *in vitro* activity against MCF-7 and Sum 1315 cell lines that have very **low** and **nearly absent** Her2 expression; 3) cryopreserved patient products mediate levels of specific cytotoxicity comparable cytotoxicity exhibited prior to cryopreservation; 4) sensitive flow cytometry methodology was developed to detect and follow the kinetics and persistence of armed ATC in the blood of patients; 5) ^{111}Indium labeled armed ATC provide information on the trafficking of armed ATC up to 4 days after infusions; 5) overlay double staining of tumor biopsies detect the presence of armed ATC in tumors 1 week to 1 month after therapy; 6) serum tumor markers exhibited impressive decreases in CEA, CA27.29, and Her2 receptors; 7) armed ATC infusions may have improved OS for the both **Her2 0-2+** (median=20 months) and **Her2 3+** patients (median=56 months); 8) infusions induced the development of BrCa specific CTL activity that parallel IFNγ EliSpots data that argue for a robust adaptive endogenous response with a statistically significant increase in the CTL activity following immunotherapy; 9) infusions polarized the immune system to a Th_1/Type 1 that was associated with a remarkable increase in IL-12 production that occurred within 2 weeks after initiation of immunotherapy; 10) injections of IL-2 and GM-CSF did not increase serum levels of IL-2 and GM-CSF during the

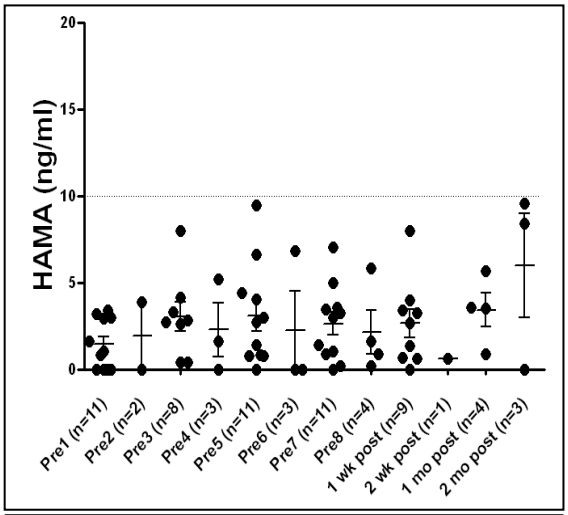


Fig-24: HAMA Responses to IgG2a Were Measured by ELISA. Human anti-mouse antibodies (HAMA) by ELISA (Antigenix, Inc. Huntington Station, NY). Individual patient samples are shown as solid circles and horizontal bars represent the mean +/- SEM for the number of patients sampled at each time point (n).

first 2 weeks of therapy (showing that levels did not accumulate) and there was a remarkable rise in all of the Th_1 cytokines after 2 weeks with levels that could not be due to IL-2 and GM-CSF injections (demonstrating that endogeneous activation was responsible for the rising levels); 11) the preclinical data showing the capacity of armed ATC to repeatedly kill, proliferate, and secrete cytokines/chemokines combined with the flow cytometry data that show circulating armed ATC and armed ATC in biopsy specimens suggest that the armed ATC persist and survive long enough to vaccinate the patient against their own tumor antigens; and 12) immunotherapy consisting of aATC, IL-2, and GM-CSF provides strong evidence for anti-breast cancer effect in phase I high risk ≥ 4 nodes breast cancer patients who received adjuvant therapy with median OS of 103.5 months with 5 of 9 women alive without disease. **Together these data provide compelling evidence for the proposed clinical trial strategy and immune evaluation function tests to identify *in vivo/in vitro* correlates.**

3.0 ELIGIBILITY

3.1 Inclusion Criteria:

Patients who satisfy all of the following conditions will be considered eligible for the study:

3.1.1 Signed and dated IRB-approved consent form

3.1.2 Patients may be male or female.

3.1.3 18 years of age or older. Women of reproductive potential must agree to use an effective non-hormonal method of contraception during therapy.

3.1.4 ECOG performance status (PS) of 0 or 1 and/or Karnofsky PS of ≥70%.

3.1.5 Diagnosis of invasive adenocarcinoma of the breast ***made by core needle biopsy***.

3.1.6 Palpable primary breast tumor measuring ≥ 2.0 cm on physical exam or imaging prior to neoadjuvant chemotherapy.

3.1.7 Patients with stage II-III breast cancer that is HER2-negative by IHC (0-2+) or FISH (HER2/CEP17 amplification ratio <2.0) who have completed "third generation" neoadjuvant chemoT (see section 4.3.1) and planned local treatment (surgery and Radiation if indicated). ER or PR receptors status can be ER negative (≤10% by IHC) or PR negative (≤10% by IHC).

3.1.8 Patients may have lymph node positive or negative disease, as long as they have clinical or pathologic stage II or III breast cancer. Patients may have the lymph nodes assessed by any method deemed appropriate by the treating physicians, including pre-neoadjuvant therapy sentinel lymph node biopsy.

3.1.9 Presence of any residual disease on final pathology following surgery will be required for immunotherapy. Patients with no residual disease at the time of surgery will be removed.

3.1.10 At the time of registration:

- Absolute neutrophil count (ANC) must be ≥ 1000/mm3.
- Platelet count must be ≥ 100,000/mm^3.
- Hemoglobin must be ≥ 9.0 mg/dL.
- There must be evidence of adequate hepatic function by these criteria:
  - Total bilirubin must be ≤ the ULN for the lab unless the patient has a grade 1 bilirubin elevation (> ULN to 1.5 x ULN) resulting from Gilbert's disease or similar syndrome due to slow conjugation of bilirubin; *and*
  - Alkaline phosphatase must be ≤ 2.5 x ULN for the lab
  - AST/ALT must be ≤ 1.5 x ULN for the lab.

- *Alkaline phosphatase and AST/ALT may not both be > the ULN*. For example, if the alkaline phosphatase is > the ULN but ≤ 2.5 x ULN, then the AST/ALT must be ≤ the ULN. If the AST/ALT is > the ULN but ≤ 1.5 x ULN, then the alkaline phosphatase must be ≤ ULN.

3.1.11 Patients with either skeletal pain or alkaline phosphatase that is > ULN must have a bone scan showing they do not have metastatic disease. Suspicious findings on bone scan must be confirmed as benign by x-ray, MRI, or biopsy.

3.1.12 Patients with AST/ALT or alkaline phosphatase > ULN must have liver imaging that does not demonstrate metastatic disease.

3.1.13 Patients with AST/ALT >ULN must have negative hepatitis studies.

3.1.14 Patients with **stage II** disease and clinical suspicion for metastatic disease based on reported symptoms, physical examination findings, or laboratory abnormalities must have staging studies demonstrating no evidence of metastatic disease (with exception of axillary lymph nodes or mammary nodes). Patients with **stage III** disease must have staging studies demonstrating no evidence of metastatic disease (with exception of axillary lymph nodes or mammary nodes), even if asymptomatic with normal physical examination and laboratory values. Such staging studies must include: chest imaging (chest X-ray, CT, or MRI), abdominal/pelvis imaging (CT or MRI), and bone imaging (bone scan or PET-scan). Abnormalities that are indeterminate and too small to biopsy should be followed with further imaging, as appropriate, but do not exclude patients from the study. Abnormalities that are suspicious and large enough to biopsy exclude patients from the study, unless a biopsy is performed and is negative for metastatic disease.

3.1.15 Serum creatinine ≤ 1.5 x ULN for the lab.

3.1.16 Left Ventricular Ejection Fraction (LVEF) ≥ 45 % (by MUGA or echocardiography)

3.2 Exclusion Criteria:

Patients with any of the following conditions will be ineligible for this study:

3.2.1 Tumor determined to be HER2-positive by immunohistochemistry (3+) or by fluorescent in situ hybridization (HER2/CEP17 amplification ratio ≥2.0). Tumors determined to be ER or PR positive by immunohistochemistry (>10%).

3.2.2 Tumors clinically staged as unresectable disease.

3.2.3 Evidence of disease progression on Neoadjuvant chemo T

3.2.4 Definitive evidence of metastatic disease with exception of axillary lymph nodes or mammary nodes.

3.2.5 Synchronous bilateral breast cancer (invasive or DCIS).

3.2.6 Treatment with biotherapy, and/or hormonal therapy for the currently diagnosed breast cancer prior to study entry.

3.2.7 Prior history of invasive breast cancer (Patients with a history of DCIS or LCIS are eligible.)

3.2.8 Other malignancies unless the patient is considered to be disease-free for 5 or more years prior to randomization and is deemed by the physician to be at low risk for recurrence.

Patients with the following cancers are eligible if diagnosed and treated within the past 5 years: carcinoma *in situ* of the cervix, carcinoma *in situ* of the colon, melanoma *in situ*, and basal cell or squamous cell carcinoma of the skin.

3.2.9 Known cardiac disease which precludes their ability to receive planned treatments:
- angina pectoris that requires the use of anti-anginal medication
- history of documented congestive heart failure
- serious cardiac arrhythmia requiring medication
- severe conduction abnormality
- valvular disease with documented cardiac function compromise; and
- uncontrolled hypertension defined as BP that is consistently > 150/90 on antihypertensive therapy at the time of registration. (Patients with hypertension that is well controlled on medication are eligible.)

3.2.10 History of myocardial infarction (MI) documented by elevated cardiac enzymes with persistent regional wall motion abnormality on assessment of LV function. (Patients with history of MI must have an echo instead of/in addition to a MUGA to evaluate LV wall motion.)

3.2.11 Other non-malignant systemic disease (cardiovascular, renal, hepatic, etc.) that would preclude treatment with any of the treatment regimens or would prevent required follow-up.

3.2.12 Chronic ongoing oral steroid use at the time of registration for any condition (such as asthma, rheumatoid arthritis, etc).

3.2.13 Administration of any investigational agents within 30 days before study entry.

3.2.14 Pregnancy or lactation at the time of registration.

3.2.15 Psychiatric or addictive disorders or other conditions that in the opinion of the investigators would preclude the patient from complying with the study protocol.

Minor changes from these guidelines will be allowed at the discretion of the research team under special circumstances. The reasons for exceptions will be documented.

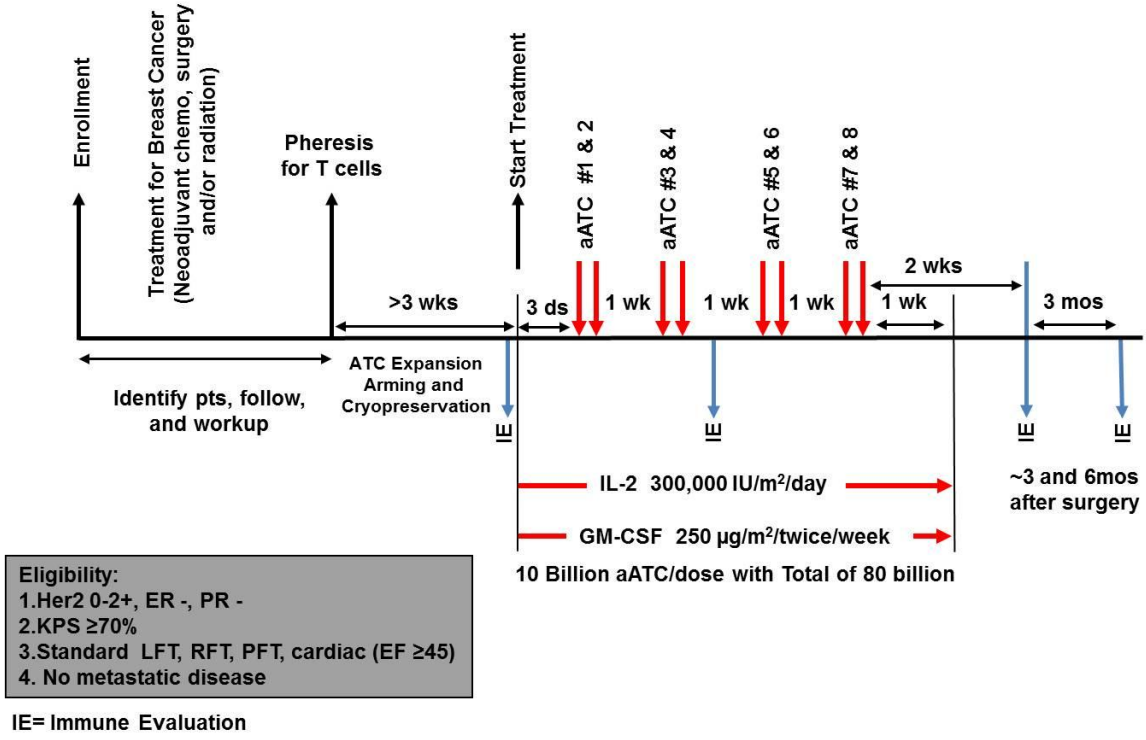
3.3 Informed consent. Each patient must be aware of the nature of his/her disease and must willingly consent to treatment after being informed of alternatives, potential benefits, side effects, and risks.

3.4 Registration. Patients may be enrolled after diagnosis if they have documented TNBC while they are undergoing neoadjuvant chemotherapy, surgery, and/or irradiation any time up to 3 months after completion of breast cancer treatment. During this time when they are undergoing standard of care treatment, they will undergo baseline studies, any staging and eligibility testing as described in section 3 prior to leukapheresis. Patients will be registered in the clinical trials office (CTO) of the Karmanos Cancer Institute (KCI) by the approved study coordinators. The study coordinators may be contacted at (313)576-8384 or (313)576-8506 or (313)576-9277.

3.5 Screening Assessments. Medical history, physical examination, EKG, CXR, breast imaging (mammography, ultrasound, or MRI) for tumor measurements, bone scans or PET-scans (if required), MUGA or echocardiography, laboratory tests (hematology, chemistry, urinalysis, creatinine clearance), and staging CT or MRI (if required) will be performed prior to registration as per section 3.1.14.

4.0 TREATMENT PLAN

4.1 Overall Treatment Plan. Patients with ER/PR negative and HER2-negative, stage II-III **operable** breast cancer with residual disease following completion of Neoadjuvant chemoT and local treatment will be entered into a phase II trial to evaluate whether infusions of HER2Bi-armed activated T cells after neoadjuvant chemoT improve PFS (primary endpoint).



- Neoadjuvant chemoT regimens using anthracycline and/or taxane-based, "third generation" chemoT regimens (dose dense AC→T or TAC), which are considered equivalent by current standards, will be used. (See section 4.3.)Dose and schedule modifications will be per treating physician's discretion.
- After planned breast cancer treatment, PBMC will be collected during 1-2 leukapheresis sessions (utilizing a central venous access catheter), activated with anti-CD3 and expanded in IL-2 to generate ATC that will be armed with HER2Bi, washed, and cryopreserved in 8 aliquots for subsequent infusions.
- Armed ATC will be thawed and **patients will receive 8 infusions of ~10-15 billion armed ATC given twice per week for 4 weeks** for a total dose of ~80 billion for each patient. The first infusions of armed ATC will be given no sooner than 3 weeks (±1 week) after completion of breast cancer treatment.
- Immune function tests will be performed before, during and after IT at the designated time points.
- Tumor samples will be obtained at diagnosis if possible (*i.e.* archived tissue from the diagnostic biopsy performed prior to study registration per routine care) and at the time of surgery. Tumors samples will be tested for CSC phenotyping and subsets of infiltrative cells.
- See the study calendar (section 8) for additional information.

4.2 Activation and Expansion of T cells with OKT3 and IL-2. Armed ATC are prepared in the Cancer Immunotherapy Laboratory's cGMP facility (***see Section 7***). 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% 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).

4.3 Neoadjuvant Chemotherapy.

4.3.1 Neoadjuvant chemoT may be administered at any location where the treating (KCI or non-KCI) oncologist normally administers chemoT. Acceptable standard, "third generation" regimens are recommended.

4.3.2 Every effort should be made to administer full doses of the standard therapy to ensure the best efficacy; however, dose modifications may be made, if necessary, as determined by the treating physician. Every effort should be made to administer the chemotherapy on the schedule to ensure the best efficacy; however, dose delays may be necessary for significant toxicity, as determined by the treating physician.

4.3.3 Treating physicians should perform regular histories/physical examinations and monitor appropriate laboratory studies during chemotherapy per good medical practice.

4.3.4 Tumor assessments will be performed by the treating physician as clinically appropriate during and after the neoadjuvant chemoT to document response to therapy.

4.3.5 Patients who discontinue any part of the neoadjuvant chemoT due to unacceptable toxicity will remain eligible to receive the aATC treatment and proceed to this followed by curative surgical resection as planned.

4.4 Immunotherapy with Armed ATC, IL-2, and GM-CSF

4.4.1 HER2Bi armed ATC Infusions

4.4.1.1 Cardiac evaluation: As a MUGA or echocardiogram is due to be repeated following chemotherapy, the LVEF must remain ≥ 45% in order to proceed with aATC infusion. If the LVEF drops below 45%, the patient will be ineligible for the study.

4.4.1.2 Armed ATC infusions will be done in the Immunotherapy outpatient clinic. All appropriate assurances for identification of product, patient, sterility, etc, will be performed prior to infusion according to the SOPs for the armed ATC IND. Frozen armed ATC will be thawed at the bedside of the patient just prior to infusion. If there is evidence of infusion-related toxicities, subsequent armed ATC will be thawed, washed, and resuspended in medium prior to infusion. The first armed ATC infusion will begin 3 weeks (±1 week) after all breast cancer treatment and subsequent doses will be administered twice weekly for a total of 8 doses. Armed ATC 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 patients will be observed for at least 4 hours after each infusion. If stable, patients will be discharged home.

4.4.1.3 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 min), 8 and 16 hours (+/- 1 hr), 24, 48, and/or 72 hours (+/- 2 hr) after each infusion, to study cytokine release. "Clinically indicated" is based on the clinical judgment of the investigator.

4.4.2 Recommended Concomitant Medications: All patients will be pre-medicated with Benadryl (50 mg IV or PO) 30 minutes prior to each Her2Bi-armed ATC infusion. This medication may be repeated every 4-6 hrs as needed. Demerol (25-50 mg IV) will also be administered if the patient develops grade 3 chills and then may be administered 30 minutes prior to subsequent armed ATC infusions dependent upon the severity of the initial reaction. Hydrocortisone (50-100 mg IV) for severe adverse reactions related to armed ATC infusions may be used, if necessary. Patients will then be treated with hydrocortisone (50-100 mg IV) at each subsequent infusion 30 minutes prior to infusion to prevent the reaction For grade 4 infusion reactions, patients will be removed from study treatment (aATC infusions), but patients 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. (See section 5.4 for required dose modifications.)

4.4.3 Subcutaneous IL-2 Injections: All patients will receive SQ IL-2 (300,000 IU/m^2/day), beginning 3 days before the first ATC infusion and ending 1 week after the last ATC infusion.

4.4.4 GM-CSF Therapy: All patients will receive SQ GM-CSF (250 µg/m^2/twice weekly), beginning 3 days before the first ATC infusion and ending 1 week after the last ATC infusion.

4.4.5 Ancillary Therapy: Patients 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. Patients will receive calcium replacement during the pheresis procedure, if needed for symptoms of hypocalcemia.

4.4.6 Steroids/Other Therapy: With the exception of steroids for adrenal failure, septic shock, pulmonary toxicity or hormonal therapies administered for pre-existing, non-disease-related conditions (e.g. insulin for diabetes), steroids and hormonal therapies will not be administered. Hydrocortisone (50-100 mg IV) for severe adverse reactions related to armed ATC infusions is allowed.

5.0 POTENTIAL TOXICITIES, DOSE MODIFICATIONS, AND MANAGEMENT

5.1 Pheresis Procedure. Reported toxicity from pheresis is minimal. Patients have a small potential for infection from the placement of central catheters used for pheresis. Use of the anticoagulant citrate dextrose may cause symptoms of mild hypocalcemia, which are controlled with calcium replacement during the procedure, as necessary.

5.2 Her2Bi-armed Activated T Cells. Severity of toxicities associated with Her2Bi-armed ATC has been minimal (**see Table 4**) and symptoms have been responsive to interventional therapy with antihistamines and analgesics. The most common side-effects are chills and headache. Other reported side-effects included mild gastrointestinal (nausea) symptoms and backache. Adverse symptoms associated with cryopreservative (i.e. DMSO) accounts for approximately 50% of all infusion-related side-effects. DMSO-related side effects include fever, nausea and fatigue. No fatalities have occurred as a result of receiving Her2Bi-armed ATC infusions.

5.3 Dose Modification for HER2Bi Armed-ATC Toxicity. All patients with treatment related, grade 4 non-hematologic toxicity will be removed from protocol. If there is clinical evidence of heart failure, a MUGA or echocardiography will be done to evaluate the **left ventricular ejection fraction (LVEF). If it falls by >10%** from the previous value (by MUGA or echocardiography) following HER2Bi armed-ATC, further therapy will be withheld and the patient will be removed from protocol treatment. If there is persistent grade 3 toxicity at any time, treatment will be held until toxicity improves to grade 0 or 1. For patients who experience grade 3 infusion reactions, the same dose of armed-ATC will be washed prior to all subsequent dose administrations to eliminate DMSO and protein. If washing does not reduce toxicity, then the treatment will be resumed with a 50% reduction in the armed ATC for all subsequent doses in that course. If a grade 3 toxicity occurs again, the armed ATC infusions will be stopped. Toxicity will be assessed via telephone contact between each aATC infusion and up to 7 days beyond

the last aATC infusion. Additionally, patients will be given contact numbers and will be instructed to call and report any urgent problems associated with the aATC infusions. Patients for whom the aATC infusions are stopped will remain on study and complete the other study procedures (tissue samples, immune studies, etc).

6.0 DRUG INFORMATION

6.1 Commercial ChemoT Doxorubicin, paclitaxel, docetaxel, cyclophosphamide, and G-CSF must be obtained from commercial sources. Please refer to the current FDA-approved package inserts provided with the chemoT or the *Physicians' Desk Reference* for information about possible side effects and instructions for preparation, handling, and storage of the drugs.

6.2 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 5 mg/5 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 patient ATC.

6.3 Anti-HER2/*neu* monoclonal antibody (Herceptin®, Trastuzumab; Genentech, Inc., CA). Herceptin is a humanized murine monoclonal antibody directed at HER2/*neu*, and is commercially available in multi-dose vials containing 440 mg of drug. For this study, Herceptin® is heteroconjugated to OKT3 to produce the Her2Bi bispecific antibody for arming patient ATC.

6.4 Anti-CD3 (OKT3) x anti-HER2/*neu* heterconjugated 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^{o}C.

6.5 Interleukin 2 (IL-2).

6.5.1 Therapeutic classification: lymphokine

6.5.2 Known Side Effects and Toxicities: Administration of low dose SQ IL-2 (2×10^5 IU/m^2/day) for 90 days resulted in no grade III toxicities. None of the patients experienced pulmonary capillary leak syndrome, severe hypotension, oliguria, azotemia, or hyperbilirubinemia. The most frequent toxicities included fatigue, fever, and nausea. None of the patients had to stop their SQ IL-2 therapy due to side effects. Therefore, it is unlikely that major toxicities associated with the low dose SQ IL-2 will occur although death due to high dose IL-2 toxicity has been reported

6.6. Granulocyte-macrophage colony stimulating factor (GM-CSF):

6.6.1 Therapeutic Classification: colony stimulating factor.

6.6.2 Known Side Effects and Toxicities: Patients receiving GM-CSF (Leukine-Sagramostim) have experienced fever 60-90 min after administration (duration 1-4 hrs); chills; nausea; vomiting; diarrhea; fatigue; weakness; headache; decreased appetite; thrombosis; rapid or irregular heartbeat or other heart problems; feeling of faintness; facial flushing; pain in the bones, muscles, chest, abdomen, or joints; local reaction at the site of injection; rashes; and kidney and liver dysfunction. Eosinophilia or other blood component abnormalities may occur. There have been infrequent reports of fluid accumulation or worsening of preexisting fluid accumulation in the extremities, in the lungs, and around the heart which may result in breathing problems or heart failure. Rarely, patients have developed acute allergic reactions. There have also been reports of low blood pressure, hypoxia, transient loss of consciousness, and difficulty in breathing after the first injection of Sargramostim. These signs may or may not recur with additional injections of

Sargramostim. Patients with prior heart, lung, kidney, or liver problems may have worsening of their symptoms following administration of Sargramostim. There may be other side effects that could occur.

7.0 PREPARATION OF HER2Bi ARMED ACTIVATED T CELLS

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

7.2 Activation, Culture, and Freezing of Armed-ATC. 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 (Centocor Ortho Biotech), and 2% human serum (Biowhitaker/Valley Biomedical). Cells will be cultured for a maximum of 14 days in medium to which no additional OKT3 will be added during the expansion period.

7.3 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.

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

7.6 Cytotoxicity Assay and IFNγ EliSpots. Cytotoxicity is measured in a 20 hr ^{51}Cr -release assay to ensure activity (minimum ≥ 10% cytotoxicity) of armed ATC 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 performed by plating SK-BR-3 targets.**

7.7 Quality Assurance of armed ATC Cell Product. Lists of suppliers of monoclonal antibodies, heteroconjugation reagents, and culture reagents will be maintained as well as lot numbers used and supplier-provided documentation of sterility and documentation that all reagents are free of endotoxin and mycoplasma. Records of all quality control measures will be maintained by the laboratory.

8.0 REGISTRATION AND REQUIRED DATA

8.1 Pre-Study. All patients enrolled will be evaluated prior to initiation of therapy. Eligibility criteria will be assessed as stated in section 3.0. Patients may be enrolled at any time after diagnosis until 3 months after completion of their breast cancer treatment. The patients will be leukapheresed after enrollment to obtain lymphocytes for expansion or when the lymphocyte population has recovered to ≥ 500 lymphocytes/mm^3

8.2 Study Calendar			
	Pre-Registration	Pre and Mid IT[1]	After IT
History & Progress Notes	X	X	2 weeks, 3 months, 6 months and 12 months post IT[2]
Physical Exam	X	X	2 weeks, 3 months, 6 months and 12 months post IT[2]
Performance Status	X	X	2 weeks, 3 months, 6 months and 12 months post IT[2]
Laboratory:			
--CBC/Plts/Diff	X	X	2 weeks, 3 months, 6 months and 12 months post IT[2]
--Lytes, Bili, AST, ALT, BUN, Cr, CrCl	X	X	2 weeks, 3 months and as clinically indicated[2]
--Urinalysis	X		As clinically indicated
--Serum Pregnancy	X		
--EKG	X		As clinically indicated
--MUGA or Echocardiography[3]	X		As clinically indicated
--HBsAg	X		As clinically indicated
--CEA, CA 15-3, or CA 27.29	As indicated		As clinically indicated
Staging:			
--Bone scan or PET-scan	As indicated		As clinically indicated
--CXR, CT or PET/CTMRI of Chest;	As indicated		Pre treatment for staging and clinically
--Mammography/, ultrasound, or MRI	X		As clinically indicated
--Others (CT Head, etc)	As indicated		As clinically indicated
Immune Evaluations[6]		X[6]	Just prior to 1st IT(within 3 days),after 4 IT infusions and 2 weeks (±3 days) or 1 month (±3 days) post starting immunotherapy; studies at 3, 6, and 12 months after IT, if immune responses are positive and continue to be of interest to the investigators[2].

[1] PreIT: (IT=armed ATC) the day of IT or up to 7 days prior.Mid IT:Preinfusion #5

[2] To be completed within the following timelines: 2 weeks (±3 days), 3 months (±1 week), 6 months (±1 months), and 12 months (±1 months) post IT.

[3] Ejection fraction by MUGA or echo should be ≥45%

[4] Sample obtained from the diagnostic biopsy if available and from surgical specimen

[5] Immune studies will be done at leukapheresis, **preinfusion #5**, and 2 weeks (±3 days) after the last armed ATC infusion or 1 month (±3 days) after the initiation of immunotherapy. Additional studies will be done at 3 months (±1 week), optional 6 months (±1 month), and optional 12 months (±1 month) after the last infusion if studies are positive. If the immune studies show changes worthy of follow-up in the opinion of the investigators, optional studies can be obtained at 6 month (±1 month) intervals, until the results become negative.

9.0 CRITERIA FOR EVALUATION (Cited criteria are based upon those established for the comparative study group)

9.1 Tumor Measurements. Tumor assessment (breast and axilla) will be measured on the final pathology from surgical specimen after completion of chemoT. Tumor measurements done clinically by physical exam and by mammogram and ultrasound in centimeters prior to neoadjuvant chemotherapy and prior to definitive surgery will be collected. These measurements will consist of the longest diameter and the perpendicular diameter at the widest portion of the tumor and will be made and recorded by the physician or his designee. An estimate of overall clinical objective response will be made and recorded at the end of treatment.

9.2 Response Definitions (RECIST)

9.2.1 Complete Pathological Response (PCR): Absence of all invasive tumor in both breast and axilla.

9.2.2 Complete clinical response (CR). The patient must be free of all symptoms. All measurable, evaluable and non-evaluable lesions and sites must be assessed and have resolved on physical exam and imaging.

9.2.2 Partial Response (PR): Thirty percent or greater decrease in the sum of the products of two perpendicular diameters of all measured lesions. No lesion may increase in size and no new lesions may appear

9.2.3 Minor Response (MR): A decrease in measurable lesion(s) which is too small or too brief to qualify as a Partial Response. MR is considered a treatment failure, but should be noted as a possible indicator of biologic activity.

9.2.4 Progressive Disease (PD): Any increase of ≥25% in the sum of the products of diameters of any measurable lesion, in the reappearance of any lesion which had previously disappeared, or appearance of an unequivocal new lesion.

9.2.5 Stable Disease (SD): No change in tumor size(s) and no evidence of progression. Patients are classified with stable disease if at the first tumor assessment after the study treatment, there is neither disease progression nor a response that is later confirmed

9.3 TIME TO PROGRESSION

The primary endpoint of this study is time to progression. Documentation of first local or distant recurrence will be defined as progression of disease after enrollment. PFS will be measured from the enrollment. The patients will be followed clinically as outlined at 3, 6, and 12 months with subsequent follow-up per standard of care guidelines as clinically indicated.

10.0 CRITERIA FOR EVALUATION OF RESPONSE AND DISCONTINUATION OF THERAPY

10.1 Response-evaluable: Participants will be considered response evaluable for immune study endpoints if they receive any aATC infusion. All patients who begin neoadjuvant chemoT and complete planned surgery will be evaluable for pathologic responses. Those with complete pathologic responses are excluded from this study.

10.2 Disease Progression: Patients who progress during the neoadjuvant chemoT and/or have residual disease after surgery are ineligible for this study.

10.3 Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, the patient shall be withdrawn from treatment. Determination of whether the patient is evaluable for response will be determined by the criteria in 10.1 and 10.2.

10.4 Patient decision to discontinue therapy

10.5 Protocol violation: Unexplained delay in delivery of treatment.

10.6 Grade 4 non-hematologic treatment related toxicity

10.7 Unexpected or Life-Threatening Toxicity:

10.7.1 Questions regarding drug therapy will be directed to the Principal Investigator.

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

10.7.2.1 Expected toxicities: All expected toxicities related to leukapheresis and after first aATC infusion, including ongoing grade 3-4 hematologic toxicity related to chemoT, will be reported as part of regular data submission.

10.7.2.2 Unexpected Toxicities: Unexpected toxicities are toxicities that are not listed in the toxicity management section of the protocol, the consent form, or the manufacturer's package insert. All unexpected toxicities related to leukapheresis and after first aATC infusion grade 3 or above will be reported per Wayne State University IRB guidelines.

10.7.2.3 ChemoT, surgery, and radiation related toxicities: Since the chemoT, surgery, and radiation are standard treatments that will be managed by the treating (KCI or non-KCI) physicians and not overseen by the protocol, toxicities attributed to the chemoT, surgery, and radiation prior to first aATC infusion and toxicities related to surgery will not be collected or reported.

10.7.2.4 All grade 5 toxicities occurring after registration and up to 30 days beyond the last aATC infusion will be reported to the Wayne State University IRB and the FDA per the standard guidelines of these organizations.

11.0 REPORTING ADVERSE REACTIONS

11.1 Investigators will notify the **FDA and IRB** of all serious and unexpected adverse drug reactions.

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

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

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

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

11.3.3 IRB: Written report within 10 working days.

11.4 Procedure for calling the FDA is as follows:

11.4.1 The research nurses or PI will call for ADRs.

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

11.4.3 The medical reviewer at the FDA to be identified:

Food and Drug Administration
Center for Biologics Evaluation and Research
Document Control Center
10903 New Hampshire Avenue
Bldg. 71 G112
Silver Spring, MD 20993-0002

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

12.0 Measurement of Functional Changes in Immune Populations: 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. Studies will be performed preIT (within 7 days or from leukaphresis product), pre-infusion #5 (within 3 days), and 2 weeks (± 3 days) after IT. Additional optional studies will be performed on specific tests if there are positive findings that warrant continued follow up for a particular patient at 2 weeks(± 3 days), 3 months (±1 week), 6 months (± 1 month), and 12 months (± 1 month) after IT. If studies are still positive at 3 months, the optional studies can be obtained at 6 month and 1 year (± 1 month). The subsequent paragraphs summarize the strategy and procedures.

12.1 Serum cytokine/chemokine levels. Serum will be stored for subsequent cytokine analysis at the indicated time points.

12.2 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.

12.3 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+ BrCa), 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 as described in statistical analysis section.

13.0 Tumor Studies and Phenotypic Evaluations:

13.1 Strategy: Standard IHC staining for ER, PR, and Her2/*neu* that will be performed by standard pathology procedures on the original diagnostic biopsy specimen by the Pathology Department at Wayne State University from the paraffin block. Patients may be identified by their treating physicians. When a patient enrolls in this study, the formalin fixed sections from the patients will be retrieved and stained with anti-CD44, anti-CD24, and anti-CD133 (or other markers when they become available). The diagnostic sample will provide the baseline pre-chemoT result to be compared to formalin fixed sections from the surgical specimen. This strategy will optimize our ability to identify the changes after chemoT and before IT.

13.2 Surgical Tumor Sample: The tumor specimen will be divided for standard pathology and an aliquot for research to be stained for T cells and ALDH1. The paraffin block will be obtained from pathology where tumor was removed. Sections from the block will be stained for T cell, T cell subsets, and tumor markers (see section 13.2).

13.3 Immunohistochemistry Staining of Diagnostic and Surgical Specimens. Standard IHC will be performed on formalin fixed tissue sections by Dr. Ali-Fehmi in pathology from the diagnostic, post chemoT, and surgical specimens. These samples will provide an assessment of the changes in CD44, CD24, CD133, and other CSC markers (when available) that occur following chemoT and the subsequent effects of IT on the final surgical specimen. ALDH1 will be the only marker not done on the diagnostic specimen.

14.0 STATISTICAL CONSIDERATIONS

14.1 Objective: The primary objective is to determine whether median PFSprovides sufficient evidence of efficacy to proceed with a larger study. . The secondary objective is to estimate functional and phenotypic changes from baseline in immune cell populations in blood and tumor samples, when accessible, as a function of IT, including phenotype and anti-tumor functions (*i.e.* specific cytotoxicity and IFNγ EliSpots) of lymphocytes. The third objective is to investigate the association between proportion of infiltrating cells and cancer stem cells in the tumor at the time of surgery with time to progressive disease..

14.2 Design: This is a one-arm phase II study.

14.3 Endpoints: The primary endpoint is PFS, defined in 9.3. Secondary endpoints include immune responses measured before IT, once during IT and at 3 times after IT.

14.4 Statistical Analyses:

PFS will be estimated under the assumption of an exponential survival distribution. It will be concluded that median PFS exceeds 18 months if the estimated median PFS exceeds 25.2 months. Kaplan-Meier methods will also be used to describe the PFS distribution

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For those immune functions measured on a continuous scale the shape of the distribution will be assessed before analysis to be sure that the assumptions of the statistical procedures are not violated. If necessary either a data transformation will be used and parametric tests employed or rank tests will be used. If the assumptions are met with either the raw or transformed data, mixed effects models will be used to evaluate changes in continuous immune functions over time. We will assess the frequency distribution of immune functions measured on an ordinal scale and collapse categories if the data are too sparse. Changes in ordinal longitudinal measures will be assessed with generalized estimating equations using a

cumulative logistic link. We will also estimate the association between measures of immune function using rank correlation methods. Association between the amount of clinical disease , pCR or not, and the number of infiltrative cells in the tumor at the time of surgery and and immune function will be assessed using two-sample tests or two-sample. Association of PFS with immune function will be explored with univariable proportional hazards models. We will evaluate the simultaneous association of the immune function parameters with PFS in a multivariable model, expecting that the lack of independence between these measures will provide different results than the univariable models.

Because of the relatively small sample size and the number of comparisons, correction for multiple comparisons will be made using Holm's procedure.

14.5 Statistical Power and Sample Size:
Using the CRAB calculator (stattools.crab.org), sample size was calculated under the assumptions of an exponential distribution of PFS, a one-arm study, a one-sided test with type I error of 0.05 and statistical power of 80% to distinguish median PFS of 18 months from median PFS of 30 months.. It was further assumed that the accrual period will be 24 months and follow-up time 36 months. Under all of these assumptions, 32 evaluable patients are required.

14.6 Accrual of Patients and Duration of Study: Patient accrual is expected to be 4 patients/year so that the accrual period will last for 8 years. Interim analysis is not possible because it is a single group study and the accrual period is quite short. Immune follow-up studies will extend 1 year beyond the last HER2 bispecific antibody infusion based on positive results seen at the 1 month post-IT time-point.

15.0 CLINICAL SAFETY MONITORING.

The Data and Safety Monitoring Committee (DSMC) provides the primary oversight of data and safety monitoring at Karmanos Cancer Institute (KCI) for Investigator-initiated trials.

15.1 *The Investigator will schedule monthly meetings or more frequently depending on the degree of risk encountered by study participants. These meetings will include the protocol investigators and research staff involved with the conduct of the protocol.*

15.2 *During these meetings the investigators review all aspects of the trial conduct related to:*

- *Safety of protocol participants*
- *Evaluate compliance with requirements regarding the reporting of AEs.*
- *Validity and integrity of the data*
- *Enrollment rate relative to expectation of target accrual*
- *Retention of participants, protocol compliance (protocol violations and deviations)*
- *Data completeness and source documentation*

These monthly meetings are recorded on the data and safety monitoring report forms and submitted for review on a quarterly basis to the DSMC.

The Data Safety Monitoring Board (DSMB) of Karmanos Cancer Institute will perform comprehensive reviews of IND clinical trials on a semiannual basis. The Study Monitor must be familiar with the protocol, the investigational product, written informed consent, Standard Operating Procedures (SOPs), Good Clinical Practices (GCPs), and Applicable Regulatory Requirements.

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