TUMOR-ASSOCIATED **A**NTIGEN (TAA)-SPECIFIC **C**YTOTOXIC **T** LYMPHOCYTES ADMINISTERED **I**N PATIENTS WITH BREAST **C**ANCER (TACTIC)

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Contents

1.0 CHECKLIST FOR PATIENT ELIGIBILITY AND NECESSARY INFORMATION

PATIENT ID _____ PATIENT NAME_____

TACTIC PROTOCOL (Procurement)

YES	NO	
Any "**NO**" answers will make a patient **ineligible** for study participation.		
		Any breast cancer patient with metastatic or locally recurrent unresectable breast cancer
		Life expectancy of ≥ 12 weeks
		Age ≥ 18 and ≤ 80 years old
		Hgb ≥ 7.0 (transfusion allowed)
		Informed Consent explained to, understood by and signed by patient. Patient given copy of informed consent.

YES	NO	
Any "**YES**" answers will make a patient **ineligible** for study participation.		
		Severe intercurrent infection
		Active infection with HIV (can be pending at this time)
		Patients with a chronic uncontrolled medical condition that, in the opinion of the principal investigator, precludes them from participation.

Signature of MD _____ Date _____

To check eligibility of a patient, telephone Dr. Rimawi at 713-798-1999. To register a patient, telephone the research coordinator, Claudette Foreman, at 713-798-7315.

TACTIC PROTOCOL (Treatment)

PATIENT ID _____ PATIENT NAME _____

YES	NO	VALUE/DATE	
Any "**NO**" answers will make a patient **ineligible** for study participation.			
			Any breast cancer patient with metastatic or locally recurrent unresectable breast cancer currently progressive after at least two prior lines of therapy in the advanced setting. Patients with HER2+ disease must have failed two or more different anti-HER2 agents.
			Patients must have measurable or evaluable disease per RECIST 1.1 criteria.
			Life expectancy of ≥ 12 weeks.
			Age ≥ 18 and ≤ 80 years old
			Pulse oximetry of >95% on room air
			ECOG Performance Status of ≤ 2 or Karnofsky score of ≥ 50.
			Bilirubin ≤ 2 x upper limit of normal
			Creatinine normal for age
			AST ≤ 3x upper limit of normal
			Hgb ≥ 7.0 (transfusion allowed)
			Off investigational therapy for 1 month prior to receiving treatment on this study.
			Off conventional therapy for at least 1 week prior to receiving treatment on this study.
			Informed Consent explained to, understood by and signed by patient. Patient given copy of informed consent.
			Sexually active patients must be willing to utilize one of the more effective birth control methods for 6 months after the T cell infusion.

YES	NO	VALUE/DATE
Any "**YES**" answers will make a patient **ineligible** for study participation.		
		Severe intercurrent infection
		Receiving systemic corticosteroids (patients off steroids for at least 48 hours are eligible)
		Pregnant or lactating
		HIV positive
		Patients with a chronic uncontrolled medical condition that, in the opinion of the principal investigator, precludes them from participation.

Signature of MD _____ Date _____

To check eligibility of a patient, telephone Dr. Rimawi at 713-798-1311. To register a patient, telephone the research coordinator, Claudette Foreman, at 713-798-7315.

2.0 OBJECTIVES

2.1 Primary endpoint

To determine the clinical efficacy associated with the administration of multiTAA-specific T cells in breast cancer patients with metastatic or locally recurrent unresectable disease as measured by clinical benefit rate (defined as overall response plus stable disease for 10 weeks or longer) according to the RECIST criteria (see Section 8.4.1).

2.2 Secondary endpoint

To evaluate the progression-free and overall survival of patients after multiTAA-specific T cell infusion

3.0 BACKGROUND AND RATIONALE

3.1 T cell therapy for non-viral tumors

From an immunotherapeutic perspective the model tumor antigen to target should be one that is exclusively and universally expressed on tumor cells in order to limit collateral damage, and ideally should be essential for the maintenance of the oncogenic phenotype of the tumor. However, the majority of antigens do not meet these criteria since they are not neo-antigens uniquely present in malignant cells but rather antigens that are also expressed in normal cells and against which peripheral blood T cells are tolerized or deleted. Tumor-specific antigens have nonetheless been identified, and these can be classified into 4 groups;

(i) Unique antigens (eg. MUM1) result from single mutations that are tumor and patient specific and therefore are only expressed in neoplastic cells[1-3]. They are often considered ideal for immunotherapy since tumor cells can be specifically targeted without destroying nearby normal tissue, and they may also be relatively strong antigens[1-4]. However, because they are also usually patient-specific, the identification of the mutated gene and then the generation of an individualized CTL product targeting the identified antigen is highly labor and cost intensive.

(ii) The shared lineage-restricted antigens, expressed on tumor cells as well as their normal tissue of origin, such as the melanoma associated antigens MART, gp100 or Melan-A. These antigens are also strongly immunostimulatory, equivalent almost to “weak” viral antigens, enabling the efficient and relatively simple generation and expansion of tumor-specific T cells from healthy donors and patients with minimal *in vitro* manipulation[5]. However, T cell mediated destruction of normal melanocytes, for example, has resulted in vitiligo as well as ocular and systemic autoimmunity in patients treated with melanoma-specific CTL or TILs[6].

(iii) Shared tumor-specific TAA (e.g. the cancer testis antigens [CTA] - MAGE, BAGE, GAGE, NY-ESO-1, SSX, PRAME) are expressed in multiple tumors including breast cancer but not in healthy organs[7-11], with the exception of germ line tissues that are immune privileged and therefore not susceptible to T cell attack. Most CTAs have heterogeneous expression in cancer tissues and are frequently expressed in high-grade or late tumor stages, with expression often correlated with a worse prognosis. Furthermore, tumors expressing one CTA are also often found to express multiple CTAs, and several have been found to be targets of spontaneous humoral or cell-mediated immune responses[12-14]. Thus, CTAs are particularly attractive as targets for tumor immunotherapy since reactive T cells can be produced on a large scale to provide broad-spectrum protection against a variety of tumors. CTAs have been targeted in both vaccine and T cell therapy protocols, with evidence of clinical efficacy[15-21].

(iv) The last group of antigens are over expressed in many different tumors but expressed at low levels in healthy tissue (eg. hTERT, CEA and Survivin). T cells targeted to these antigens carry the risk of inducing collateral damage to normal tissues co-expressing the antigen (e.g. CEA and normal biliary epithelium), and there are limited clinical data available regarding the safety of targeting these antigens *in vivo*. However, Survivin- and CEA-specific T cells have been isolated from the peripheral blood of patients who have cleared their tumors, and increases in Survivin-specific T cells in patients receiving oncolytic viruses have been reported, suggesting that they can have efficacy without toxicity in patients[22-27].

3.2 Clinical experience targeting non-viral TAAs

3.2.1 Melanoma-targeted therapies

T cell immunotherapies for non-viral tumor antigens have been described, with promising clinical results in some studies. Rosenberg and colleagues reported that infusion of melanoma-specific tumor-infiltrating lymphocytes (TILs) together with high-dose interleukin 2 (IL-2) produced clinical responses in approx. 35% of patients with metastatic melanoma [28-30]. The specificity of the infused cells was not analyzed but it is likely that they targeted multiple epitopes/tumor associated antigens. More promising results were subsequently achieved using a modified treatment protocol which incorporated a lymphodepletion step prior to CTL infusion, in order to improve the expansion and persistence of adoptively-transferred cells. Ninety three patients with metastatic melanoma refractory to standard therapies received immunodepleting chemotherapy -/+ total body irradiation followed by the adoptive transfer of highly selected, TIL-derived, tumor-reactive T cells and high-dose IL-2 (720,000 IU/kg q 8h to tolerance). Fifty two of the 93 patients had objective clinical responses to treatment (39 PR, 12 CR), including regression of large bulky tumors[31,32]. However, the collection of autologous TILs is not possible for most tumors. Furthermore the *in vitro* expansion of large numbers of tumor-specific T cells (>10^{10} CTL) is a complex and expensive procedure.

To extend applicability and generate a more defined tumor-specific product with respect to specificity and function, a number of groups have investigated the clinical use of tumor-specific T cells isolated from peripheral blood and selectively activated and expanded ex vivo. Mitchell and colleagues used insect cells modified to express HLA-A2, CD80 and CD54 and loaded with an HLA-A2-restricted epitope peptide derived from the melanoma-expressed tyrosinase antigen to repetitively stimulate and selectively expand tyrosinase peptide-specific T cells. Infusion of 5x10^8 cells (of which between 10-30% were tyrosinase-specific) to 10 patients with tyrosinase-positive melanoma tumors was associated with clinical responses in two[33]. Other groups have used dendritic cells (DCs) to activate a tumor-directed product. For example, Yee and colleagues generated CD8+ T cell clones specific for HLA-A2 peptides derived from MART1 and gp100 using peptide-loaded autologous DCs as stimulators. Four infusions (3.3x10^9/m^2/infusion) of the tumor-directed clones without and subsequently with low dose IL2 produced clinical benefit in 8 of 10 patients with metastatic melanoma and IL2 was found to be crucial for in vivo persistence[34]. A follow-up study from the same group incorporating a pre-conditioning step with high dose cyclophosphamide +/- low or high dose IL2 was shown to further increase proliferation and persistence[35]. However, the toxicities associated with pre-conditioning followed by T cells with high dose IL2 were found to be substantial and recruitment on this arm of the study was halted. Finally, MART1 was also targeted by Mackensen et al, who prepared tumor-directed T cell lines using HLA-A2 peptide-

loaded DCs to stimulate selected CD8+ T cells. Eleven patients received at least 3 and up to 10 infusions of specific CTLs (median 2.11 x 10^8 cells/infusion) with low dose IL2, with evidence of T cell homing to tumor sites detected and favorable clinical responses reported in 3 (1 complete, 1 partial, and 1 mixed response)[36].

Thus, while the generation and adoptive transfer of CD8+ melanoma peptide-specific T cells from peripheral blood has proven to be feasible, as in the TIL setting clinical benefit appears to be tightly linked with in vivo persistence, which has proven challenging. One potential strategy to overcome this obstacle may be to infuse CD8+ peptide-directed T cells with antigen-specific CD4+ T cell populations, which not only mediate tumor killing directly against HLA class II positive targets[37], but can support the survival and maintain the effector function of the transferred CD8+ T cells via the production of immunostimulatory cytokines and other signals upon antigen encounter. Perhaps a more general limitation of this peptide stimulation approach, however, is its limitation to individuals with a restricted HLA genotype due to the small number of class I-restricted epitopes used for T cell activation. Finally, one emerging concern is the potential for such a targeted therapy to evade the immune system as was reported by Mackensen et al who reported the evolution of MART1 negative tumors in 2 patients infused with MART1-targeted CD8+ T cells[36].

3.2.2 T cell therapy to prevent leukemic relapse

Adoptive immunotherapy has also proven to be an effective strategy in preventing leukemic relapse in the post allogeneic HSCT setting. The first adoptive T cell transfer protocols were based on the premise that donor peripheral blood contained T cells that were able to mediate antitumor activity in vivo. Accordingly, unmanipulated donor lymphocyte infusions (DLI) have been extensively used to provide anti-tumor immunity[38]. However, the efficacy of this approach is limited by the low frequency of tumor-specific T cells and the relatively high frequency of alloreactive T cells, resulting in frequent and severe GVHD. One strategy to enhance the “graft vs leukemia” effect without promoting GVHD is to selectively target tumor-expressed antigens using selectively-expanded T cell populations and clinically the antigens which have been targeted fall into two categories: (i) minor histocompatibility antigens (mHAgs) expressed by leukemia progenitors, and (ii) TAAs overexpressed by the leukemic cells with limited expression on normal cells.

mHAgs are HLA-binding peptides derived from endogenous proteins in cells of the stem cell transplant recipient that differ from those of the donor due to genetic polymorphisms, thus representing a unique class of antigens that can only be targeted after allogeneic HSCT to promote both GVL and GVHD effects in vivo.[39] However, much of the current research in this area is focused on identifying and selectively targeting mHAgs expressed exclusively on malignant cells. Warren and colleagues recently evaluated the safety of adoptively transferring donor-derived CD8+ CTL clones recognizing minor histocompatibility antigens (mHAgs) preferentially expression on hematopoietic cells to patients with relapse of acute leukemia after myeloablative allogeneic HSCT[40]. The highest doses administered to each patient ranged from 2.25 x 10^9 - 6.6 x 10^9 cells. Pulmonary toxicity was seen in three of the seven treated patients, and was severe in one, and correlated with the level of expression of the mHAg-encoding genes in lung tissue. However, the administration of steroids coincided with a rapid reversal in pulmonary symptoms. Thus the associated toxicity could be rapidly and effectively controlled[40].

In both the autologous and donor-specific setting adoptive transfer approaches are being developed against a number of leukemia-associated antigens including Wilms Tumor Gene 1 (WT1), Proteinase 3 (Pr3), human neutrophil elastase (NE), melanoma associated antigen

A3 (MAGE-A3) and preferentially expressed antigen in melanoma (PRAME)[13,14]. Of these WT1 is perhaps the most extensively characterized with several groups having defined CD4+ and CD8+ epitope peptides, thus confirming the immunogenicity of this target. Its clinical relevance is evidenced by the fact that disease control or remission in several vaccine studies has been associated with the induction of WT1-specific T cells, and an increased frequency of circulating WT1-specific T cells post HSCT has been associated with sustained disease remission. Based on these and other studies, O'Reilly and colleagues have recently initiated a clinical trial of donor-derived WT1 peptide-specific T cells, activated using DCs loaded with an overlapping peptide library (15mers overlapping by 11 amino acids) spanning the entire sequence of the antigen, and infused as treatment of persistent minimal residual disease or recurrence of WT1+ AML, ALL, or MDS following allogeneic HSCT. In preliminary reports, T cell infusions at the lowest dose levels were reported to be safe, well tolerated, and associated with clinical benefit[41,42].

3.3 Targeting EBV negative lymphomas using adoptive T cell transfer

To target lymphoma we have developed a strategy to target multiple tumor expressed antigens including PRAME, SSX2, MAGE-A4, NY-ESO-1 and Survivin.

To date we have infused a total of 18 patients: 9 with Hodgkin lymphoma, 8 with aggressive non-Hodgkin lymphoma, and 1 with a composite lymphoma. Patients have received 2 infusions of cells ranging from 0.5-2x10^7 multiTAA-T cells/m^2 without adverse events. Of 11 patients who were infused as adjuvant therapy all but 1 remain in remission (range 1-24 months post-infusion). Seven patients have received multiTAA-specific T cells to treat active disease. Of these, 1 had transient disease stabilization followed by disease progression, 3 have stable disease 3-6 months post-multiTAA-specific T cells while the remaining 3 (all with HL) have had complete responses, as assessed by PET. Notably, since patients received no pre-infusion cytoreductive chemotherapy, these clinical responses can be attributed to the cells infused. Indeed, clinical benefit correlated with the detection of tumor-reactive T cells in patient peripheral blood post-infusion directed against both targeted antigens as well as non-targeted TAAs including MAGEA2B and MAGE C1, indicating antigen spreading. Thus, infusion of autologous multiTAA-targeted T cells directed to PRAME, SSX2, MAGEA4, NY-ESO-1 and Survivin has been safe and provided clinical benefit to patients with lymphomas.

Additionally, we have administered these multi-antigen-targeted T cell lines to patients with multiple myeloma (n=5) and a spectrum of solid tumors including squamous cell carcinoma, lung cancer and osteosarcoma (n=4).

3.4 Extending our approach to treat breast cancer

Given our demonstration that (i) targeting NY-ESO-1, PRAME, MAGE-A4, SSX2 and Survivin simultaneously is feasible and safe, and (ii) these antigens are expressed in different stages of breast cancer including triple-negative breast cancer[7-11], we now propose to administer multiTAA-specific CTLs to patients with breast cancer. The CTL generation protocol will be identical to that used in our lymphoma study. In the current phase II study, we propose testing the safety and antitumor activity of these multiTAA-specific cells in patients with a breast cancer diagnosis at a fixed dose of 2x10^7 cells/m^2.

3.5 Potential adverse events

Although no toxicity has been associated with CTL infusion in patients with Hodgkin and non-Hodgkin lymphoma, we will actively look for evidence of potential side effects such as:

Cytokine Release Syndrome:

There have been several reported SAEs associated with cytokine release syndrome (CRS) in patients who received T cells[43] or bispecfic T-cell engagers[44]. The majority of CRS have been reported after the infusion of CAR T cells[45-47], but CRS can also occur after the infusion of conventional antigen-specific T cells[48] or tumor infiltrating lymphocytes[49]. Patients will be monitored closely as per study calendar and assessed for evidence of incipient CRS (onset of fever, malaise and dyspnea) and treated promptly. Management of CRS will follow published guidelines[43,50], and is described in more detail in SOP F 05.11.XX and includes treatment options based on the clinical severity of the symptoms, such as oxygen, inotropic agents, IL-6 receptor antibody (4-8 mg/kg),TNF-α antibody (5-10 mg/kg), and/or steroids (1-2 mg/kg/day of methylprednisolone or equivalent).

4.0 PATIENT ELIGIBILITY

4.1 Procurement Inclusion Criteria

4.1.1 Any breast cancer patient with metastatic or locally recurrent unresectable disease.

4.1.2 Patients with life expectancy $\geq$12 weeks.

4.1.3 Age $\geq$ 18 and $\leq$ 80 years old

4.1.4 Hgb ≥7.0 (transfusions allowed -see Section 7.3).

4.1.5 Informed Consent explained to, understood by and signed by patient. Patient given copy of informed consent.

4.2 Procurement Exclusion Criteria

4.2.1 Patients with severe intercurrent infection.

4.2.2 Patients with active HIV infection at time of procurement

4.2.3 Patients with a chronic uncontrolled medical condition that, in the opinion of the principal investigator, precludes them from participation.

4.3 Treatment Inclusion Criteria

4.3.1 Any breast cancer patient with metastatic or locally recurrent unresectable breast cancer currently progressive, after at least two prior lines of therapy in the advanced setting. Patients with HER2+ disease must have failed two or more different anti-HER2 agents.

4.3.2 Patients must have measurable or evaluable disease per RECIST 1.1 criteria.

4.3.3 Patients with life expectancy $\geq$ 12weeks

4.3.4 Age $\geq$ 18 and $\leq$ 80 years old

4.3.5 Pulse oximetry of >95% on room air

4.3.6 Patients with an ECOG score of ≤ 2 or Karnofsky score of ≥ 50 as described below:

ECOG Performance Status	
Grade	ECOG
0	Full active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

Performance Status Criteria Karnofsky performance scores are intended to be multiples of 10	
Karnofsky	
Score	Description
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity, minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or do active work.
60	Required occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.

40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.

4.3.7 Patients with bilirubin ≤2x upper limit of normal, AST ≤3x upper limit of normal, and Hgb ≥7.0 (transfusion allowed - see Section 7.3).

4.3.8 Patients with a creatinine normal for age

4.3.9 Patients should have been off other investigational therapy for one month prior to receiving treatment on this study.

4.3.10 Patients should have been off conventional therapy for at least 1 week prior to receiving treatment on this study.

4.3.11 Sexually active patients must be willing to utilize one of the more effective birth control methods for 6 months after the T cell infusion.

4.3.12 Informed Consent explained to, understood by and signed by patient. Patient given copy of informed consent.

4.4 Treatment Exclusion Criteria

4.4.1 Patients with severe intercurrent infection.

4.4.2 Patients receiving systemic corticosteroids (Patients off steroids for at least 48 hours are eligible)

4.4.3 Pregnant or lactating

4.4.4 Patients with a chronic uncontrolled medical condition that, in the opinion of the principal investigator, precludes them from participation.

4.4.5 HIV positive

5.0 STUDY DESIGN

This protocol will be discussed with eligible patients and informed consent for participation in the study will be obtained for the generation of the cell lines. The protocol will be discussed with patients undergoing treatment at other medical Institutions at the time they are referred to Houston Methodist Hospital (HMH).

All cell culture manipulations will be carried out in the Center for Cell and Gene Therapy GMP facility using current standard operating procedures (SOPs). After quality assurance testing of the manufactured cell product is complete, a certificate of analysis will be issued.

5.1 Blood procurement for CTL and antigen-presenting cell (APC) generation

Generation of tumor-specific CTL lines requires the generation of several different components from peripheral blood mononuclear cells (PBMC). The CTL line will be derived from patient peripheral blood T cells, by stimulation with antigen-presenting cells (APCs) pulsed with pepmixes spanning the tumor-associated antigens SSX2, MAGEA4, Survivin, PRAME, and NY-ESO-1. The initial stimulation will be performed in the presence of the Th1/pro-proliferative cytokines interleukin (IL) 7, IL-12, IL-15, and IL6 and cells will be expanded in the presence of IL-2 or IL15. The APCs used to stimulate and expand the tumor-specific T cells will be dendritic cells derived from patient mononuclear cells.

For CTL generation, we will either use:
* a mononuclear cell-apheresis collection procedure (for patients with ALCs <500) or
* a maximum blood draw of 390 ml total, obtained in 1-3 draws over a 2 month period for CTL generation, testing for infectious viruses and HLA typing. PBMC will be separated from whole blood using ficoll gradients. T cells and monocyte-derived dendritic cells (DCs) can be prepared from fresh or cryopreserved PBMC.

To initiate tumor-specific CTL lines we will make DCs by culture of PBMC-derived monocytes with cytokines (GM-CSF, IL-4) followed by maturation with a standard DC maturation cocktail (IL-1β, IL6, TNFα and PGE1). These mature DCs will be pulsed for 30-60 mins with a mastermix of pepmixes spanning the target antigens PRAME, SSX2, MAGEA4, NY-ESO-1 and Survivin. For dilution 1ul of each pepmix (200ng/peptide) will be added to 200ul Cell Genix/RPMI media and 50-100ul will be used to pulse the DCs (≤5x10e6 DCs - 50ul; >5x10e6 DCs - 100ul). Subsequently the DCs will be washed once and used to stimulate PBMC-derived T cells in the presence of a T cell activating cocktail, IL7, IL15, IL12 and IL6 at a responder:stimulator ratio of at least 10:1. For initiation, DCs will be prepared from about 100mL of blood and the T cells will be derived from the monocyte-depleted PBMC fraction.

To expand the tumor-specific T cells we will use pepmix-pulsed DCs for the second and subsequent stimulations and cells will be cultured in the presence of IL-2 or IL-15. At least 100 x 10^6 mononuclear cells are required to generate the second batch of DCs.

At the end of the culture period, CTLs will be cryopreserved and aliquots tested for phenotype, function, specificity, identity and sterility. The frequency of tumor-specific CTLs will be determined using intracellular cytokine staining, ELIspot assay, and HLA-peptide tetramers if available. Effector memory phenotype and T cell subsets will be analyzed by flow cytometry.

Products that meet study specific release criteria, as detailed on the CofA, will be infused as per Section 5.2.

If a positive sterility testing result is reported after the product was infused, the FDA and other relevant parties would be notified as per manufacturing SOP B01.03.XX (Product Quality Assurance Program and Release and Return of Clinical GMP/GTP Products) and clinical research SOP J02.06.XX (Serious Adverse Experience and Unanticipated Problem

Reporting). Our management of such a situation is further described in our SOP F05.09.XX (Management of Culture Positive Cell Therapy Products).

We will use pepmixes produced by JPT Technologies as an antigen source. These pepmixes are overlapping peptide libraries (15 mers overlapping by 11 amino acids) spanning the entire sequence of each of the antigens of interest as per our lymphoma (TACTAL) study.

5.2 Administration and Monitoring

Patients will be evaluated in the clinic and 2 fixed doses of CTL will be administered 4 weeks apart. Patients will be monitored for clinical toxicity by the CTEP NCI Common Toxicity Criteria Scale (Version 4.X, See Section 13.3) with the exception of CRS toxicities that are related to T-cell infusions. CRS toxicities will be graded according to Appendix I. We will also analyze immunological parameters including phenotype and CTL frequencies by ELIspot and tetramer studies in patients who have HLA types for which tetramers are available. This information will be used to determine the in vivo persistence of the infused product and whether multiTAA-specific T cells will be able to recruit the patient's endogenous immune system as measured by epitope spreading. Functional analyses (antigen-specific cytotoxicity and cytokine release) will be performed by chromium release assays and ELIspot/intracellular cytokine staining, respectively. The levels of serum cytokines before and following infusion will be compared. If needed, we will compare the infused product with the patient's peripheral blood using TCR deep-sequencing. A time period of 6 weeks post the 2^{nd} infusion will constitute the time for clinical efficacy and safety monitoring. If patients have had a partial response or have stable disease they will be eligible to receive up to 6 further doses of CTLs, each of which will consist of the same cell dose or less (if there is not enough product available for the subject's original dose) than their second infusion

6.0 TREATMENT PLAN

This study will evaluate the clinical efficacy of T cells specific for all 5 tumor associated antigens in a fixed dose study.

6.1 Dose Levels

The patients will be treated 2 times 4 weeks apart at a fixed dose of $2x10^7$ cells/m^2.

6.2 Dose Schedule

This protocol is designed as a phase II fixed-dose study.

Each patient will receive 2 injections at a fixed dose, 28 days apart, according to the following dose schedule: The expected volume of infusion will be 1 to 10 cc.

Dose schedule:

Day 0	2×10^7 cells/m^2
Day 28	2×10^7 cells/m^2

If patients have stable disease or a partial response by the Response Evaluation Criteria in Solid Tumors (RECIST) criteria (see 8.4.1) at their 6 week evaluation after the 2nd cell dose, they will be eligible to receive up to 6 additional doses of CTLs, At least one month

should have passed before each additional dose.. Each additional infusion will consist of the same cell number or less (if there is not enough product available for the subject's original dose) than their second infusion. Patients will not be able to receive additional doses until the initial safety profile is completed at 6 weeks following the second infusion.

6.3 Premedication

Patients may be premedicated with diphenhydramine (Benadryl) up to 1mg/kg IV (max 50mg) and acetaminophen (Tylenol) 10mg/kg po (max 650mg), though this is not mandatory.

6.4 Cell Administration:

Tumor-specific T cells will be given by intravenous injection over 1-10 minutes through either a peripheral or a central line.

6.5 Monitoring

Monitoring will be undertaken according to institutional standards for administration of blood products with the exception that the injection will be given by a physician.

6.6 Supportive Care

Patients will receive supportive care for acute or chronic toxicity, including blood components or antibiotics, and other intervention as appropriate.

6.7 Treatment Center

All treatments will be administered at the Center for Cell and Gene Therapy in Houston Methodist Hospital. Patient follow up and regular care will be at the Breast Center at Baylor Clinic or Smith Clinic.

6.8 Other Treatment

Patients should not receive other treatment for their cancer between the first and second CTL doses or for at least 6 weeks post 2nd CTL (for purposes of evaluation). If a patient receives other treatment for their cancer between the first and second CTL doses, that patient will be taken off treatment.

7.0 PATIENT EVALUATION

7.1 Follow up Interval

Patients shall be seen in clinic prior to the first and second infusions and evaluated (seen in clinic or contacted by a research coordinator) at 1 -2 week intervals for up to six weeks after the 2nd infusion and then at 3, 6, 9 and 12 months (post 2nd infusion). We will then continue to follow patients clinically for up to 4 additional years (total of 5 years follow-up) to evaluate long-term disease responses. If persistence of cells is noted at the 12 month visit, additional follow-up will added. Additional visits will be obtained as clinically indicated or if the patient is having more than 2 infusions.

7.2 History and Physical Examination

A complete history will be performed at pre-infusion, at week 2 post CTL #1, at Week 4/Day 0 (CTL infusion #2) and Weeks 1, 2, 4, 6 post CTL infusion #2, at 3, 6, 9 and 12 months, then annually for up to 4 additional years for a total of 5 years follow-up. Physical examinations will be conducted at pre-infusion for CTL #1 and on days of subsequent CTL infusions.

7.3 Other Studies

7.3.1 The following investigations will be obtained at pre-infusion for CTL #1 and on days of subsequent CTL infusions

CBC and differential and Complete Metabolic Panel (CMP).

7.3.2 Pregnancy testing is required on female patients of childbearing potential prior to CTL infusion #1. Pregnancy testing is not required prior to CTL infusion #2 for patients treated.

7.3.3 Diagnostic imaging (CT scans, MRI, nuclear imaging) and/or blood tests (serum cytokines) to document measurable disease and response to therapy at pre-infusion and 6 weeks following the second infusion. The time may vary by 1-2 weeks due to scheduling issues and may be done earlier if clinically indicated. The choice of imaging will depend on what studies have been most informative in following the patient's disease. If diagnostic imaging studies are performed at other times either during or after treatment on this study, that data will be collected and information gained will be used for this study.

7.3.4 The following investigations will be obtained pre-infusion, and at 1, 2, 4 weeks after the first infusion and then at 1, 2, 4, 6 weeks and 3, 6, 9, and 12 months following the 2nd infusion. 20-40ml of peripheral blood will be collected in preservative-free heparin anticoagulant. This blood will be used for analysis of specificity of CTL response using HLA-peptide tetramer analysis and immune function assays including ELIspot, intracellular cytokine staining and cytotoxicity assays. Serum will be batched for analysis of blood cytokine levels. These studies will be done on patients on whom the appropriate reagents are available.

7.3.5 If the patient has additional injections of cells after the first 2 infusions, the tests listed in 7.2.1 and 7.2.4 will also be obtained pre each infusion, and at 1, 2, 4, and 6 weeks post each infusion. Follow up will then continue at 3, 6, 9 and 12 months after the last infusion. No study specific blood tests will be done after two years (if the patients have had additional injections) but we will continue to follow patients clinically once a year for up to 4 additional years (total of 5 years follow-up) to evaluate long term disease responses.

7.3.6 Other Tissues: Should the patient undergo a tumor biopsy at any time while they are on study, a sample of this will be used to assess the tumor antigen expression profile.

If a patients' hemoglobin is less than 7.0g/dl at any of the evaluation times, the amount of blood drawn for the evaluation will be reduced and may be obtained over more than one venipuncture, if necessary.

All pre-study exams must be done within 72 hours of eligibility.

7.4 Summary of Monitoring

Study Calendar: A summary of monitoring studies is provided in the following table:

Study Procedure	Treatment													Follow-up			
	Pre-infusion	Day 0	Wk 1	Wk 2	Wk 4/ Day 0 [#2}	Wk 1 [#2]	Wk 2 [#2]	WK 4 [#2]	Wk 6 [#2]	M 3	M 6	M 9	M 12	Y 2	Y 3	Y 4	Y 5
STUDY DRUG ADMINISTRATION																	
mTAA CTL infusion		X			X												
DATA/TESTS/SPECIMENS TO BE OBTAINED																	
Blood Procurement[a]	X																
Concomitant Medications		X															
PE	X				X												
Hx	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test[b]	X																
Blood Tests [Hematology, Comprehensive Metabolic Panel][c]	X				X												
Imaging studies[d]	X								X								
Immune function studies[e]	X		X	X	X	X	X	X	X	X	X	X	X				
TCR deep-sequencing[f]																	
Tumor Biopsy[g]																	
DATA REVIEW																	
Eligibility Assessment for Procurement	X																
Eligibility Assessment for Therapy		X															
Breast Cancer Information	X																
AE Assessment		X[h]															
Response Assessment									X[i]								
Follow-up										X	X	X	X	X	X	X	X
Follow-up Cancer																	
Follow-up Pregnancy																	
Relapse and Progression Assessment																	
Death																	

a- Blood will be collected for infectious disease testing, HLA typing, and mTAA CTL generation

b- Pregnancy testing will be done pre-infusion in patients of childbearing potential. Either serum or urine is acceptable

c- Hematology labs for CBC with differential WBC count; Comprehensive Metabolic Panel includes BUN, creatinine, bilirubin, SGOT, SGPT, alkaline phosphatase, sodium, potassium, chloride, carbon dioxide, albumin.

d- Imaging studies required per section 7.3.3; imaging studies will be performed on a case by case basis to assess disease status at the discretion of the treating physician.

e- Blood will be used for immune function assays including ELISPOT and cytotoxicity assays per section 7.2.4

f- If required, TCR deep-sequencing will be performed to compare the infused product with the patient's peripheral blood per section 5.2

g- Should the patient undergo a tumor biopsy at any time on the study, a sample will be used to determine the tumor antigen expression profile per section 7.2.6

h- Adverse events are assessed throughout the clinical safety monitoring timeframe

i- Time point for evaluation clinical efficacy, and eligibility for additional doses per section 5.2

Additional Treatment

Study Procedure	Treatment									Follow-Up			
	Day 0	Wk 1	Wk 2	Wk 4	Wk 6	M 3	M 6	M 9	M 12	Y 2	Y 3	Y 4	Y 5
STUDY DRUG ADMINSITRATION													
mTAA CTL infusion	X												
DATA/TESTS/SPECIMENS TO BE OBTAINED													
Concomitant Medications	X												
PE	X												
Hx	X		X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test	X												
Blood Tests [Hematology, Comprehensive Metabolic Panel][a]	X												
Imaging studies[b]					X								
Immune function studies[c]	X	X	X	X	X	X	X	X	X				
TCR deep-sequencing[d]													
Tumor Biopsy[e]													
DATA REVIEW													
AE Assessment	X[f]												
Response Assessment					X[g]								
Follow-up						X	X	X	X	X	X	X	X
Follow-up Cancer													
Follow-up Pregnancy													
Relapse and Progression Assessment													
Death													

a- Hematology labs for CBC with differential WBC count; Clinical chemistry panel includes sodium, potassium, carbon dioxide, chloride, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, aspartate transaminase, alanine transaminase)

b- Imaging studies are not required with additional doses but if performed the data will be collected.

c- Blood will be used for immune function assays including ELISPOT and cytotoxicity assays

d- If required, TCR deep-sequencing will be performed to compare the infused product with the patient's peripheral blood per section 5.2

e- Should the patient undergo a tumor biopsy at any time on the study, a sample will be used to determine the tumor antigen expression profile per section 7.2

f- Adverse events are assessed throughout the clinical safety monitoring timeframe

g- Data pertaining to clinical efficacy and safety monitoring will be obtained 6 weeks after the final injection per section 9.2

8.0 RESPONSE CRITERIA

A 6-week period after the second infusion will constitute a course, which will be evaluated for critical toxicity, and a 6-week period after the second CTL infusion will be required for evaluation for antitumor activity.

Patients with measurable disease will be assessed by standard criteria to determine clinical response. Evaluations of tumor size will be performed pre-infusion and 6 weeks after the second injection. Additional imaging performed as part of standard clinical care will also be evaluated. All patients who receive CTL infusion and continue to be enrolled on the study will be evaluable for response. Patient long term overall and progression free survival will also be evaluated at 1 year and then annually for up to an additional 4 years (total of 5 years follow-up).

8.1 Definitions

This study will use the RECIST 1.1 criteria

Definitions
Response and progression will be evaluated in this study using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) Committee[51]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >10 mm with CT scan, MRI, or clinically using calipers. The investigator will identify up to 5 measurable lesions to be followed for response. Serial measurements are to be done with CT or MRI. The same method of assessment is to be used to characterize each identified and reported lesion at baseline and during follow-up.

Complete Response: Disappearance of all target lesions

Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

9.0 STATISTICAL CONSIDERATIONS

This is a phase II study evaluating the safety and clinical efficacy of multiTAA-specific CTLs administered in patients with breast cancer. All patients will receive 2 injections at a fixed dose, 28 days apart, of T cells specific for all 5 tumor associated antigens.

9.1 Study Design and Sample Size

9.1.1 Sample Size

The primary endpoint is clinical benefit rate at 6 weeks after the second dose. Clinical benefit rate is defined as the proportion of evaluable patients with complete response or partial response or stable disease for ≥10 weeks from the first infusion (6 weeks after the second infusion) according to the RECIST criteria (see Section 8.4.1). This study is designed to detect an increase in clinical benefit rate from 5% to 30%. A Simon's two-stage optimal design will be employed to test the null hypothesis that clinical benefit rate is 5% vs. the alternative target rate of 30%. A sample of 9 evaluable patients will be accrued during the first stage. If none of these 9 patients shows clinical benefit at the analysis of the first stage, the study will be stopped early for lack of efficacy. If at least 1 patient shows clinical benefit, then a second stage will enroll additional evaluable patients. If we observe at least 3 clinical benefit responses out of the total of 17 evaluable patients enrolled into the study, we will consider further investigation is warranted. The proposed sample size is based on testing the above hypotheses with 90% power and 5% significance level. This design has 63% probability of stopping early and an expected sample size of 12 if the true clinical benefit rate is only 5%. Taking into account a 20% drop-out rate for non-evaluable patients, we propose to accrue a total of 22 patients.

9.2 Data Analysis

Safety and toxicity outcomes, and laboratory evaluations will be summarized using descriptive statistics. Information on the expansion, persistence and anti-tumor effects of the adoptively transferred tumor-specific CTL will be analyzed for the immunological parameters based on multimer analysis, intracellular cytokine staining and ELIspot assays to assess the frequency of cells secreting IFNγ using the descriptive statistics such as mean, median, standard deviation at each time point. Comparison of diagnostic imaging studies from pre-infusion to 6 weeks following the second infusion will be summarized. Frequencies, proportions and their 95% confidence intervals of clinical benefit responders (adjusted to account for the two-stage nature of the design) will be summarized overall. Overall survival and progression free survival will be estimated using the Kaplan-Meier survival curve and summarized by median and 95% confidence interval.

Growth curves of immune response over time within a patient will be generated to visualize general patterns of immune response. Pairwise comparisons will compare changes of these immunological parameters from pre-infusion to each time point of post-infusion measurements using paired t-tests or Wilcoxon signed-ranks tests. Longitudinal analysis is employed to model repeatedly-measured immunologic parameters. This will allow us to model patterns of immune response per patient while allowing for varying intercepts and slopes for a patient. The normality assumption will be assessed and transformations to achieve approximate normality will be carried out if necessary.

If a patient has stable disease or a partial response as assessed by the RECIST criteria (see Section 8.4.1) after the first two infusions, they may receive up to 6 additional infusions at monthly intervals. We will collect data on the survival, immunological efficacy and anti-tumor activity of cytotoxic T-lymphocyte lines after these additional infusions so it may be compared with results obtained after the initial two infusions.

To obtain preliminary data on the clinical efficacy and safety monitoring, patients will be monitored for 6 weeks after the final injection for toxicity using standard NIH criteria. If a patient develops Grade III-IV toxicity attributable to the CTL infusions at any time during the extended dosing regimen, they will not be able to receive any more CTL infusions.

9.3 Analysis Set

Evaluable for response: Only patients who receive both infusions will be evaluable for response.

Evaluable for adverse events: All patients who received the first infusion will be evaluable for adverse events.

9.4 Stopping Rule

If there are 2 patients with grade 4 events considered possibly related to the study drug, the study will be halted for review by the Data Safety Monitoring Committee and the FDA.

10.0 MODIFIED FOLLOW-UP AND OFF STUDY CRITERIA

10.1 Criteria for Modified Follow-Up

The following criteria will result in the patient being ineligible for further treatment on the protocol, although response data will continue to be collected as applicable.

10.1.1 Any patient who develops grade $\geq$ III infusional and/or organ toxicity that is NOT (1) pre-existing, or (2) due to infection or (3) due to underlying malignancy and is considered to be possibly, probably, or definitely related to the study drug. In such patients, the toxicities will be followed until resolution or their off-study date. Although no toxicity has been associated with the infusion of this product in patients with Hodgkin and non-Hodgkin lymphoma, we will actively look for evidence of any adverse events, such as cytokine release syndrome.

10.1.2 Any patient who receives any other hematopoietic progenitor cell product. In such patients, adverse event data collection will cease.

10.1.3 Any patient who receives therapy for progression of their breast cancer. In such patients, adverse event data collection will cease.

10.1.4 Any patient who desires to withdraw from the study or if the physician feels that it is in the best interest of the patient, treatment will be discontinued.

Patients who meet modified follow-up criteria remain on long-term follow-up as per the Summary of Monitoring (see table in Section 7.3.1)

10.2 OFF STUDY

10.2.1 Completion of study-specified procedures.
10.2.2 Refusal of study follow up by patient
10.2.3 Lost to follow up
10.2.4 Death

Any questions regarding patients on this study should be addressed to Dr. Rimawi at 713-798-1999.

11.0 RECORDS TO BE KEPT

The research nurse/coordinator will maintain a database documenting on study information, adverse events, off study notification and death information. The dates and doses of therapy as well as clinical chemistries, hematologic parameters, the clinical status and occurrence of any adverse events and subsequent interventions are to be kept on all patients.

Imaging reports
Surgical summaries
Autopsy summaries, where appropriate
Informed consent documents

All required clinical evaluation records will be the responsibility of Principal Investigator who will also be responsible for analysis of the clinical outcome and toxicity.

The laboratory evaluation of immunological efficacy will be the responsibility of Dr. Tzannou.

12.0 REPORTING REQUIREMENTS

12.1 Register all patients with the research nurse/research coordinator.

12.2 Enter all patients by phoning Dr. Rimawi. The following data will be captured:

Eligibility
Pre-study
Concomitant Medication
Adverse event
CRS Adverse Event (if applicable)
Off study
Death

12.3 Drug Toxicity and/or Adverse Reactions

12.3.1 Toxicity Grading: The criteria listed in the CTEP (Version 4.X) of the NCI Common Toxicity Criteria Scale will be used in grading toxicity with the exception of CRS

toxicities that are related to T-cell infusions. CRS toxicities will be graded according to Appendix I.

12.3.2 The CTEP CTCAE (Version 4.X) is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

12.3.3 Adverse events will be collected as per SOP J 02.05.XX and J 02.75.XX. Data on adverse experiences/toxicities regardless of seriousness, must be collected for documentation purposes only for 6 weeks after the last dosing of study drug/biologic.

12.3.4 Serious adverse events will be collected as per SOP J 02.06.XX.

13.0 INFORMED CONSENT

All patients must sign a document of informed consent consistent with local institutional and Federal guidelines stating that they are aware of the investigational nature of this protocol and of the possible side effects of treatment. Further, patients must be informed that no efficacy of this therapy is guaranteed, and that unforeseen toxicities may occur. Patients have the right to withdraw from this protocol at any time. No patient will be accepted for treatment without such a document signed by his or her legal guardian. Full confidentiality of patients and patient records will be provided according to institutional guidelines

14.0 CLINICAL TRIAL OVERSIGHT AND MONITORING

This protocol will be conducted in accordance with the Cell and Gene Therapy Monitoring Plan on file with the FDA.

This protocol will be monitored in accordance with the current Data Safety Monitoring Plan for investigator-initiated Phase I and II studies in the Dan L Duncan Comprehensive Cancer Center at Baylor College of Medicine.

The conduct of this clinical trial will be evaluated in accordance with Center for Cell and Gene Therapy Quality Assurance Policy and Procedure Plan.

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16.0 Appendix I - Grading of CRS

CRS Grading Scale

Grade	Symptoms
1	• Symptoms are not life threatening and require symptomatic treatment only (e.g. fever, nausea, fatigue, headache, myalgia, malaise)
2	• Symptoms require and respond to moderate intervention • Oxygen requirement <40% or hypotension responsive to fluids or • low dose of one vasopressor or Grade 2 organ toxicity
3	• Symptoms require and respond to aggressive intervention • Oxygen requirement ≥ 40% or hypotension requiring high dose or multiple vasopressors or • Grade 3 organ toxicity or Grade 4 transaminitis
4	• Life-threatening symptoms • Requirements for ventilator support or Grade 4 organ toxicity (excluding transaminitis)
5	• Death