

**Abbreviated Title:** Anti-BCMA-CAR T cells

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- A. Obtain information by intervening or interacting with living individuals for research purposes
- B. Obtaining identifiable private information about living individuals
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- D. Makes decisions about subject eligibility
- E. Studying, interpreting, or analyzing identifiable private information or data/specimens for research purposes
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- G. Some/all research activities performed outside NIH

**Investigational Agents:**

Drug Name:	Anti-BCMA-CAR- transduced autologous peripheral blood lymphocytes (PBL)
IND Number:	16041
Sponsor:	Center for Cancer Research

**Commercial Agents: Cyclophosphamide, Fludarabine**

## PRÉCIS

### Background:

- Multiple myeloma (MM) is a malignancy of plasma cells.
- MM is nearly always incurable.
- T cells can be genetically modified to express chimeric antigen receptors (CARs) that specifically target malignancy-associated antigens.
- Autologous T cells genetically modified to express CARs targeting the B-cell antigen CD19 have caused complete remissions in a small number of patients with leukemia or lymphoma. These results demonstrate that CAR-expressing T cells have anti-malignancy activity in humans.
- B-cell maturation antigen (BCMA) is a protein expressed by normal plasma cells and the malignant plasma cells of multiple myeloma.
- BCMA is not expressed by normal cells except for plasma cells and some mature B cells.
- We have constructed an anti-BCMA CAR that can specifically recognize BCMA-expressing target cells in vitro and eradicate BCMA-expressing tumors in mice.
- Anti-BCMA-CAR-expressing T cells have not been previously tested in humans.
- We hypothesize that anti-BCMA-CAR-expressing T cells will specifically eliminate BCMA-expressing MM cells in patients
- Possible toxicities include cytokine-associated toxicities such as fever, hypotension, and neurological toxicities. Elimination of normal plasma cells and unknown toxicities are also possible.

### Objectives:

#### Primary

- Determine the safety and feasibility of administering T cells expressing an anti-BCMA CAR to patients with MM.

#### Secondary

- Evaluate the in vivo persistence of anti-BCMA CAR T cells
- Assess for evidence of anti-myeloma activity by anti-BCMA CAR T cells

### Eligibility:

- Patients must have measurable MM defined as a serum M-protein  $\geq 0.4$  g/dL or a urine M-protein  $\geq 200$  mg/24 hours or an involved serum free light chain (FLC) level  $\geq 10$  mg/dL (provided FLC ratio is abnormal) or a biopsy-proven plasmacytoma .
- Patients must have previously received at least 3 different treatment regimens for MM.
- Patients must have a normal creatinine and a normal cardiac ejection fraction.
- An ECOG performance status of 0 -2 is required.

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- Patients on any anticoagulant medications except aspirin are not eligible.
- No active infections are allowed.
- Absolute neutrophil count  $\geq 1000/\mu\text{L}$ , platelet count  $\geq 45,000/\mu\text{L}$ , hemoglobin  $\geq 8\text{g/dL}$
- ALT and AST  $\leq 2.5$ -fold higher than the upper limit of normal
- At least 14 days must elapse between the time of any prior systemic treatment (including corticosteroids) and the required leukapheresis.
- At least 14 days must elapse between the time of any prior systemic treatment (including corticosteroids) and initiation of protocol treatment.
- Bone marrow plasma cells must be 30% or less of total bone marrow cells 30 days or less prior to the start of protocol treatment.
- The patient's MM will need to be assessed for BCMA expression by flow cytometry or immunohistochemistry performed at the NIH. If unstained, paraffin-embedded bone marrow or plasmacytoma sections are available from prior biopsies, these can be used to determine BCMA expression by immunohistochemistry; otherwise patients will need to come to the NIH for a bone marrow biopsy or other biopsy of a plasmacytoma to determine BCMA expression. The sample for BCMA expression can come from a biopsy obtained at any time before enrollment.

**Design:**

- This is a phase I dose-escalation trial
- Patients will undergo leukapheresis
- T-cells obtained by leukapheresis will be genetically modified to express an anti-BCMA CAR
- Patients will receive a lymphocyte-depleting chemotherapy conditioning regimen with the intent of enhancing the activity of the infused anti-BCMA-CAR-expressing T cells.
- The chemotherapy conditioning regimen is cyclophosphamide  $300\text{ mg/m}^2$  daily for 3 days and fludarabine  $30\text{ mg/m}^2$  daily for 3 days. Fludarabine will be given on the same days as the cyclophosphamide.
- Two days after the chemotherapy ends, patients will receive an infusion of anti-BCMA-CAR-expressing T cells.
- The initial dose level of this dose-escalation trial will be  $0.3 \times 10^6$  CAR+ T cells/kg of recipient bodyweight.
- The cell dose administered will be escalated until a maximum tolerated dose is determined for patients in which less than 50% of total bone marrow cells are plasma cells. With Amendment C, all patients with 50% or greater bone marrow plasma cells will receive  $3 \times 10^6$  anti-BCMA CAR T cells/kg.
- Following the T-cell infusion, there is a mandatory 9-day inpatient hospitalization to monitor for toxicity.

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- Outpatient follow-up is planned for 2 weeks, and 1, 2, 3, 4, 6, 9, and 12 months after the CAR T-cell infusion.
- Repeat treatments are possible for patients with residual MM and no greater than grade 2 toxicity with an initial treatment.
- Re-enrollment will be allowed for a small number of subjects.

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## 1 INTRODUCTION

### 1.1 STUDY OBJECTIVES

#### 1.1.1 Primary Objective

Determine the safety and feasibility of administering T cells expressing an anti-B-cell maturation antigen (BCMA) chimeric antigen receptor (CAR) to patients with multiple myeloma (MM).

#### 1.1.2 Secondary Objectives

- Evaluate the *in vivo* persistence and *ex vivo* function of T cells expressing an anti-BCMA CAR.
- Assess infusions of anti-BCMA-CAR-expressing T cells for anti-MM activity.

### 1.2 BACKGROUND AND RATIONALE

#### 1.2.1 Introduction

Multiple myeloma (MM) is a malignancy of plasma cells that is almost always incurable. New therapies are needed for MM. Regressions of MM occurring in the setting of allogeneic stem cell transplantation (alloHSCT), and particularly after allogeneic donor lymphocyte infusions (DLIs), provide evidence that cellular immune responses can have a clinically significant anti-myeloma effect, but alloHSCT is associated with a significant transplant-related mortality and by chronic graft-versus-host disease.<sup>1,2</sup> Several patients have obtained lengthy complete remissions of lymphoma or chronic lymphocytic leukemia after infusions of autologous T cells that were genetically modified to express chimeric antigen receptors (CARs) targeting the B-cell antigen CD19.<sup>3,4</sup> The lasting complete remissions of MM that sometimes occur in the setting of alloHSCT and the encouraging early results obtained treating patients with anti-CD19-CAR-expressing T cells provide a rationale for attempting to develop CAR-T-cell therapies for MM. B-cell maturation antigen (BCMA) is a protein that is expressed by MM cells.<sup>5</sup> BCMA is also expressed by normal plasma cells and by some normal B cells, but BCMA is not expressed by other normal cells.<sup>5</sup> The very limited expression of BCMA in normal tissues makes BCMA a very promising target for CAR-T-cell therapies. We have developed an anti-BCMA CAR, and we have demonstrated that T cells expressing this CAR have BCMA-specific activity *in vitro* and *in vivo*.<sup>5</sup> Anti-BCMA-CAR-expressing T cells can eradicate MM tumors in mice.<sup>5</sup> We propose to conduct a phase I clinical trial of anti-BCMA-CAR-expressing T cells. This clinical trial will enroll patients with advanced MM that is progressive despite at least 3 prior therapies. Patients enrolled on the trial will receive a single cycle of chemotherapy that is designed to decrease endogenous lymphocyte counts because extensive evidence exists that depleting endogenous lymphocytes, and possibly other cells, with chemotherapy or total body irradiation dramatically increased the anti-tumor activity of adoptively transferred T cells.<sup>6-8</sup> After the lymphocyte-depleting chemotherapy, patients will receive an infusion of autologous anti-BCMA-CAR T cells. The T cell dose will escalate with sequential cohorts of patients until a maximum tolerated dose is determined.

#### 1.2.2 Multiple myeloma epidemiology and standard treatment

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Multiple myeloma (MM) is a neoplasm of monoclonal plasma cells.<sup>9,10</sup> For MM to be diagnosed, a patient must have a proliferation of monoclonal plasma cells plus other abnormalities such as anemia, renal insufficiency, hypercalcemia, and lytic bone lesions.<sup>10</sup> MM is the second most frequently occurring hematologic cancer in the United States (U.S.).<sup>10</sup> An estimated 22,350 new cases of MM will be diagnosed in the U.S. in 2013, and an estimated 10,710 patients will die from MM in the U.S. in 2013.<sup>11</sup> Recent improvements in the therapy of MM have occurred.<sup>9,10,12</sup> These improved treatments have increased the median survival of patients with newly-diagnosed MM from 3 years to slightly over 5 years, although the prognosis of newly-diagnosed MM patients varies widely.<sup>10,12</sup>

Current standard non-transplant therapies for MM include various combinations of dexamethasone, bortezomib and its analogs, lenalidomide and its analogs, prednisone, melphalan, and cyclophosphamide.<sup>9,10,12,13</sup> Myeloablative doses of chemotherapy followed by autologous stem cell transplantation is a standard therapy for MM patients with good performance status, and adequate bone marrow stem cells.<sup>10,12</sup> Compared to standard doses of chemotherapy for first treatment of MM, administration of myeloablative doses of chemotherapy followed by autologous stem cell transplantation improved progression-free survival in most trials and overall survival in some trials.<sup>10,12</sup> Myeloablative chemotherapy followed by autologous transplantation can also be used as a treatment for relapsed MM in some cases.<sup>10,12,14</sup> Use of the International Uniform Response Criteria for Multiple Myeloma is the most common approach for assessing clinical outcomes in MM.<sup>15</sup> Despite the recent improvements in treatment, MM remains an almost always incurable disease.<sup>12,16,17</sup> Patients obtaining remissions of MM almost always relapse.<sup>12</sup> The median overall survival of patients with relapsed MM 3 years or less.<sup>16</sup> Survival is shorter for patients treated with lenalidomide plus dexamethasone who have received at least 2 prior lines of therapy compared to patients who received only one prior line of therapy.<sup>18</sup> In patients with MM that was refractory to bortezomib, the median overall survival was only 9 months when the patient was also either ineligible for thalidomide or lenalidomide, or the patient had MM that was relapsed or refractory to thalidomide or lenalidomide.<sup>17</sup> Utilizing the immune system to treat MM is one possible way to improve therapy of MM. So far, the clearest evidence of activity of the immune system against MM comes from allogeneic transplantation studies.

### 1.2.3 Allogeneic transplantation for MM

Allogeneic stem cell transplantation (alloHSCT) can cure a fraction of patients with MM.<sup>19,20</sup> Myeloablative alloHSCT can induce long-term complete remissions of MM, but also has a transplant-related mortality rate (TRM) of 20% to 50%.<sup>19,20</sup> The high TRM of myeloablative alloHSCT led investigators to test nonmyeloablative alloHSCT for MM.<sup>19-21</sup> A commonly used transplantation strategy is to administer myeloablative chemotherapy plus an autologous stem cell transplant and then to conduct a nonmyeloablative alloHSCT a short time later.<sup>1,19,20</sup> In some studies, this strategy has been shown to yield higher rates of progression-free and overall survival when compared to the strategy of 2 sequential autologous stem cell transplants.<sup>1,21</sup> One recent trial showed an 8-year progression-free survival rate of 22% for patients receiving an autologous transplant followed by a nonablative alloHSCT compared to an 8-year progression-free survival of 12% for patients receiving sequential autologous transplants.<sup>1</sup> These results

showed a statistically significant advantage for the autologous followed by alloHSCT strategy, but also point out that the vast majority of patients are not cured by either approach.

Because many nonmyeloablative transplant regimens that have been used to treat MM include very low doses of radiation or chemotherapy, nonmyeloablative alloHSCTs depend on an immunologic graft-versus-myeloma effect, and remissions of MM that occur after nonmyeloablative alloHSCT provide evidence that immune responses can be effective at eliminating MM.<sup>1,19,20</sup> Direct evidence that lymphocytes can eliminate MM comes from donor lymphocyte infusions (DLIs).<sup>2,20</sup> Twenty-two to twenty-eight percent of patients receiving DLIs to treat persisting MM after alloHSCT have achieved complete remissions (CRs).<sup>2,19,20</sup> Of note, some of these patients obtaining CRs after DLIs did not receive any other therapies around the time of their DLI.<sup>2</sup> These results demonstrate the ability of the immune system to eradicate MM. Unfortunately, both alloHSCT and DLIs utilize allogeneic lymphocytes; therefore, they are associated with the sometimes fatal complication of graft-versus-host disease<sup>2,19,20</sup>; in addition most patients receiving DLIs for MM do not obtain CRs, so developing of an effective autologous cellular immune therapy for MM would be a major advance.

#### 1.2.4 T-cell gene therapy

T cells can be prepared for adoptive transfer by genetically modifying the T cells to express receptors that specifically recognize tumor-associated antigens.<sup>22-29</sup> Genetic modification of T cells is a quick and reliable process, and clinical trials of genetically modified T cells targeting a variety of malignancies have been carried out.<sup>3,4,26,30,31</sup> Genetically modified antigen-specific T cells can be generated from peripheral blood mononuclear cells (PBMC) in sufficient numbers for clinical treatment within 10 days.<sup>3</sup> Genetically modifying T cells with gammaretroviruses consistently causes high and sustained levels of expression of introduced genes without in vitro selection<sup>4,30,32,33</sup>, and genetic modification of mature T cells with gammaretroviruses has a long history of safety in humans.<sup>34-36</sup> There are two general approaches for generating antigen-specific T cells by genetic modification: introducing genes encoding natural  $\alpha\beta$  T cell receptors (TCRs) or introducing genes encoding chimeric antigen receptors (CARs).<sup>23,25,26,28</sup> CARs are fusion proteins incorporating antigen recognition moieties and T cell activation domains.<sup>27,37-39</sup> The antigen-binding domains of most CARs currently undergoing clinical and preclinical development are antibody variable regions.<sup>23,27,37,39</sup> TCRs recognize peptides presented by human leukocyte antigen (HLA) molecules; therefore, TCRs are HLA-restricted, and a particular TCR will only be useful in patients expressing certain HLA molecules<sup>23,25,26,37</sup>, which limits the number of patients who could be treated with T cells genetically modified to express a TCR. In contrast, CARs recognize intact cell-surface proteins and glycolipids, so CARs are not HLA-restricted, and CARs can be used to treat patients regardless of their HLA types.<sup>23,26,40-42</sup>

#### 1.2.5 Chimeric antigen receptors

Preclinical experiments evaluating CAR-expressing T cells as cancer therapy were initiated in 1993.<sup>43,44</sup> These experiments led to a clinical trial of CAR-transduced T cells targeting the  $\square$  folate receptor on ovarian cancer cells; no tumor regressions were observed during this clinical trial.<sup>45</sup> CARs that were capable of recognizing a variety of tumor-associated antigens have been

evaluated in many centers.<sup>23,37</sup> Preclinical studies have assessed a wide variety of factors that could affect in vivo function of CAR-expressing T cells. Multiple approaches for inserting CAR genes into T cells by using gammaretroviruses<sup>4,30,32-34,46-48</sup>, lentiviruses<sup>3,49-52</sup>, or transposon systems<sup>53,54</sup> have been assessed. Because all methods of T-cell genetic modification require a period of in vitro culture, various T-cell culture techniques have been evaluated.<sup>3,46,55</sup> Different portions of CARs including antigen-recognition moieties, extracellular structural components, costimulatory domains such as the cytoplasmic portion of the CD28 protein, and T-cell-activation moieties such as the signaling domains of the CD3 $\zeta$  protein can all be important to the in vivo function of CAR-expressing T cells, and all of these portions of CARs remain the subject of intensive investigation.<sup>37,46,50,56-58</sup> Much of the preclinical work evaluating CARs has been performed with CARs targeting the B-cell antigen CD19.<sup>7,46-48,53,59,60</sup> Data suggesting that T-cell costimulation played an important role in the activity of CAR-expressing T cells in vivo led investigators to add signaling moieties from the costimulatory molecule CD28 to CARs.<sup>48,57</sup> These studies showed that adding CD28 moieties to CARs enhanced antigen-specific cytokine production and proliferation by anti-CD19 CAR T cells.<sup>57,61,62</sup> T cells expressing CARs with CD28 signaling moieties and CD3 $\zeta$  signaling domains were more effective than T cells expressing CARs without CD28 moieties at eradicating human leukemia cells from mice.<sup>61,62</sup> Subsequently, CARs incorporating other signaling domains from costimulatory molecules such as 4-1BB (CD137) were developed.<sup>49</sup> Anti-CD19 CARs containing the signaling domains of both 4-1BB and CD3 $\zeta$  were superior to CARs containing the signaling domains of CD3 $\zeta$  without any costimulatory domains at eradicating human malignant cells from mice.<sup>50,56</sup> Similar to CD28, including 4-1BB signaling moieties in CARs led to increased CD19-specific proliferation and enhanced in vivo persistence.<sup>50</sup> In contrast to T cells expressing a CAR with a CD28 moiety, the increased in vitro proliferation and prolonged in vivo persistence of T cells expressing a 4-1BB-containing CAR occurred whether or not the T cells were exposed to the antigen that the CAR recognized.<sup>50,56</sup>

Results from several clinical trials of anti-CD19 CAR T cells have been reported to date in peer-reviewed papers; these papers have all reported results from patients receiving autologous CAR-modified T cells.<sup>3,4,30,32,33,63,64</sup> The first evidence of antigen-specific activity of anti-CD19 CAR T cells in humans was generated during a clinical trial at the National Cancer Institute in a patient who experienced a dramatic regression of advanced follicular lymphoma.<sup>32</sup> This clinical trial utilized a gammaretroviral vector to introduce an anti-CD19 CAR containing the signaling domains of the CD28 and CD3 $\zeta$  molecules.<sup>32</sup> The anti-CD19 CAR-transduced T cells were prepared by using a 24-day in vitro culture process. The clinical treatment regimen consisted of lymphocyte-depleting chemotherapy followed by an infusion of anti-CD19 CAR T cells and a course of high-dose interleukin-2 (IL-2). The first patient treated on this protocol had a large disease burden of follicular lymphoma. This first patient experienced no acute toxicities except for a low grade fever that lasted for 2 days, and he obtained a partial remission (PR) that lasted for 32 weeks.<sup>32</sup> Bone marrow biopsies revealed a complete elimination of extensive bone marrow lymphoma that was present before treatment; in addition, normal B-lineage cells were completely eradicated from the bone marrow.<sup>32</sup> The bone marrow B-cell eradication was confirmed by flow cytometry, and it persisted for over 36 weeks.<sup>32</sup> B cells were also completely absent from the blood during this time, while T cells and other blood cells recovered rapidly.<sup>32</sup> Seven months after the anti-CD19 CAR T cell infusion, progressive lymphoma was detected in the patient's cervical lymph nodes. The lymphoma remained CD19 $^+$ , so the patient was treated a

second time with anti-CD19 CAR T cells. The first and second treatment regimens were the same except the patient received a higher dose of cells with the second treatment. After the second treatment, the patient obtained a second ongoing partial remission.<sup>4</sup>

Seven more patients were subsequently treated with the same regimen of chemotherapy, anti-CD19 CAR T cells, and high-dose IL-2.<sup>4</sup> In 4 of 7 evaluable patients on the trial, administration of anti-CD19 CAR T cells was associated with a profound and prolonged B-cell depletion.<sup>4,32</sup> In all 4 patients with B-cell depletion, the depletion lasted for over 36 weeks. The B-cell depletion could not be attributed to the chemotherapy that was administered because blood B-cells recovered to normal levels in 8 to 19 weeks in patients receiving the same chemotherapy plus infusions of T cells targeting NY-ESO or gp100, which are antigens that are not expressed by B cells.<sup>32</sup> Because normal B cells express CD19, prolonged normal B-cell depletion after anti-CD19 CAR T-cell infusions demonstrated that CAR-expressing T cells had a powerful ability to eradicate CD19<sup>+</sup> cells in humans. All of the patients with long-term B-cell depletion obtained either complete or partial remissions of their malignancies, and the 4 patients with long-term B cell depletion also developed hypogammaglobulinemia. Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins. Of the eight patients treated, seven patients were evaluable for malignancy response; the one patient who was not evaluable died with pneumonia caused by influenza A.<sup>4</sup> Six of the seven evaluable patients had remissions of their malignancies. Two of the remissions were complete remissions (CRs) of CLL.<sup>4</sup> Both of these CRs were confirmed by multicolor flow cytometry of bone marrow cells.<sup>4</sup> One of these CRs lasted 24 months, and the other is ongoing at 30 months.<sup>4</sup> Most patients treated with this regimen of chemotherapy, anti-CD19 CAR T cells, and IL-2 experienced significant acute toxicities including fever, hypotension, and neurological toxicities such as delirium and obtundation.<sup>4</sup> All of these toxicities peaked within 10 days after the cell infusion and resolved less than 3 weeks after the cell infusion.<sup>4</sup> These acute toxicities correlated with serum levels of the inflammatory cytokines tumor necrosis factor and interferon- $\gamma$ , and T cells producing these inflammatory cytokines in a CD19-specific manner were detected in the blood of patients after the anti-CD19 CAR T cell infusions.<sup>4</sup>

### 1.2.6 BCMA

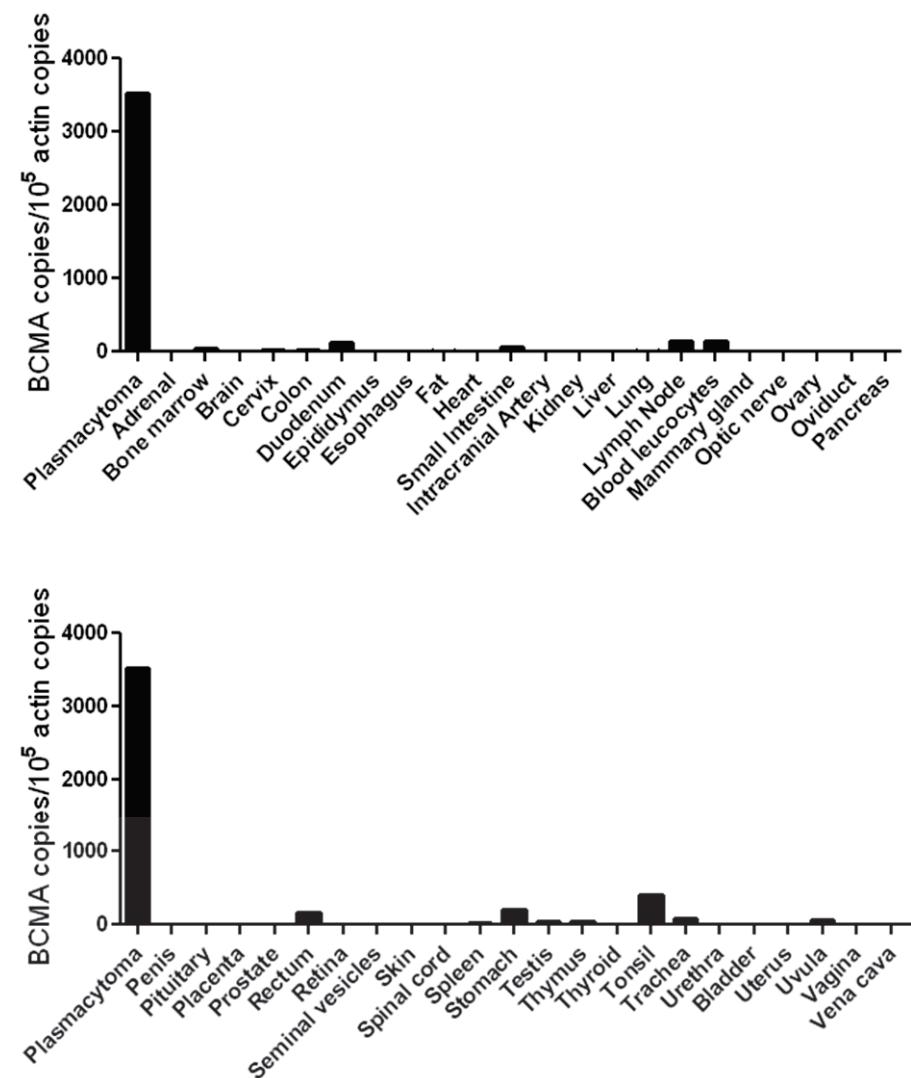
One candidate antigen for immunotherapies of MM is B-cell maturation antigen (BCMA, CD269)<sup>65,66</sup>. BCMA RNA was detected universally in MM cells, and BCMA protein was detected on the surface of plasma cells from multiple myeloma patients by several investigators<sup>67-70</sup>. BCMA is a member of the tumor necrosis factor receptor (TNF) superfamily<sup>71,72</sup>. BCMA binds B-cell activating factor (BAFF) and a proliferation inducing ligand (APRIL)<sup>72-74</sup>. Among nonmalignant cells, BCMA has been reported to be expressed mostly by plasma cells and subsets of mature B cells<sup>65,66,73,75,76</sup>. Mice deficient in BCMA were healthy and had a normal physical appearance<sup>77,78</sup>. BCMA-deficient mice had normal numbers of B cells, but survival of long-lived plasma cells was impaired<sup>75,77</sup>.

A critical factor for any antigen being considered as a target for immunotherapies is the antigen's expression pattern in normal tissues. BCMA has been reported to be expressed in plasma cells and in some B cells but to otherwise have limited expression<sup>65-67,76</sup>. To more completely assess the expression pattern of BCMA, we performed quantitative polymerase chain reaction (qPCR) on a panel of cDNA samples from a wide range of normal tissues (Figure 1).<sup>5</sup> As a positive control, we performed qPCR on cDNA from cells of a plasmacytoma that was resected from a

patient with advanced MM.<sup>5</sup> Ninety-three percent of the cells from the plasmacytoma sample were plasma cells as determined by flow cytometry. The BCMA expression of the plasmacytoma sample was dramatically higher than the BCMA expression of any other tissue (Figure 1). Not surprisingly, BCMA cDNA copies were detected in several hematologic tissues such as blood leukocytes, bone marrow, spleen, lymph node, and tonsil. Low levels of BCMA cDNA copies were detected in the samples of testis and trachea. In addition, low levels of BCMA cDNA copies were detected in most gastrointestinal organs such as duodenum, rectum, and stomach (Figure 1). One possible explanation for BCMA expression in gastrointestinal organs and the trachea was the known presence of plasma cells and B cells in tissues such as lamina propria and Peyer's Patches<sup>79,80</sup>.

In Figure, 1 actin cDNA copies and BCMA cDNA copies were measured by qPCR in all of the samples, and the results were expressed as the number of BCMA cDNA copies per  $10^5$  actin cDNA copies.<sup>5</sup>

**Figure 1**



After demonstrating a very restricted expression pattern of BCMA RNA, we carried out an assessment of BCMA protein expression by immunohistochemistry (IHC). As expected, our anti-BCMA IHC staining procedure yielded strong staining of BCMA-K562 cells, which expressed high levels of BCMA after being transduced with the gene encoding BCMA. We found a lack of BCMA staining with NGFR-K562 negative control cells.<sup>5</sup> We went on to evaluate BCMA protein expression in normal human organs. Except for plasma cells, we did not detect BCMA protein expression by the cells of any of the organs that we stained. We detected plasma cells expressing cell-surface BCMA in gastrointestinal organs. BCMA expression by normal plasma cells probably accounts for the low levels of BCMA RNA detected in these organs because we did not detect BCMA expression by any of the other cells in these organs. We detected BCMA-expressing plasma cells in the tonsil. The organs assessed by IHC and found to lack BCMA expression except for plasma cells are shown in Table 1.<sup>5</sup> We concluded that BCMA expression detected at low levels in some organs by qPCR (Figure 1) was due to plasma cells in these organs.

**Table 1**

**Organs stained for BCMA by immunohistochemistry and found to lack of BCMA expression except on plasma cells**

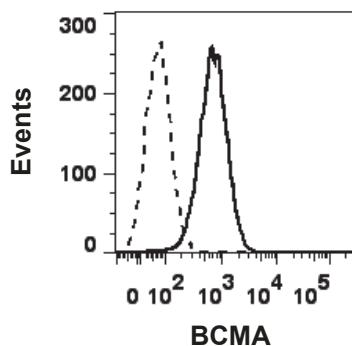
adrenal	lung
bladder	ovary
bone	pancreas
breast	parathyroid
cerebellum	pituitary
cerebral cortex	placenta
duodenum	prostate
eye	skin
fallopian tube	spinal cord
esophagus	spleen
stomach	skeletal muscle
small intestine	testis
colon	thymus
rectum	thyroid
heart	trachea
kidney	cervix
liver	uterine endometrium

For a protein to be an appropriate target for CAR-expressing T cells aimed at MM, the protein must be expressed on the surface of MM cells. By flow cytometry of bone marrow samples from

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patients with MM, we found that 6/10 tested samples uniformly expressed high levels of BCMA by flow cytometry. An example of this flow cytometry staining is shown in Figure 2.

**Figure 2**



As shown in Figure 2, flow cytometry for BCMA (solid line) and isotype-matched control staining (dashed line) revealed BCMA expression on the surface of MM cells from a plasmacytoma of a patient with MM.<sup>5</sup> The plot is gated on plasma cells, which made up 93% of the total plasmacytoma cells. In addition to the flow cytometry studies, we stained tissue sections from plasmacytomas of 3 patients with MM. In all 3 of the samples, the neoplastic plasma cells expressed cell-surface BCMA.<sup>5</sup>

#### 1.2.7 Anti-BCMA CAR development and preclinical testing

Due to the expression pattern of BCMA, we reasoned that BCMA would be an appropriate target for CAR-expressing T cells. To further assess the suitability of BCMA as a target for CAR-expressing T cells, we designed an anti-BCMA CAR. The anti-BCMA CAR contained an scFv derived from one of two mouse anti-human-BCMA monoclonal antibodies<sup>81</sup>. The anti-BCMA CAR also contained the hinge and transmembrane regions of the human CD8 $\alpha$  molecule, the signaling moiety of the CD28 costimulatory molecule, and the signaling domains of the CD3 $\zeta$  molecule (Figure 3).

**Figure 3**



We also designed a negative-control CAR named SP6. The SP6 CAR contained the variable regions of the hapten-specific SP6 monoclonal antibody<sup>39</sup>. Except for the different variable regions, the sequence of the SP6 CAR was identical to the sequence of the anti-BCMA CAR. We ligated DNA encoding each of the CARs that we designed into a gammaretroviral vector that has been used in previously in many clinical trials of T-cell gene therapies.<sup>4,24,26,32</sup> After transductions, we found high levels of cell surface expression of the anti-BCMA CAR on the transduced T cells (Figure 4).

**Figure 4**

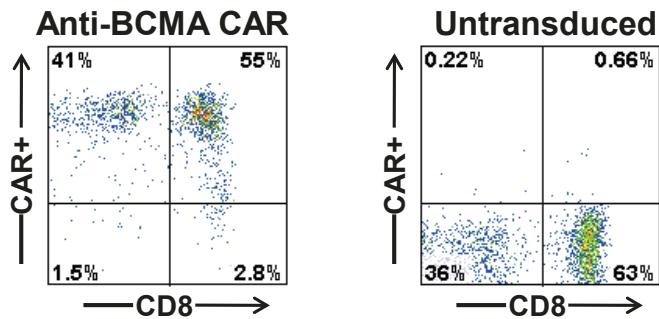


Figure 4 shows anti-BCMA CAR expression on T cells from Donor 1 6 days after transduction with gammaretroviruses encoding the anti-BCMA CAR. Transductions were carried out 2 days after the cultures were started, so the T cells had been in culture for a total of 8 days at the time of this analysis. The plots are gated on live, CD3<sup>+</sup> lymphocytes.

We also performed a series of in vitro assays to assess the function of anti-BCMA-CAR-expressing T cells, and we found that BCMA-CAR-expressing T cells exhibit BCMA-specific activities including CD107a upregulation and cytokine production in vitro. These experiments showed that anti-BCMA-CAR-expressing T cells are activated in a BCMA-specific manner.

Figure5

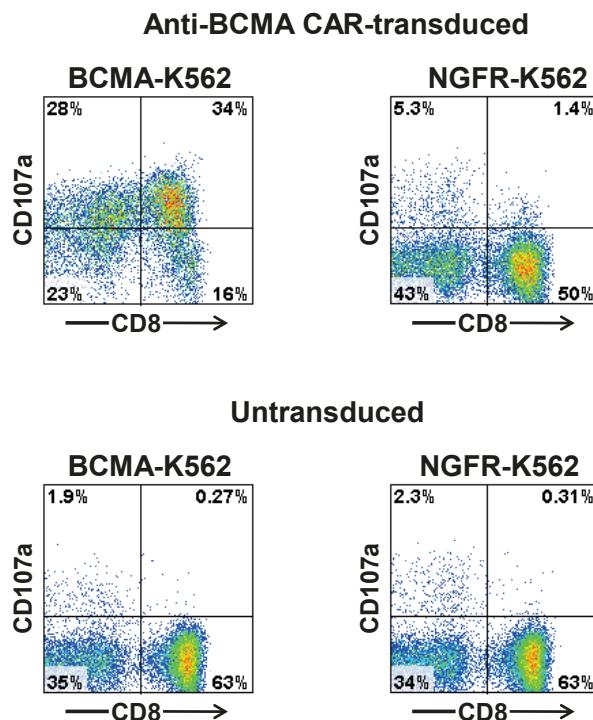
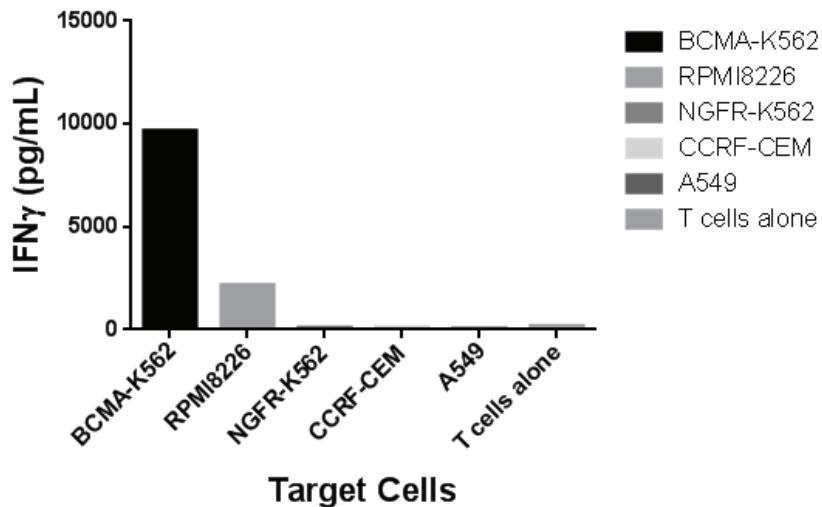


Figure 5 shows upregulation of CD107a, which indicates degranulation and correlates with cytotoxicity<sup>82</sup>, when anti-BCMA CAR-expressing T cells from Donor 1 were cultured with the BCMA-expressing cell line BCMA-K562. CD107a was not upregulated when anti-BCMA-CAR-expressing T cells from Donor 1 were cultured for 4 hours with the BCMA-negative control cell line NGFR-K562. Untransduced T cells from Donor 1 did not upregulate CD107a when cultured with either BCMA-K562 or NGFR-K562. The plots are gated on live CD3<sup>+</sup> lymphocytes. The T cells depicted in Figure 5 are from the same cultures as the cells shown in Figure 4 and were used in this experiment on the same day of culture as the cells shown in Figure 4.

T cells transduced with the anti-BCMA CAR produced large amounts of IFN $\gamma$  when they were cultured overnight with the BCMA-expressing cell lines BCMA-K562 and RPMI8226 (Figure 6). In contrast, the anti-BCMA CARs produced only background levels of IFN $\gamma$  when they were cultured with the BCMA-negative target cell lines NGFR-K562, CCRF-CEM, and A549. The anti-BCMA-CAR-transduced T cells also made minimal IFN $\gamma$  when cultured without any target cell. The experiment depicted in Figure 6 consisted of culturing the anti-BCMA-CAR-transduced T cells with the indicated target cell lines overnight and then performing a standard IFN $\gamma$  enzyme-linked immunosorbant assay (ELISA).

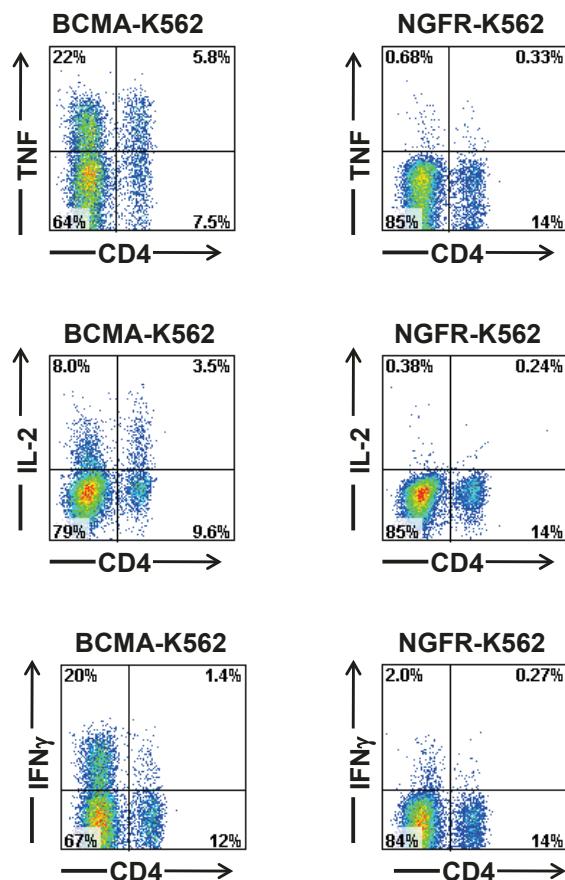
**Figure 6**



Large fractions of anti-BCMA-CAR-transduced T cells produced the cytokines IFN $\gamma$ , tumor necrosis factor (TNF), and interleukin-2 (IL-2) when cultured with the BCMA $^+$  target cell BCMA-K562 but not when cultured with the BCMA-negative cell line NGFR-K562 (Figure 7). For the assay described in Figure 7, T cells and target cells were cultured together for 6 hours, and an intracellular cytokine staining assay was performed to detect the cytokines within the T cells as described previously.<sup>5</sup> The plots shown in Figure 7 are gated on CD3 $^+$  lymphocytes.

**Figure**

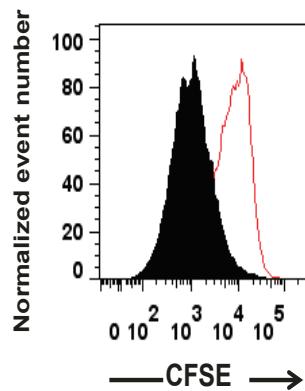
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Anti-BCMA-CAR-transduced T cells also proliferated in a BCMA-specific manner. Figure 8 shows a carboxyfluorescein diacetate,succinimidyl ester (CFSE) proliferation assay in which anti-BCMA-CAR-transduced T cells were cultured for 4 days with either BCMA-K562 cells or BCMA-negative NGFR-K562 cells. CFSE was diluted to a greater degree, indicating more proliferation, when the T cells were cultured with BCMA-K562 target cells (solid histogram in Figure 8) compared to when anti-BCMA-CAR T cells were cultured with BCMA-negative NGFR-K562 target cells. The assay was conducted as described previously.<sup>5</sup>

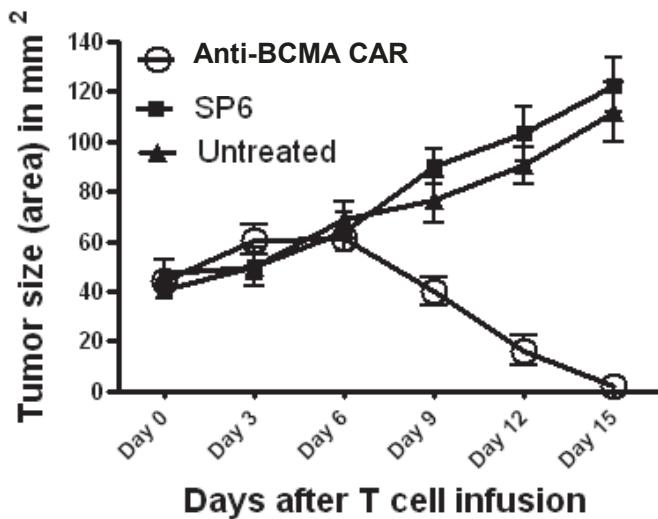
**Figure 8**

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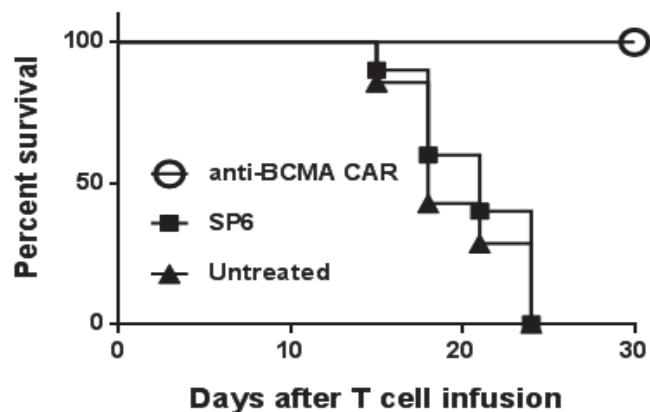
We established RPMI8226 human multiple myeloma cell line tumors in immunodeficient mice.<sup>5</sup> We allowed sizable tumors to develop over 17 to 19 days then we treated the mice with a single intravenous infusion of anti-BCMA-CAR-transduced human T cells. After infusion of anti-BCMA-CAR T cells, dramatic regressions of all tumors occurred between day 6 and day 15 after the T cell infusion, and 100% of mice receiving anti-BCMA-CAR T cells were cured (Figures 9 and 10).<sup>5</sup> In contrast, tumors continued to increase in size in all mice receiving infusions of T cells expressing a negative-control CAR designated SP6. The mice receiving infusions of anti-BCMA-CAR-transduced T cells had no signs of toxicity during this experiment.

**Figure 9**



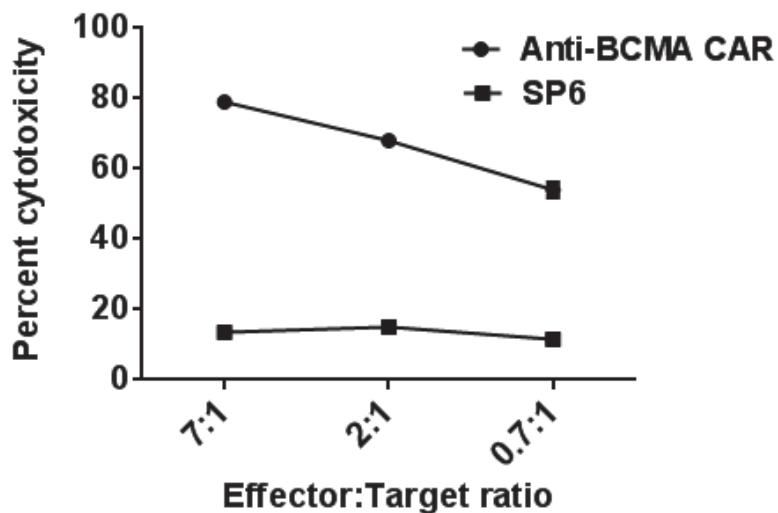
In the experiments depicted in Figures 9 and 10, anti-BCMA-CAR T cells were infused on Day 0, and no other treatments were administered. In the experiments reported in Figures 9 and 10, there were 10 mice in the anti-BCMA CAR and SP6 groups. There were 7 mice in the untreated group.

**Figure 10**



Myeloma cells from a plasmacytoma of a patient with MM were specifically killed by autologous anti-BCMA-CAR-transduced T cells at low effector: target ratios while autologous T cells transduced with the negative control CAR SP6 caused only a low level of cytotoxicity of the myeloma cells in a 4-hour cytotoxicity assay (Figure 11).<sup>5</sup> For all effector: a target ratio, the cytotoxicity was determined in duplicate, and the results are displayed as the mean +/- the standard error of the mean.

**Figure 11**



### 1.2.8 Rationale for immunosuppressive chemotherapy and selection of lymphocyte-depleting chemotherapy regimen

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We plan to administer a conditioning chemotherapy regimen of cyclophosphamide and fludarabine before infusions of anti-BCMA-expressing T cells because substantial evidence demonstrates an enhancement of the anti-malignancy activity of adoptively-transferred T cells when chemotherapy or radiotherapy are administered before the T cell infusions.<sup>6,8,83</sup> In mice, administering chemotherapy or radiotherapy prior to infusions of tumor-antigen-specific T cells dramatically enhanced the anti-tumor efficacy of the transferred T cells.<sup>6,8,25,26,83,84</sup> Administering chemotherapy or radiotherapy enhances adoptive T-cell therapy by multiple mechanisms including depletion of regulatory T cells and elevation of T-cell stimulating serum cytokines including interleukin-15 (IL-15) and interleukin-7 (IL-7), and possibly depletion of myeloid suppressor cells and other mechanisms.<sup>6,8,84,85</sup> Removal of endogenous “cytokine sinks” by depleting endogenous T cells and natural killer cells caused serum levels of important T-cell stimulating cytokines such as IL-15 and IL-7 to increase, and increases in T-cell function and anti-tumor activity were dependent on IL-15 and IL-7.<sup>6</sup> Experiments in a murine xenograft model showed that regulatory T cells could impair the anti-tumor efficacy of anti-CD19 CAR T cells.<sup>86</sup> Myeloid suppressor cells have been shown to inhibit anti-tumor responses.<sup>85</sup> Experiments with a syngeneic murine model showed that lymphocyte-depleting total body irradiation (TBI) administered prior to infusions of anti-CD19-CAR-transduced T cells was required for the T cells to cure lymphoma.<sup>7</sup> In these experiments, some mice received TBI, and other mice did not receive TBI. All mice were then challenged with lymphoma and treated with syngeneic anti-CD19-CAR T cells. Mice receiving TBI had a 100% cure rate and mice not receiving TBI had a 0% cure rate.<sup>7</sup>

Strong suggestive evidence of enhancement of the activity of adoptively-transferred T cells has been generated in humans.<sup>30,87,88</sup> Very few clinical responses have occurred and very little evidence of in vivo activity has been generated in clinical trials of anti-CD19-CAR T cells administered without lymphocyte-depleting chemotherapy.<sup>30,33</sup> In contrast, many regressions and evidence of long-term B-cell depletion have occurred in clinical trials in which patients received anti-CD19-CAR T cells after lymphocyte-depleting chemotherapy.<sup>3,4,32,51</sup> The chemotherapy regimen that best increases the anti-malignancy efficacy of CAR-expressing T cells is not known, but the most commonly used chemotherapy regimens that have been used in clinical trials and that convincingly demonstrate persistence and in vivo activity of adoptively transferred T cells have included cyclophosphamide and fludarabine.<sup>3,4,31,32,87,88</sup> Both cyclophosphamide and fludarabine are highly effective at depleting lymphocytes.<sup>87,88</sup> One well-characterized and commonly used regimen is the combination of 300 mg/m<sup>2</sup> of cyclophosphamide administered daily for 3 days and fludarabine 30 mg/m<sup>2</sup> administered daily for three days on the same days as the cyclophosphamide.<sup>89</sup> Multiple cycles of this regimen can be tolerated by heavily pretreated leukemia patients.<sup>89</sup>

### 1.2.9 Rationale for dose-escalation

The clinical trial described in this protocol is planned as a dose escalation in which the number of anti-BCMA-CAR T cells administered to patients will be increased with sequential dose levels. The rationale for conducting a dose-escalation trial of a cellular therapy is based on two main sources of evidence. First, the anti-tumor efficacy of adoptively-transferred T cell treatments increases as the dose of T cells administered to mice increases.<sup>90-92</sup> Second, in the setting of allogeneic transplantation, relapsed malignancy is often treated with infusions of allogeneic donor lymphocytes (DLIs).<sup>93,94</sup> The incidence of graft-versus-host disease, which is

caused by T cells attacking allogeneic antigens on host tissues, increases as the dose of T cells administered in DLIs increases'.<sup>93,94</sup>

#### 1.2.10 Summary of risks and potential benefits

This clinical trial is being performed to evaluate a genetically-modified T-cell therapy for multiple myeloma, which is an almost always incurable disease.<sup>12</sup> Only patients with multiple myeloma who have progressive or relapsed myeloma despite at least 3 prior lines of therapy will be enrolled. The risks of the study fall into 3 general categories. First, chemotherapy that could cause cytopenias is part of the protocol. As with any chemotherapy that causes neutropenia and thrombocytopenia, this chemotherapy could cause toxicities such as infections and bleeding. The second category of toxicity is cytokine-release-type toxicities such as high fevers, hypotension and neurological toxicities such as delirium, obtundation, myoclonus, seizures, headache, and transient focal neurological toxicities including aphasia and focal paresis. These cytokine-release-type toxicities have been detected in other clinical trials of CAR T cells during the first 2 weeks after anti-CD19 CAR T cells were infused.<sup>4,95</sup> In the anti-CD19 CAR trials, these toxicities have been transient with toxicities generally resolving within 2 days to 2 weeks. The third main category of toxicity is direct damage to normal tissues by the CAR T cells. This could happen because of unexpected expression of BCMA on normal cells or because of unexpected cross-reactivity of the anti-BCMA CAR with proteins other than BCMA *in vivo*. A potential toxicity caused by anti-BCMA CAR T cells damaging normal cells is hypogammaglobulinemia due to depletion of plasma cells and a subset of mature B cells. Hypogammaglobulinemia has been a complication for many patients on clinical trials of anti-CD19 CAR-expressing T cells.<sup>4,32</sup> Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins.<sup>4</sup>

The potential benefits to subjects enrolling on this trial include the possibility that the anti-BCMA-CAR T cells can cause a significant anti-myeloma effect. Many patients enrolled on early trials of anti-CD19 CAR T cells obtained prolonged complete remissions of advanced malignancies<sup>3,4,96</sup>, so there is a chance that recipients of anti-BCMA CAR T cells could also derive a direct benefit from participation in this trial. Patients might also derive a benefit from knowing that they are contributing to the development of new cellular therapies for cancer. Aiding in the development of new therapies might help future patients.

## 2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

### 2.1 ELIGIBILITY CRITERIA

Note: if a patient meets an eligibility requirement as outlined below, and is enrolled on the protocol but then is found to no longer meet the eligibility requirement after enrollment but before the start of protocol treatment, the treatment will be aborted.

#### 2.1.1 Inclusion Criteria

##### 2.1.1.1 Multiple Myeloma criteria

- Clear BCMA expression must be detected on greater than 50% of malignant plasma cells from either bone marrow or a plasmacytoma by flow cytometry or

immunohistochemistry. These assays must be performed at the National Institutes of Health. It is not required that the specimen used for BCMA determination comes from a sample that was obtained after the patient's most recent treatment. BCMA expression will need to be documented on the majority of malignant plasma cells at some time after the original anti-BCMA CAR T-cell infusion in all patients undergoing a second anti-BCMA CAR T-cell infusion. If paraffin embedded unstained samples of bone marrow involved with MM or a plasmacytoma are available, these can be shipped to the NIH for BCMA staining, otherwise new biopsies will need to be performed for determination of BCMA expression.

- Bone marrow plasma cells must make up 30% or less of total bone marrow cells based on a bone marrow biopsy performed within 30 days of the start of protocol treatment.
- Patients must have received at least 3 different prior treatment regimens for multiple myeloma
- Patients must have measurable MM as defined by at least one of the criteria below.
  - One or more of these abnormalities defines measurable disease:
  - Serum M-protein greater or equal to 0.4 g/dl (10 g/l).
  - Urine M-protein greater or equal to 200 mg/24 h.
  - Serum free light chain (FLC) assay: involved FLC level greater or equal to 10 mg/dl (100 mg/l) provided serum FLC ratio is abnormal.
  - A biopsy-proven plasmacytoma

#### **2.1.1.2 Other inclusion criteria:**

- Greater than or equal to 18 years of age and less than or equal to age 73.
- Able to understand and sign the Informed Consent Document.
- Clinical performance status of ECOG 0-2
- Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for four months after receiving the preparative regimen.
- Women of child bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the preparative chemotherapy on the fetus.
- Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive can have decreased immune -competence and thus are less responsive to the experimental treatment and more susceptible to its toxicities.)
- Seronegative for hepatitis B antigen, positive hepatitis B tests can be further evaluated by confirmatory tests, and if confirmatory tests are negative, the patient can be enrolled.

- Seronegative for hepatitis C antibody unless antigen negative. If hepatitis C antibody test is positive, then patients must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.
- Absolute neutrophil count greater than or equal to 1000/mm<sup>3</sup> without the support of filgrastim or other growth factors.
- Platelet count greater than or equal to 45,000/mm<sup>3</sup> without transfusion support
- Hemoglobin greater than 8.0 g/dL.
- Less than 5% plasma cells in the peripheral blood leukocytes
- Serum ALT and AST less or equal to 2.5 times the upper limit of the institutional normal.
- Serum creatinine less than or equal to 1.3 mg/dL.
- Total bilirubin less than or equal to 2.0 mg/dL, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dL.
- At least 14 days must have elapsed since any prior systemic therapy at the time the patient starts the cyclophosphamide and fludarabine conditioning regimen, and patients' toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo).
- Because this protocol requires collection of autologous blood cells by leukapheresis in order to prepare anti-BCMA-CAR T cells, systemic anti-myeloma therapy including systemic corticosteroid steroid therapy of greater than 5 mg/day of prednisone or equivalent dose of another corticosteroid are not allowed within 2 weeks prior to the required leukapheresis.
- Normal cardiac ejection fraction (greater than or equal to 50% by echocardiography) and no evidence of hemodynamically significant pericardial effusion as determined by an echocardiogram within 6 weeks of the start of the treatment protocol.
- Patients should not take corticosteroids including prednisone, dexamethasone or any other corticosteroid for any purpose at doses higher than 5 mg/day of prednisone or equivalent dose of another corticosteroid 2 weeks before apheresis and within 2 weeks prior to CAR T-cell infusion, and at any time after the CAR T cell infusion.

## 2.1.2 Exclusion criteria:

Patients on any anticoagulants except aspirin are not eligible.

- Patients that require urgent therapy due to tumor mass effects or spinal cord compression.
- Patients that have active hemolytic anemia.
- Patients with second malignancies in addition to multiple myeloma are not eligible if the second malignancy has required treatment within the past 3 years or is not in complete remission. There are two exceptions to this criterion: successfully treated non-metastatic basal cell or squamous cell skin carcinoma.
- Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the preparative chemotherapy on the fetus or infant.

- Active systemic infections (defined as infections causing fevers or requiring antimicrobial treatment), active coagulation disorders or other major uncontrolled medical illnesses of the cardiovascular, respiratory, endocrine, renal, gastrointestinal, genitourinary or immune system, history of myocardial infarction, active cardiac arrhythmias, active obstructive or restrictive pulmonary disease.
- Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).
- Systemic corticosteroid steroid therapy of greater than 5 mg/day of prednisone or equivalent dose of another corticosteroid are not allowed within 2 weeks prior to either the required leukapheresis or the initiation of the conditioning chemotherapy regimen.
- History of severe immediate hypersensitivity reaction to any of the agents used in this study.
- History of allogeneic stem cell transplantation
- Patients with CNS metastases or symptomatic CNS involvement (including cranial neuropathies or mass lesions and spinal cord compression).
- Patients with active autoimmune skin diseases such as psoriasis or other active autoimmune diseases such as rheumatoid arthritis.

## 2.2 SCREENING EVALUATION

The following assessments must be completed within 30 days prior to starting the chemotherapy conditioning regimen unless otherwise noted (if not, then the evaluation must be repeated):

- Complete history and physical examination, including, weight and vital signs, noting in detail the exact size and location of any lesions that exist.
- Confirmation of diagnosis of MM by the NCI Laboratory of Pathology and confirmation of clear BCMA expression on the majority of malignant plasma cells from either bone marrow or a plasmacytoma by flow cytometry or immunohistochemistry. The sample used for this BCMA expression analysis can come from any time prior to enrollment on the protocol.
- Bone marrow aspirate and biopsy specifically ask for BCMA immunohistochemistry staining of the bone marrow biopsy. Order cytogenetics with interphase FISH and flow cytometry on the bone marrow aspirate. Cytogenetics studies are not required if a written report of cytogenetics studies performed at any time are available from an outside institution. The bone marrow biopsy must take place at some time after the patient's most recent myeloma treatment. If the patient agrees to future use of specimens when screening bone marrow is obtained, reserve one tube of bone marrow aspirate to be sent to Fran Hakim's lab for research purposes. Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594 5339. (A bone marrow biopsy must also be performed within 30 days of starting the protocol treatment to assess for disease burden. See section **3.1.4** for details.)

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- All patients must have flow cytometry staining of bone marrow or plasmacytoma cells for BCMA by Dr. Stetler-Stevenson's lab (NCI Laboratory of Pathology).
- EKG
- MRI of spine and pelvis (only if clinically indicated in patients with back pain or pelvic pain or a history of plasmacytomas of spine or pelvic bones are present)
- MRI of the brain-within 6 weeks of protocol treatment start.
- If necessary to document progressive disease obtain a skeletal X-ray survey
- Donor venous assessment
- Antibody screen for Hepatitis B and C; HIV, HTLV-I/II, T. cruzi (Chagas agent), West Nile, and syphilis (RPR)
- Cardiac echocardiograph-within 6 weeks of protocol treatment start.
- (Sodium (Na), Potassium (K), Chloride (Cl), Total CO<sub>2</sub> (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid)
- CBC with differential and platelet count
- Serum immunofixation electrophoresis
- Serum immunoglobulin free light chains
- Glucose-6-phosphate dehydrogenase (G6PD) deficiency screening
- Thyroid panel
- Serum Cortisol
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential

## 2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) must be completed and sent via encrypted email to: NCI Central Registration Office [ncicentralregistration-1@mail.nih.gov](mailto:ncicentralregistration-1@mail.nih.gov).

After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

## 3 STUDY IMPLEMENTATION

### 3.1 STUDY DESIGN

#### 3.1.1 General study plan

*Abbreviated Title: Anti-BCMA-CAR T cells*

*Version Date: September 21, 2017*

This protocol is a phase I dose-escalation study of autologous T cells that are genetically modified to express an anti-BCMA CAR.

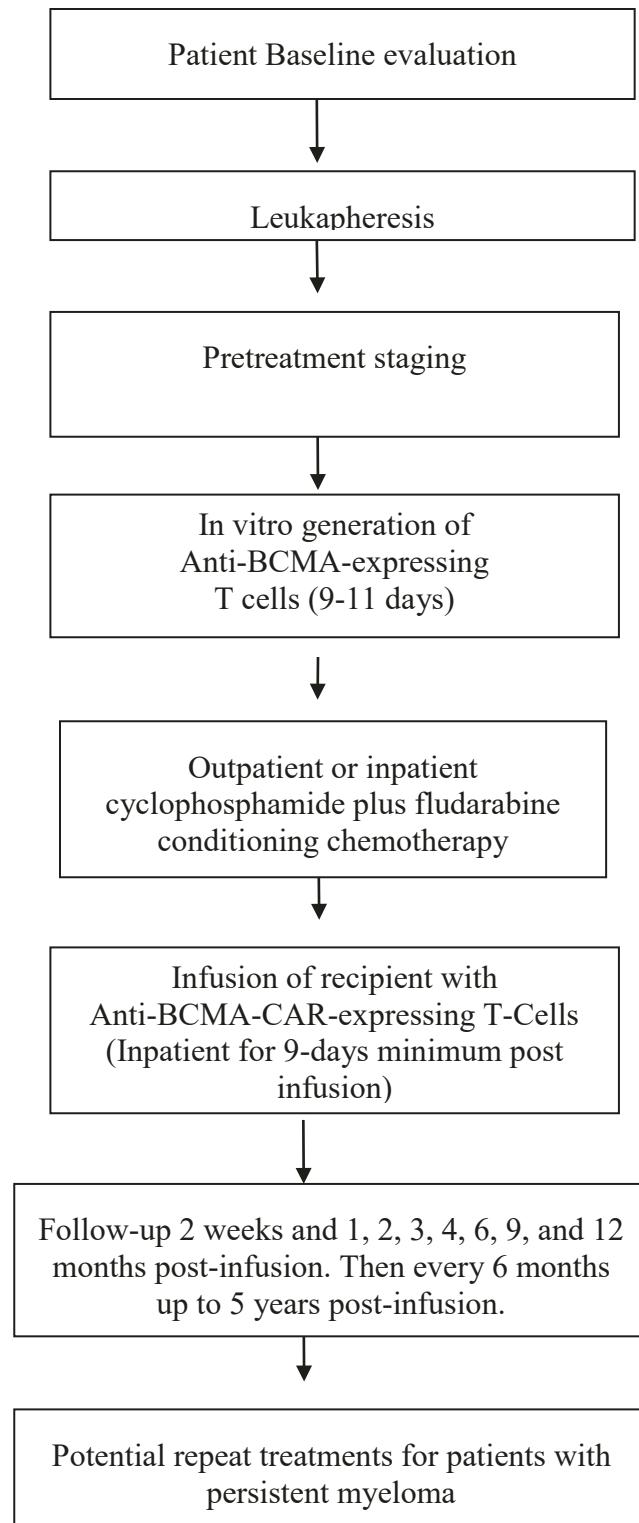
The protocol will enroll patients with multiple myeloma that is progressive or relapsed despite at least 3 prior lines of therapy. Patients will be evaluated for general health, and multiple myeloma staging will be carried out. An assessment of BCMA expression will be an important part of the eligibility screening. Patients enrolled on the study will undergo leukapheresis, and anti-BCMA-CAR-expressing T cells will be generated by transducing the patient's T cells with a gammaretrovirus encoding the anti-BCMA CAR. During the time of T-cell preparation, the patients will receive a conditioning chemotherapy regimen of cyclophosphamide 300 mg/m<sup>2</sup> daily for 3 days and fludarabine 30 mg/m<sup>2</sup> IV daily for 3 days on the same days. This is an extensively-used chemotherapy regimen that can be easily administered on an outpatient basis. Two days after the end of the conditioning chemotherapy, patients will receive a single infusion of anti-BCMA-CAR-expressing T cells. A minimum 9-day hospitalization will be required after the cell infusion to monitor closely for acute toxicities. Patients are required to stay within 60 minutes driving time from the Clinical Center until day 14 after the CAR T-cell infusion. Patients will then be evaluated for toxicity and multiple myeloma will be staged 2 weeks and 1, 2, 3, 4, 6, 9, and 12 months after the infusion.

A small number of subjects may be eligible for re-enrollment if a patient is removed from the protocol BEFORE receiving any protocol therapy, and would be required to meet all eligibility criteria at the time of re-enrollment. Patients will be assigned a new sequential study number for the re-enrollment study period. Any cryopreserved cells produced from a patient who was removed from the study can be used to treat that patient after re-enrollment. We do not anticipate changes in the risk profile for the initial versus re-enrollment.

*Abbreviated Title: Anti-BCMA-CAR T cells*

*Version Date: September 21, 2017*

### 3.1.2 Protocol schema



### 3.1.3 Dose Limiting Toxicity

Dose-limiting toxicities are defined as follows:

- Grade 3 toxicities possibly or probably related to either the anti-BCMA CAR T cells or the fludarabine and cyclophosphamide chemotherapy and lasting more than 7 days
- Grade 4 toxicities possibly or probably related to the study interventions

The following specific toxicities will not be dose-limiting toxicities:

- Neutropenia (ANC<500/ $\mu$ L) lasting 9 days or less
- Anemia (Hgb<8 g/dL) lasting 7 days or less
- Grade 4 thrombocytopenia lasting 25 days or less
- Grade 3 thrombocytopenia
- Hypotension requiring treatment with vasopressors for 72 hours or less. The 72 hours is measured from the first institution of vasopressors even if vasopressors are temporarily discontinued and then re-started..
- Fever
- All cytopenias except neutropenia, anemia, and thrombocytopenia
- Asymptomatic electrolyte disturbances regardless of grade

### 3.1.4 Dose Escalation

The trial will be a dose-escalation with 4 dose levels based on the patient's actual bodyweight. As of amendment C, the number of anti-BCMA-CAR-expressing T cells transferred for each dose level will be as shown in the Dose Escalation Schedule below. for patients in which less than 350% of total bone marrow cells are plasma cells.

Dose Escalation Schedule	
Dose Level	Dose of IND Agent *
Level 1	0.3x10 <sup>6</sup> CAR+ T cells per kg of recipient bodyweight
Level 2	1.0x10 <sup>6</sup> CAR+ T cells per kg of recipient bodyweight

Level 3	3.0x10 <sup>6</sup> CAR+ T cells per kg of recipient bodyweight
Level 4	9.0x10 <sup>6</sup> CAR+ T cells per kg of recipient bodyweight

Each dose level will include a minimum of 3 patients. There will be a minimum of 9 days between the CAR T-cell infusion of a patient and the start of the conditioning chemotherapy regimen for the next patient. This will cause a 14 day delay between sequential CAR T-cell infusions.

Patients will be enrolled sequentially; therefore, enrollment will not proceed to a higher dose level until all patients have been treated in the prior cohort. If sufficient cells cannot be grown to meet the criteria for the assigned dose level but sufficient cells can be grown to meet the requirements of one dose level lower than the assigned dose level, the patient will be enrolled in the appropriate dose level for the number of cells infused. If a DLT occurs in an additional patient entered at a lower dose due to cell growth limitations, accrual will continue at this level as described in the dose-escalation scheme below. Accrual will be halted at the higher level until the cohort at the lower level is complete as described below. If sufficient cells cannot be produced to infuse the number of cells called for by one dose level lower than the called for dose level, the treatment will be aborted. A second attempt will be made to prepare cells for the patient if the patient agrees and if the patient still meets all eligibility criteria.

Should none of the first 3 patients treated on a dose level experience a DLT accrual can start on the next higher dose level after a 14-day delay following CAR T-cell infusion of the third patient. Should 1 of 3 patients experience a dose limiting toxicity a particular dose level, three more patients would be treated at that dose level to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher level. If 1/6 patients have a DLT at a particular dose level, accrual can proceed to the next higher dose level after a 14-day delay following treatment of the 6<sup>th</sup> patient. If a level with 2 or more DLTs in 3-6 patients has been identified, 3 additional patients will be accrued at the next-lowest dose for a total of 6, in order to further characterize the safety of the maximum tolerated dose.

Starting with Amendment E, only patients with less than 30% bone marrow plasma cells documented on a bone marrow biopsy will be enrolled. We have already treated 4 patients on dose level 4 (9x10<sup>6</sup> CAR+ T cells/kg). One of 4 low-dose patients experienced a DLT on dose level 4. If 2 more patients can be treated on this dose level without a DLT, we will declare dose level 4 the maximum tolerated dose and proceed to treat 8 patients on an expansion cohort as described on page 35 of the protocol. If 2 more patients cannot be treated on Dose Level 4 without a DLT, we will hold discussions with the FDA and NCI IRB to ask to proceed with a

lower dose to be determined at that time. Regarding the expansion cohort of 8 patients, if more than 1 patient on this expansion cohort has a DLT, enrollment on the expansion cohort will stop.

The maximum tolerated dose is the dose at which a maximum of 1 of 6 patients has a DLT. After a maximum tolerated dose is defined, additional patients can be treated on this trial. Up to 8 total additional recipients can be treated after an MTD is established. If cell growth limitations preclude administration of the maximum tolerated dose, the patient will receive as many cells as possible up to the maximum tolerated dose. This patient will be replaced in the dose escalation by the next enrolled patient. If it proves to be technically impossible or impractical to achieve the higher dose levels due to cell production constraints and a maximum tolerated dose has not been reached, the highest achievable dose level will be declared the maximum feasible dose.

Dose escalation will follow the rules outlined in the Table below.

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter up to 3 patients at the next dose level
$\geq 2$	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	<p>Enter up to 3 more patients at this dose level.</p> <ul style="list-style-type: none"> <li>• If 0 of these 3 patients experience DLT, proceed to the next dose level.</li> <li>• If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. UP to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.</li> </ul>
$\leq 1$ out of 6 at highest dose level below the maximally administered dose	This is the MTD and is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

### 3.2 DOSE MODIFICATIONS/DELAY

Other Toxicity:

- Patients may be removed from further treatment if they have active infections defined as infections causing fevers or infections requiring anti-microbial therapy that arise while patients are on-study but before the CAR T-cell infusion; however such patients are eligible for treatment if they meet all eligibility criteria after the infection resolves.
- If a patient experiences a grade 3 or greater toxicity (with the exception of cytopenias including neutropenia, lymphopenia, anemia, or thrombocytopenia) while on-study before the CAR T-cell infusion, the CAR T-cell infusion must be delayed until the toxicity improves to a grade 2 or less.

### 3.3 DRUG ADMINISTRATION

#### 3.3.1 Leukapheresis

The patient will undergo a 10-15 liter leukapheresis (generally, 12 liters will be processed to target a yield of 6-10 x10<sup>9</sup> lymphocytes) in the Department of Transfusion Medicine (DTM) Dowling Apheresis Clinic according to DTM standard operating procedures. The procedure requires dual venous access, and takes approximately 3-4 hours to complete. A central line will be placed if peripheral venous access is not sufficient.

#### 3.3.2 Anti-BCMA-CAR-expressing T-cell preparation

After cells are obtained by apheresis, further cell processing to generate anti-BCMA CAR-expressing T cells will occur in the DTM according to standard operating procedures (SOPs). Either freshly-collected cells or cryopreserved cells can be used to initiate the cell-preparation process. Peripheral blood mononuclear cells will be isolated. Sufficient cells for 3 complete cell productions will be retained in the Department of Transfusion Medicine; the excess cells will be sent to the ETIB Preclinical Core for cryopreservation at 100 to 200 million PBMC per vial. Contacts in the ETIB preclinical core are Fran Hakim and Jeremy Rose (301-594-5339). The anti-CD3 monoclonal antibody OKT3 will be used to stimulate T-cell proliferation. Two and 3 days after the start of the T-cell cultures, the cells will be transduced by exposing them to a supernatant containing replication-incompetent gammaretroviruses encoding the anti-BCMA CAR. The cells will continue to proliferate in culture until the day of infusion. Anti-BCMA-CAR T cells will be infused between day 8 and day 11 of culture. Ten vials of the infused cells will be cryopreserved and stored in the ETIB preclinical core. Each vial will contain 20 million cells.

Before infusion, the percentage of T cells expressing the anti-BCMA CAR will be determined by flow cytometry, and this percentage of BCMA+ T cells will be used in calculating the total number of cells to be infused to meet the dose requirements of the dose-escalation plan described in 3.1.4. In addition to flow cytometry, further testing of the cells will take place prior to infusion to evaluate for microbial contamination, replication-competent retroviruses, and viability. Details of this testing can be found in the appropriate DTM SOPs. When a patient is no longer eligible for retreatment on this protocol due to meeting any of the off-study criteria listed in section 3.7.2, any remaining cryopreserved pretreatment PBMC collected on this protocol will be transferred from the Department of Transfusion Medicine to the Principle Investigator of this protocol for storage in the ETIB preclinical core and possible use in research.

3.3.3 Conditioning chemotherapy and anti-BCMA CAR T-cell administration-this can be either inpatient or outpatient

3.3.3.1 Overall summary of the treatment plan

Drug	Dose	Days
Cyclophosphamide	300 mg/m <sup>2</sup> IV infusion over 30 minutes	Daily x 3 doses on days -5, -4, -3
Fludarabine	30 mg/m <sup>2</sup> IV infusion over 30 minutes administered immediately following the cyclophosphamide on day -5, -4, -3	Daily x 3 doses on days -5, -4, -3
Anti-BCMA CAR T cells	Variable.	One time dose on day 0

3.3.3.2 Detailed treatment plan

Retreatment decisions will be decided on a case-by-case basis to include discussion with FDA.

Day -5, -4, and -3: Patients will receive pre-hydration with 1000 mL 0.9% sodium chloride I.V. over 1 to 3 hours.

Patients will receive anti-emetics following NIH Clinical Center guidelines, but **dexamethasone will not be administered**. One suggested regimen is ondansetron 16 to 24 mg orally on days -5, -4, and -3 before chemotherapy (I.V. ondansetron can be substituted). Patients should be provided with anti-emetics such as lorazepam and prochlorperazine to use at home.

Next on days -5, -4, and -3, cyclophosphamide at a dose of 300 mg/m<sup>2</sup> I.V. will be diluted in 100 ml 5% dextrose solution and infused over 30 minutes. After the cyclophosphamide on days -5, -4, and -3, patients will receive 30 mg/m<sup>2</sup> I.V. fludarabine in 100 mL 0.9% sodium chloride over 30 minutes. **Note: in patients with a creatinine clearance of 30- 70 ml/minute/1.73m<sup>2</sup> , the daily dose of fludarabine will be reduced by 20%.**

Following the fludarabine infusion, patients will receive 1000 mL 0.9% sodium chloride I.V. over 1-2 hours. Furosemide will be given if needed.

Days -2 and -1: No interventions except as needed for general supportive care such as anti-emetics. To minimize bladder toxicity, patients should increase normal oral fluid intake to at least 2 L/day.

Day 0: Anti-BCMA CAR T cells will be administered. Premedication for the cell infusion will be given approximately 30 minutes prior to the infusion. The premedications are acetaminophen 650 mg orally and diphenhydramine 12.5 mg IV. Cells are delivered to the patient care unit from the Department of Transfusion Medicine. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), and identification of the product and

documentation of administration are entered in the patient's chart as is done for blood banking protocols. The cells are to be infused intravenously over 20 to 30 minutes via a central line with non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping. Cells may arrive on the unit in a syringe instead of a bag. In this case, the cells can be administered to the patient by pushing the cells through a free flowing normal saline line per nursing SOP, but still must be infused over 20-30 minutes. Details of the infusion procedure are included in section **13.3**.

Days 1 to 9: Mandatory hospitalization for observation and treatment as necessary. In addition, patients are required to stay within 60 minutes driving time from the Clinical Center until day 14 after the CAR T-cell infusion.

Guidelines for dealing with toxicities that often occur after CAR T cell infusions including hypotension, fever and tachycardia are given in Appendix 5.A CBC will be obtained daily. If the absolute neutrophil count becomes less than 500/microliter, filgrastim will be initiated at a dose of 300 micrograms daily subcutaneously for patients under 70 kg in weight and at a dose of 480 micrograms daily for patients over 70 kg in weight. Filgrastim will be given daily and then discontinued as soon as the absolute neutrophil count recovers to 2000/microliter.

### 3.3.4 Potential repeat treatment

Patients obtaining any response except progressive disease are potentially eligible for a repeat treatments consisting of conditioning chemotherapy followed by an infusion of anti-BCMA CAR T cells. Retreatment will be at least 2 months after the original treatment. The dose of anti-BCMA CAR T cells administered during repeat treatments will be the dose level currently enrolling patients receiving initial CAR T-cell infusions. The repeat treatments will include the same conditioning chemotherapy as the initial treatment.

To be eligible for repeat treatments, patients must have not experienced a DLT with their first treatment. Patients must also meet the same eligibility requirements listed in Section **2.1**. BCMA expression must be documented after the first anti-BCMA CAR infusion and before the second BCMA CAR T-cell infusion. The patients must undergo screening evaluation as listed in Section **2.2** except infectious disease serology, and brain MRI are not required to be repeated unless clinically indicated. In addition, serum will be screened for human anti-mouse antibodies (HAMA) by ELISA in all patients being considered for retreatment, and patients with HAMA reactivity that first appears after an initial anti-BCMA T-cell infusion will not be eligible for re-treatment. Follow-up testing for retreatment will be the same as for the first treatment. The patient maybe re-enrolled on the the study as a new patient to allow this re-treatment and these patients will be considerd in the total sample size for this study. A maximum of 2 total treatments can be administered to any one patient, and at least 2 months must elapse between the first treatment and the second treatment.

## 3.4 PROTOCOL EVALUATION

### 3.4.1 Baseline evaluations and interventions

The following tests must be completed within 14 days of the start of the conditioning chemotherapy regimen:

- Patients must have a central venous access before the time of cell infusion. This might require placement of a non-valved P.I.C.C line or other device.

- Physical exam with vital signs and oxygen saturation
- CT scan of chest, abdomen, and pelvis or PET scan (only if necessary for staging)
- Skeletal survey. Additional scans and x-rays may be performed if clinically indicated based on patients' signs and symptoms.
- If necessary for staging: serum immunofixation electrophoresis
- 250 microgram cosyntropin stimulation test if suspicious for adrenal insufficiency based on low serum sodium or high serum potassium or hypotension or a history of adrenal insufficiency or other clinical indications
- 24-hour urine collection with immunofixation electrophoresis timed urine, and creatinine for creatinine clearance calculation
- Serum immunoglobulin free light chains
- Anti CMV antibody titer, HSV serology, and EBV panel, T cruzi serology, toxoplasmosis serology (Note: patients who are known to be positive for any of the above do not need to be retested; may be performed within 3 months of chemotherapy start date)
- Serum will be sent for measles IgG, Rubella IgG, VZV IgG, and pneumococcal antibodies (Ab) 23 serotypes
- Blood will be collected for research purposes. Draw 12 CPT tubes (8 mL each of blood will be collected prior to initiation of the conditioning chemotherapy regimen. This is a total of 96 mL of blood. Some of this blood will be used for immunology assays and some will be used for RCR assays. This blood can be collected on different days as long as a total of 12 CPT tubes are collected prior to cell initiation of the chemotherapy. Send to Dr. Fran Hakim's, Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.
- In addition to the CPT tubes, draw 16 mL of blood to obtain serum for research purposes (2 SST tubes, 8 mL per tube) within 3 days prior to the start of the chemotherapy. Send to Dr. Fran Hakim's, Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.

The following tests must be completed within 7 days of the start of the conditioning chemotherapy regimen:

- TBNK
- Sodium (Na), Potassium (K), Chloride (Cl), Total CO<sub>2</sub> (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid (to be repeated on the first day of the chemotherapy at the discretion of the PI)
- β2-microglobulin
- Serum quantitative immunoglobulins
- ABO typing

- Ionized calcium
- CBC with differential and platelet count(to be repeated on the first day of the chemotherapy at the discretion of the PI)
- PT/PTT
- Urinalysis; if results are abnormal, send for urine culture
- $\beta$ -HCG pregnancy test (serum or urine) on all women of child-bearing potential

3.4.2 Studies to be performed during the mandatory 9-day inpatient admission after cell infusion

- Vital signs including pulse oximetry will be monitored q1h x 4 hours after completion of the CAR T cell infusion and every 4 hours otherwise unless otherwise clinically indicated.
- Daily physical exam
- CBC twice a day from day 0 until day 10 with differential every 24 hours. After day 10 do a CBC with differential daily until discharge.
- TBNK on the day of CAR T-cell infusion (day 0) and day 7 after infusion
- Chemistries twice a day starting from day 0 to day 10 .After day 10 do chemistries once a day until discharge: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO<sup>2</sup> (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Uric Acid )
- Serum C-reactive peptide (CRP) within 7 days of day -5 before initiation of chemotherapy and daily while hospitalized.
- PT/PTT 7 days after cell infusion
- Other tests will be performed, as clinically indicated.
- Research blood: Every Monday, Wednesday, and Friday during hospitalization, starting on the first Monday, Wednesday, or Friday after the day of CAR T-cell infusion and lasting up to 14 days after infusion of anti-BCMA-CAR-transduced T cells, 56 mL of patient peripheral blood will be obtained (6 CPT tubes 8 mL each and 1 SST tube 8 mL). Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.

3.4.3 Post-infusion outpatient evaluation

After completion of therapy the patient will be followed for potential complications related to anti-BCMA-CAR T-cell infusion. The patient will be seen at the NIH in follow-up to evaluate disease status and late problems related to anti-BCMA –CAR T-cell infusion at days +14, +30, +60, +90, and +120; and at 6, 9, and 12 months after anti-BCMA –CAR T-cell infusion. After 12 months, the patient will be seen every 6 months up to five years. At these times patients will have the following tests performed to determine clinical response:

- 6 CPT tubes of Research Blood (48 mL) will be collected to obtain blood for immunological testing. Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.
- 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.

**Note: after the first year of follow-up, research blood will be reduced to 4 CPT tubes (32 mL total) during required protocol visits.**

- CT scan of chest, abdomen, and pelvis or PET scan at outpatient follow-up appointments starting 1 month after infusion (only if a plasmacytoma was detected before treatment)
- Physical exam with vital signs and oxygen saturation
- (Sodium (Na), Potassium (K), Chloride (Cl), Total CO<sub>2</sub> (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid)
- β2-microglobulin
- TBNK
- Blood for serum quantitative immunoglobulins
- Ionized calcium
- CBC with differential
- Urinalysis: if results are abnormal, send for urine culture
- Serum immunofixation electrophoresis
- 24-hour urine collection with immunofixation electrophoresis timed urine (can be omitted if not needed for staging after day 30. This is required for all patients pretreatment and at the day 14 and day 30 follow-up appointments). Serum immunoglobulin free light chains
- At the 2 month follow-up, a bone marrow aspirate and biopsy will be collected. Aspirate must be sent for flow cytometry to the lab of Dr. Maryalice Stetler-Stevenson. BCMA staining must be requested for the flow cytometry. BCMA immunohistochemistry should also be requested on the bone marrow biopsy. This may also be performed at day +30 if clinically indicated.
- At the day +180, day +270, and +1 year time points only, serum will be sent for measles IgG, Rubella IgG, VZV IgG, and pneumococcal antibodies (Ab) 23 serotypes
- For patients with ongoing responses a second bone marrow aspirate and biopsy will be performed at the 6-month follow-up appointment. Aspirate must be sent for flow cytometry to the lab of Dr. Maryalice Stetler-Stevenson. BCMA staining must be requested for the flow cytometry. BCMA immunohistochemistry should also be requested on the bone marrow biopsy.

*Abbreviated Title: Anti-BCMA-CAR T cells*

*Version Date: September 21, 2017*

- For each bone marrow aspirate performed, send one tube of bone marrow aspirate to Fan Hakim's lab for research purposes. Attention Jeremy Rose, Bldg 10, room 12C316 contact phone: 301-594 5339.
- Gene-therapy-specific follow-up must be carried out as described in section **3.6**

### 3.5 STUDY CALENDAR

Procedures <sup>a</sup>	Baseline/ Screening/ Pre-cell infusion / Day 0	Follow up				Every 6 month after day 365(+/- 30 days) up to 5 years
		Day+14 and Day+30 (+/- 5 days)	Day +60 (+/- 7 days)	Day +90 (+/- 7 days)	Day +120 (+/- 10 days)	
<b>Clinical Assessments</b>						
<i>History and PE</i>	X	X	X	X	X	X
<i>Vital signs, O2 saturation</i>	X	X	X	X	X	X
<i>Height, weight</i>	X	X				
<i>Performance Score</i>	X	X				
<b>Laboratory Assessments</b>						
<i>CBC with differential</i>	X	X	X	X	X	X
<i>Sodium, Potassium Chloride, CO2, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Inorganic Phosphorus, Alkaline Phosphatase, ALT, AST, T. Bilirubin, D. Bilirubin, LDH, Total Protein, CK, Uric Acid</i>	X	X	X	X	X	X
<i>Serum C-reactive peptide PT/PTT</i>	X <sup>h</sup>	X <sup>m</sup>	X <sup>m</sup>			
<i>Ionized calcium</i>	X <sup>h</sup>		X	X	X	X
<i>Thyroid panel</i>	X <sup>K</sup>					

Procedures <sup>a</sup>	Baseline Screening	Pre-cell infusion / Day 0	Follow up			
			Day+14 and Day+30 (+/- 5 days)	Day +60 (+/- 7 days)	Day +90 (+/- 7 days)	Day +120 (+/- 10 days)
<i>β2-microglobulin</i>	X <sup>h</sup>		X	X	X	X
<i>Quantitative immunoglobulins</i>	X <sup>h</sup>		X	X	X	X
<i>Anti-CMV, HSV, EBV, t.cruzi,toxoplasma</i>	X <sup>h,j</sup>					
Antibody screen for Hepatitis B and C; HIV, HTLV-I/II, T. cruzi (Chagas agent), West Nile, and syphilis (RPR)	X					
<i>TBNK</i>	X <sup>h</sup>	X	X	X	X	X
<i>G6PD</i>	X <sup>K</sup>					
<i>Urimalysis (culture prn)</i>	X		X	X	X	X
<i>24 hr urine with immunofixation electrophoresis timed urine</i>	X <sup>h</sup>		X	X <sup>f</sup>	X <sup>f</sup>	X <sup>f</sup>
<i>24 hr urine Creatinine Clearance</i>	X <sup>h</sup>					
<i>ABO typing</i>	X <sup>h</sup>					
<i>Serum immunoglobulin free light chains</i>	X		X	X	X	X

Procedures <sup>a</sup>	Pre-cell infusion / Day 0	Follow up				Every 6 month after day 365 (+/- 30 days) up to 5 years
		Day +14 and Day +30 (+/- 5 days)	Day +60 (+/- 7 days)	Day +90 (+/- 7 days)	Day +120 (+/- 10 days)	
<i>Serum immunofixation electrophoresis</i>	X		X	X	X	X
<i>Radiological Assessments</i>						
<i>Skeletal Survey</i>	X		X <sup>i</sup>	X	X	X
<i>CT chest, abdomen, pelvis<sup>f</sup></i>	X <sup>h</sup>		X <sup>i</sup>	X	X	X
<i>PET<sup>f</sup></i>	X <sup>h</sup>		X <sup>i</sup>	X	X	X
<i>Brain MRI</i>	X					
<i>Spine and Pelvis MRI<sup>e</sup></i>	X					
<i>Other Specific Assessments</i>						
<i>Serum Cortisol</i>	X <sup>k</sup>					
<i>250 microgram cosyntropin test<sup>c</sup></i>	X <sup>h</sup>					
<i>EKG, echocardiogram</i>	X					
<i>RCR</i>	X				X	X <sup>g</sup>
<i>Central Venous catheter placement</i>						
<i>Donor Venous Assessment</i>						
<i>Response Evaluation</i>						
<i>Bone marrow aspirate/biopsy<sup>d</sup></i>	X		X <sup>l</sup>	X	X <sup>e</sup>	

Procedures <sup>a</sup>	Pre-cell infusion / Day 0	Day+14 and Day+30			Day+90			Day+120			Day+180			Day+270			Day+365			Every 6 month after day 365 (+/- 30 days) up to 5 years
		Day +7 <sup>b</sup>	Day +60 (+/- 7 days)	Day +30 (+/- 5 days)	Day +7 <sup>c</sup>	Day +10 (+/- 7 days)	Day +10 (+/- 5 days)	Day +14 (+/- 10 days)	Day +14 (+/- 10 days)	Day +14 (+/- 14 days)	Day +14 (+/- 10 days)	Day +14 (+/- 14 days)	Day +14 (+/- 10 days)	Day +14 (+/- 14 days)						
<i>Research Blood</i>		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
<i>Adverse Events</i>		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
<i>Concomitant Medications</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
<i>Measles IgG, Rubella IgG, VZV IgG, Pneumococcal antibodies 23 serotypes</i>		X <sup>d</sup>																		

<sup>a</sup> see section 2.2 and section 3.3.1 for details

<sup>b</sup> see section 3.4.2 for details of testing during hospitalization

<sup>c</sup> only if cortisol<18 mg/dL or clinical suspicion of adrenal insufficiency

<sup>d</sup> bone marrow biopsy will include flow cytometry, BCMA expression, cytogenetics, amyloid staining, and molecular studies of each sample.

<sup>e</sup> only if clinically indicated

<sup>f</sup> if useful for staging

<sup>g</sup> only at 2, 3, 4, 5 year point, see section 3.6 for details

<sup>h</sup> only at baseline

<sup>i</sup> only D+30, no CTs or PETs indicated at D+14

<sup>j</sup> Patients who are known to be positive for any of these tests do not need to be retested; may be performed within 3 months of chemotherapy start date

<sup>k</sup> only at Screening

<sup>l</sup> only Day 30 if clinically indicated

<sup>m</sup> Done Daily during hospitalization

### **3.6 GENE-THERAPY-SPECIFIC FOLLOW-UP**

- Long-term follow up of patients receiving gene transfer is required by the FDA and must continue even after the patient comes off the study. Physical examinations will be performed and documented annually for 5 years following cell infusion to evaluate long-term safety. After 5 years, health status data will be obtained from surviving patients via telephone contact or mailed questionnaires for 10 additional years for a total of 15 years after cell infusion. Blood will need to be collected at least annually for long-term follow-up of gene therapy.
- Persistence of CAR transduced cells will be assessed by quantitative PCR and/or flow cytometry at 1, 2, 3, 4, 6 and 12 months after cell infusion, or until CAR-expressing cells are no longer detectable. If any patient shows a high level of persistence of CAR gene transduced cells or an increasing population of CAR gene transduced T cells at month 6 or later (by FACS staining or qPCR), the previously archived samples will be subjected to techniques that would allow the identification of predominant clonal populations of transduced cells.
- Patients' blood samples will be obtained and undergo analysis for detection of replication competent retroviruses (RCR) by PCR prior to cell infusion and at 3, 6, and 12 months post cell administration. Blood samples will be archived annually thereafter if all previous testing has been negative with a brief clinical history.

### **3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA**

Prior to documenting removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

#### **3.7.1 Criteria for removal from protocol therapy**

Note that the treatment consists of a conditioning chemotherapy regimen followed by a T-cell infusion, so off-treatment criteria mainly apply to eligibility for potential repeat treatments and cancellation of cell infusion for toxicity arising during the conditioning chemotherapy. Patients will be taken off treatment for the following:

- Greater than grade 3 toxicity attributable to anti-BCMA CAR T cells makes patients ineligible for repeat treatments.
- The patient no longer meets the eligibility criteria for the protocol after enrolling but before start of the chemotherapy conditioning regimen. If the reason that the patient is not eligible can be rapidly resolved within 2 weeks, the treatment can proceed if not, the patient will be off-study.
- The patient receives any other treatment for multiple myeloma except bisphosphonates and repeat treatment on this protocol. Bisphosphonates are allowed when administered to prevent osteopenia more than 2 months after the anti-BCMA-CAR T-cell infusion. If a patient receives corticosteroids in doses greater than 5 mg/day of prednisone or an equivalent dose of another corticosteroid, the patient will be off-treatment.

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- General or specific changes in the patient's condition render the patient unacceptable for further treatment on this study in the judgment of the investigator.
- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Positive pregnancy test

### 3.7.2 Off-Study Criteria

Patients will be taken off study for the following:

- The patient voluntarily withdraws
- There is significant patient noncompliance
- Death
- PI decision to end this study
- The patient no longer meets the eligibility criteria for the protocol after enrolling but before start of the chemotherapy conditioning regimen.
- Development of progressive or relapsed multiple myeloma after anti-BCMA CAR T cell infusion in patients not desiring or not eligible for re-treatment on this protocol.
- The patient receives any anti-myeloma therapy except bisphosphonates. Bisphosphonates are allowed 2 months or more after the CAR T-cell infusion.
- Taking corticosteroids for any reason after CAR T-cell infusion in a total cumulative dose of more than 30 mg of prednisone or equivalent

Note: Patients must be followed until all adverse events have resolved to grade 2 or less with the exception of lymphopenia and alopecia. If an adverse event is not expected to resolve to grade 2 or less this will be noted in the patient medical record and the patient will be taken off study. In addition, all patients must be followed for the gene therapy specific follow up as outlined in section [3.6](#) prior to being removed from study.

### 3.7.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Update Form from the web site(<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-1@mail.nih.gov.

## 4 CONCOMITANT MEDICATIONS/MEASURES

### 4.1 ANTIBIOTIC PROPHYLAXIS

- Patients with a CD4 T-cell count less than 200 will be maintained on pneumocystis prophylaxis with trimethoprim-sulfamethoxazole 1 double-strength tablet every Monday-

Wednesday-Friday. If patients cannot tolerate trimethoprim sulfamethoxazole, an alternative pneumocystis prophylaxis will be used.

- Patients with serum IgG level less than 400 mg/dL will receive intravenous immunoglobulin replacement as needed to maintain an IgG level above 400 mg/dL, if the patient has serious bacterial infections. An example of an intravenous immunoglobulin infusion to be used for this purpose would be Gammunex 500 mg/kg given as a single dose. Intravenous immunoglobulin infusions should be preceded by premedication with diphenhydramine and acetaminophen, and rate of infusion should be started at low rates and escalated in a step-wise manner.
- Empiric Bacterial Antibiotics
- Neutropenic patients will start on broad spectrum antibiotics with a first fever of 38.30C or greater or two fevers of 38.0 separated by at least 1 hour and concomitant ANC < 500/ml.
- Aminoglycosides will be avoided unless clear evidence of sepsis.

#### **4.2 BLOOD PRODUCT SUPPORT**

- Leukocyte filters will be utilized for all blood and platelet transfusions with the exception of the CAR-transduced T cell infusions to decrease sensitization to transfused WBC and decrease the risk of CMV infection.
- Patients who are seronegative for CMV should receive CMV-negative blood products whenever possible.
- Using daily CBC's as a guide, the patient will receive platelets and packed red blood cells (PRBC's) as needed. Attempts will be made to keep Hgb >8.0 gm/dl, and platelets >10,000/mm<sup>3</sup>. All blood products with the exception of the CAR-transduced T cells will be irradiated. Leukocyte filters will be utilized for all PRBC and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection.

#### **4.3 ANTI-EMETICS**

Anti-emetics will follow NIH Clinical Center Guidelines (except that corticosteroids will be avoided).

#### **4.4 GRANULOCYTE COLONY-STIMULATING FACTOR**

A CBC will be obtained daily while the patient is inpatient. If the absolute neutrophil count becomes less than 500/microliter, Filgrastim will be initiated at a dose of 300 micrograms daily for patients under 70 kg in weight and a dose of 480 micrograms daily for patients over 70 kg in weight only in patients with absolute neutrophil counts less than 500/microliter. Filgrastim will be discontinued as soon as the absolute neutrophil count recovers to 1500/microliter.

#### **4.5 AVOIDANCE OF CORTICOSTEROIDS**

Patients should not take corticosteroids including prednisone, dexamethasone or any other corticosteroid at a dose equivalent to 5 mg/day or more of prednisone for any purpose without approval of the Principle Investigator.

## 4.6 GUIDELINES FOR MANAGEMENT OF COMMON ACUTE TOXICITIES THAT OCCUR AFTER CAR T CELL INFUSIONS

These are guidelines only. It is understood that treatment of these toxicities must be individualized for each patient. Not following the exact recommendations in Section 13.3 is not a protocol deviation.

## 5 BIOSPECIMEN COLLECTION

Bio specimen collection on this protocol will consist of blood draws and acquisition of bone marrow aspirates and possible biopsies of plasmacytomas for research purposes.

### 5.1 CORRELATIVE STUDIES FOR RESEARCH

#### 5.1.1 Biospecimen collection before the start of the conditioning chemotherapy:

- One heparinized syringe containing 5 to 8 mL of bone marrow aspirate to be sent to Fran Hakim's lab. Attention Jeremy Rose, Bldg 10, room 12C316 contact phone: 301-594-5339. It will be used in functional assays to see if anti-BCMA-CAR T cells can recognize the patient's multiple myeloma cells.
- Blood will be collected for research purposes. A total of 12 CPT tubes (8 mL each of blood will be collected prior to initiation of the conditioning chemotherapy regimen. This is a total of 96 mL of blood. Some of this blood will be used for immunology assays and some will be used for RCR assays. This blood can be collected on different days as long as a total of 12 CPT tubes are collected prior to the start of the chemotherapy and within 14 days of the start of the chemotherapy. Send to Dr. Fran Hakim's, Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.
- 16 mL of blood will be drawn to obtain serum for research purposes (2 SST tubes, 8 mL per tube) within 14 days prior to the start of the chemotherapy. Send to Dr. Fran Hakim's, Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.
- An apheresis is required to obtain cells used to prepare the anti-BCMA CAR T cells that are administered on this protocol. After sufficient cells are processed for all possible clinical needs, the left over apheresis cells can be cryopreserved for research use. Note that these cells would simply be discarded if not used for research.
- Specimens will be cryopreserved and assays will be performed retrospectively.

#### 5.1.2 Biospecimen collection after anti-BCMA-CAR T-cell infusion during the required hospitalization

Every Monday, Wednesday, and Friday during hospitalization, starting on the first Monday, Wednesday, or Friday after the CAR T-cell infusion and lasting up until 14 days after infusion of anti-BCMA-CAR-transduced T cells, 56 mL of patient peripheral blood will be obtained (6 CPT tubes 8 mL each and 1 SST tube 8 mL). Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.

Additional research blood may be collected at any time during the clinical course at the discretion of the PI (within the volume restriction limits) allowing for the research studies already outlined in this protocol to be performed at the time of an unanticipated clinical event, if necessary to address the objectives of the study.

#### 5.1.3 Biospecimen collection during outpatient follow-up

- Patients will return for outpatient follow-up clinic visits 2 weeks, 1 month, 2 months, 3 months, 4 months, 6 months, 9 months and 12 months after the anti-BCMA- CAR T-cell infusion. After the 12-month follow-up appointment patients will return for follow-up every 6 months. The specimens listed below will be performed at each outpatient clinic visit during the first year of follow up.
  - 6 CPT tubes of Research Blood (48 mL) will be collected to obtain blood for immunological testing. Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.
  - 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. Attention Jeremy Rose, Bldg 10, room 12C316 contact phone: 301-594-5339.

After 1 year research blood collected will be reduced to 4 CPT tubes at each visit.

- At the 2 month follow-up only, bone marrow aspirate and biopsy will be performed and the aspirate will be cryopreserved.
- Only for patients with ongoing responses, a second bone marrow aspirate and biopsy will be performed at the 6-month follow-up appointment, and the aspirate will be cryopreserved.

#### 5.1.4 Immunological Testing

- T-cell assays: Direct immunological monitoring will consist of quantifying CD3+ T cells that express the anti-BCMA CAR by quantitative PCR, and/or by flow cytometry. These assays will be performed to measure the persistence and estimate the proliferation of the infused CAR+ T cells. A quantitative PCR assay or a flow cytometry assay will be used to quantitate CAR+ T cells at all post-infusion time-points up to at least 3 to 6 months after infusion, and CAR+ T cell analysis will continue until the CAR+ T cell level drops to undetectable levels unless a stable low level of CAR+ T cells is present at more than a year after infusion. The absolute number of CAR+ PBMC will be estimated by multiplying the percentage of CAR+ PBMC by the absolute number of lymphocytes plus monocytes per microliter of blood. Ex vivo immunological assays will be used to measure the BCMA-specific functional activity of the CAR+ T cells and will consist of assays such as intracellular cytokine staining and anti-CD107a degranulation assays. Immunological assays will be standardized by the inclusion of pre-infusion recipient PBMC and in some cases an aliquot of the engineered T cells cryopreserved at the time of infusion.
- Serum cytokine levels will also be measured by enzyme-linked immune sorbent assays.

- Gene expression studies will be performed on patient multiple myeloma cells and on the infusion CAR T cells of each patient. Methods used will be either Nanostring and/or RNAseq (RNA sequencing).
- Patients' blood samples will be obtained and undergo analysis for detection of replication competent retroviruses (RCR) by PCR **prior to cell infusion and at 3 and 6 months, and at one year post cell administration**. Blood samples will be archived annually thereafter if all previous testing has been negative with a brief clinical history. If a patient dies or develops neoplasms during this trial, efforts will be made to assay a biopsy sample for RCR. If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up will be undertaken, in consultation with the FDA. RCR PCR assays detect the Gibbon Ape Leukemia Virus (GALV) envelop gene and are performed under contract by the National Gene Vector Laboratory at Indiana University. The results of these tests are maintained by the contractor performing the RCR tests and by the ETIB research team.
- Due to nature of these studies, it is expected that expansion of specific T-cell clones will be observed as T-cell proliferate in response to the targeted antigen. Therefore, care will be taken to track T-cell persistence, but presence of an oligoclonal T cell population does not indicate an insertional mutagenesis event. If any patient shows a high level of persistence of CAR gene transduced cells or an increasing population of CAR gene transduced T cells at month 6 or later (by FACS staining or qPCR), the previously archived samples will be subjected to techniques that would allow the identification of predominant clonal populations of transduced cells. Such techniques may include T cell cloning or LAM-PCR. If a predominant or monoclonal T cell clone derived from CAR gene transduced cells is identified during the follow-up, the integration site and sequence will be identified and subsequently analyzed against human genome database to determine whether the sequences are associated with any known human cancers. If a predominant integration site is observed, the T cell cloning or LAM-PCR test will be used at an interval of no more than three months after the first observation to see if the clone persists or is transient. In all instances where monoclonality is persistent and particularly in instances where there is expansion of the clone, regardless of whether or not the sequence is known to be associated with a known human cancer, the subject should be monitored closely for signs of malignancy, so that treatment, if available, may be initiated early.

### 5.1.5 Additional biopsies and additional blood draws

Patients might be asked to undergo biopsies or additional blood draws for research purposes. Additional blood draws might be necessary to investigate T cell responses and serum cytokine levels in cases of clinical events such as rapid regressions of malignancy or toxicity. These research biopsies are optional and patients can participate in this trial whether or not they agree to undergo biopsies for research purposes. These biopsies will only be performed if minimal morbidity is expected based on the procedure performed and the granulocyte and platelet count. Biopsy tissue will be processed in the NIH Department of Pathology. Studies will be performed to evaluate the antigen expression by the tumor and to evaluate the reactivity of lymphocyte from these biopsies. In addition the presence of transduced cells may be quantitated.

### 5.1.6 Future studies

Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study the patient provides consent for this. However, this research may only be done if the risks of the new questions were covered in the consent document. If new risks are associated with the research (e.g. analysis of germ line genetic mutations) a protocol amendment will be required and informed consent will be obtained from all research subjects to whom these new studies and risks pertain.

## 5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

### 5.2.1 Storage/Tracking in the Preclinical Development and Clinical Monitoring Facility (PDCMF)

- Samples will be ordered in CRIS and tracked through Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.
- Patient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the Experimental Transplantation and Immunology Branch, may be archived by the ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF). All data associated with archived clinical research samples is entered into the ETIB PDCMF's Microsoft Excel databases on frozen cells and serum. These databases are stored on the NCI group drive in the ETIB 'PRECLINSERVICE' folder. Access to this folder is limited to PDCMF staff and ETIB clinical staff, requiring individual login and password. All staff in the PDCMF laboratory receive annually updated NIH/CIT training and maintain standards of computer security.
- The data recorded for each sample includes the patient ID, trial name/protocol number, date drawn, treatment cycle/post-transplant time point, cell source (e.g. peripheral blood, marrow,) as well as box and freezer location. Patient demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI/ETIB clinical records. As of January 2007, all newly received samples receive a unique bar code number, which is included in the sample record in the PDCMF database. Only this bar code is recorded on the sample vial and the vials will not be traceable back to patients without authorized access to the PDCMF database. All non-coded samples previously archived will be stripped of identifiers prior to distribution for any use other than as a primary objective of the protocol under which they were collected.
- Samples are stored in freezers. . All samples will be labeled solely with a bar code (which includes the date, and serially determined individual sample identifier). The key will be available to a restricted number of ETIB investigators and associate investigators on the protocol. Coded samples will be stored frozen at -20°, -80° or liquid nitrogen vapor phase to -180 C according to the stability requirements in a single location under the restricted control of the PDCM Facility of ETIB.
- Access to samples from a protocol for research purposes will be by permission of the Principal Investigator of that protocol in order to be used (1) for research purposes associated with protocol objectives for which the samples were collected, or (2) for a new

research activity following submission and IRB approval of a new protocol and consent, or (3) for use only as unlinked or coded samples under the OHSRP Exemption Form guidelines stipulating that the activity is exempt from IRB review. Unused samples must be returned to the PDCMF laboratory. Samples, and associated data, will be stored permanently unless the patient withdraws consent. If researchers have samples remaining once they have completed all studies associated with the protocol, they must be returned to the PDCMF laboratory.

- These freezers are located onsite at the Preclinical Service laboratory (12C216) (-85° freezer) or in ETIB common equipment space (CRC/3-3273).

### 5.2.2 Protocol Completion/Sample Destruction

- Once research objectives for the protocol are achieved, researchers can request access to remaining samples, providing they have both approval of the Principal Investigator of the original protocol under which the samples or data were collected and either an IRB approved protocol and patient consent or an OHSRP exemption indicating that the activity is exempt from IRB review.
- The PDCMF staff will report to the Principal Investigators any destroyed samples, if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container), lost in transit between facilities or misplaced by a researcher.
- The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

### 5.2.3 Samples for Genetic/Genomic Analysis

Samples will use for gene expression analysis will be RNAseq and/or Nanostring<sup>TM</sup>. These studies will be used to determine gene expression in multiple myeloma cells and infusion CAR T cells. The purpose of these studies is to assess gene expression at the RNA level not to study germline mutations.

#### 5.2.3.1 Description of the scope of genetic/genomic analysis

RNAseq and/or Nanostring<sup>TM</sup> will be used to determine gene expression in multiple myeloma cells and infusion CAR T cells. The purpose of these studies is to assess gene expression at the RNA level not to study germline mutations. One purpose of these studies is to determine if different levels of gene expression in malignant cells are associated with response to CAR T-cell therapy. Another purpose of these studies is to determine if different levels of gene expression in infusion CAR T cells are associated with anti-malignancy responses caused by CAR T cells or persistence of CAR T cells.

### 5.2.3.2 Certificate of Confidentiality

As part of study efforts to provide confidentiality of subject information, this study has obtained a Certificate of Confidentiality, which helps to protect personally identifiable research information. The Certificate of Confidentiality allows investigators on this trial to refuse to disclose identifying information related to the research participants, should such disclosure have adverse consequences for subjects or damage their financial standing, employability, insurability or reputation. The informed consent includes the appropriate coverage and restrictions of the Certificate of Confidentiality.

### 5.2.3.3 Management of Results

The analyses that we perform in our laboratory are for research purposes only; they are not nearly as sensitive as the tests that are performed in a laboratory that is certified to perform genetic testing. Changes that we observe unrelated to our research may or may not be valid. Therefore, we do not plan to inform participants of the results of testing on the tissue and blood that is performed in our research lab. However, subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: <https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists>) Subjects who still remain on the study will be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory. The CLIA testing may be funded by the PI or the CCR. If the research findings are verified in the CLIA certified lab, the subject will be referred to the NCI Genetics Branch for genetic counseling on the implications of the results. Subjects that do not wish to return to the NCI will be referred to a local genetics health care provider (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

Note: Subjects must remain on the study in order to maintain up to date contact information and to have results returned. Subjects opting for voluntary withdrawal from the study are to be informed at the time that results cannot be returned once this has been done.

## 6 DATA COLLECTION AND EVALUATION

### 6.1 DATA COLLECTION

Data will be prospectively collected and entered in real time into the Cancer Center Clinical Data System database (NCI C3D database) (information at <http://ccrtrials.nci.nih.gov>). It is expected that clinical data be entered into C3D no later than after 10 business days of the occurrence. The NCI PI and research nurse will have access to these data via web access.

The medical record will maintain complete records on each patient including any pertinent supplementary information obtained from outside laboratories, outside hospitals, radiology reports, laboratory reports, or other patient records. The NCI C3D will serve as the primary source from which all research analyses will be performed.

Data collection will include the eligibility criteria checklist, patient history, specialty forms for pathology, radiology, toxicity monitoring, and relapse data and an off-study summary sheet, including a final assessment by the treating physician. After patients are seen in clinic at each scheduled follow up, the database will be updated in real-time.

All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

**End of study procedures:** Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

**Loss or destruction of data:** Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

#### 6.1.1 Adverse event recording:

- Grade 1 adverse events will not be recorded.
- Grade 2 adverse events that will be recorded:
  - a. Unexpected events that are possibly, probably, or definitely related to the research.
  - b. Expected events that are probably or definitely related to the study interventions will be recorded only for the first year after the infusion..
  - c. All Infections proven by culture, PCR, antigen detection or other laboratory methods will be recorded for the first year after infusion regardless of attribution.
  - d. Any serious events that are deemed clinically significant by the PI
- All grade 3, 4, and 5 adverse events will be recorded regardless of attribution.

#### 6.2 GENOMIC DATA SHARING PLAN

Unlinked genomic data will be deposited in the database of genotypes and phenotypes (dbGaP) in compliance with the NIH Genomic Data Sharing Policy.

#### 6.3 RESPONSE CRITERIA

Responses will be categorized by using the International Uniform Response Criteria for Multiple myeloma.<sup>15</sup> Multiple myeloma staging will be conducted at the 2 week follow-up appointment and at each subsequent follow-up appointment. The appropriate staging studies will need to be determined for each patient because of the variability in multiple myeloma.

##### 6.3.1 Important Considerations on response criteria

- Response criteria for all categories and subcategories of response except CR and sCR are applicable only to patients who have ‘measurable’ disease by at least one of the three measurements as defined below
- All responses must be confirmed to be stable in two evaluations made at any time.

###### 6.3.1.1 Definition of measurable disease

Any one or combination of these abnormalities defines measurable disease:

- Before amendment E: Serum M-protein greater or equal to 1 g/dl (10 g/l).
- After amendment E: Serum M-protein greater or equal to 0.4 g/dl (10 g/l).
- Urine M-protein greater or equal to 200 mg/24 h.
- Serum FLC assay: involved FLC level greater or equal to 10 mg/dl (100 mg/l) provided serum FLC ratio is abnormal.
- Presence of a biopsy-proven plasmacytoma

#### 6.3.1.2 Laboratory tests for measurement of M-protein

- Serum M-protein level is quantitated using densitometry on SPEP except in cases where the SPEP is felt to be unreliable such as in patients with IgA monoclonal proteins migrating in the beta region. If SPEP is not available or felt to be unreliable (e.g., in some cases of IgA myeloma) for routine M-protein quantitation during therapy, then quantitative immunoglobulin levels on nephelometry or turbidometry can be accepted. However, this must be explicitly reported, and only nephelometry can be used for that patient to assess response and SPEP and nephelometric values cannot be used interchangeably.
- Urine M-protein measurement is estimated using 24-h UPEP only. (Random or 24 h urine tests measuring kappa and lambda light chain levels are not reliable and are not recommended)

#### 6.3.1.3 Suggested follow-up to meet response criteria

- Patients with measurable disease by both SPEP and UPEP need to be followed by both SPEP and UPEP for response assessment and categorization;
- Except for assessment of sCR, CR, and VGPR, patients with “measurable disease” restricted to the SPEP will need to be followed routinely only by SPEP;
- Patients with “measurable disease” restricted to the UPEP will need to be followed routinely only by UPEP;
- Patients with “measurable disease” in either SPEP or UPEP or both will be assessed for response only based on these two tests and not by the FLC assay;
- FLC response criteria are only applicable to patients without measurable disease in the serum or urine, and to fulfill the requirements of the category of sCR;
- Bone marrow is required only for categorization of CR
- For good clinical practice patients should be periodically screened for light chain escape with UPEP or serum FLC assay.
- Plasmacytomas can be staged with CT scans

#### 6.3.2 International Myeloma Working Group uniform response criteria:

#### 6.3.2.1 Stringent Complete Remission (sCR)

- CR as defined below plus
  - Normal FLC ratio and
  - Absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence or flow cytometry (only 1 bone marrow evaluation is needed).

#### 6.3.2.2 Complete Remission (CR)

- Negative immunofixation on the serum and urine **and**
- Disappearance of any soft tissue plasmacytomas **and**
- 5% or less plasma cells in bone marrow (only 1 bone marrow evaluation is needed)
- No evidence of progressive or new bone lesions if radiographic studies were performed  
(X-Rays not required in absence of clinical indication)

Comments: To be considered a CR,

- **Both** serum and urine immunofixation must be carried out and be negative regardless of the size of baseline M-protein in the serum or urine;
- Patients with negative UPEP values pretreatment still require UPEP testing to confirm CR and exclude light chain or Bence-Jones escape

#### 6.3.2.3 Very Good Partial Remission (VGPR)

- Serum and urine M-protein detectable by immunofixation but not on electrophoresis **or**
- 90% or greater reduction in serum M-protein **plus** urine M-protein level <100mg per 24 h
- No evidence of progressive or new bone lesions if radiographic studies were performed  
(X-Rays not required in absence of clinical indication)

#### 6.3.2.4 Partial Remission (PR)

- 50% or greater reduction of serum M-protein **and**
- 90% or greater reduction in 24-h urinary M-protein (or to less than 200mg per 24 h) **and**
- 50% or greater reduction in the size of soft tissue plasmacytomas, if present at baseline
- No evidence of progressive or new bone lesions if radiographic studies were performed  
(X-Rays not required in absence of clinical indication)

Only if the serum and urine M-protein are not measurable (as per definition in section **6.2.1.1**),

- 50% or greater decrease in the difference between involved and uninvolved FLC levels is required (in lieu of the serum and urine M-protein criteria).

If serum and urine M-protein are not measurable and serum FLC assay is also not measurable,

- Before amendment E: 50% or greater reduction in plasma cells is required in lieu of M-protein evaluation (provided baseline bone marrow plasma cell percentage was 30% or greater)
- After amendment E: bone marrow plasma cell will not be used for PR evalution.

#### 6.3.2.5 Stable Disease (SD)

- Not meeting criteria for CR, VGPR, PR or progressive disease

(Not recommended for use as an indicator of response; stability of disease is best described by providing the time to progression estimates)

Comments:

- All response categories require two consecutive assessments made at **any time** before the institution of any new therapy;
- Confirmation with repeat bone marrow biopsy not needed.
- Presence/absence of clonal cells is based upon the  $\kappa/\lambda$  ratio. An abnormal  $\kappa/\lambda$  ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is  $\kappa/\lambda$  ration of greater than 4:1 or less than 1:2.

#### 6.3.2.6 Progressive Disease (PD)<sup>a</sup>

Requires one or more of the following:

- Increases of greater or equal to 25% from the lowest post-treatment (nadir) value in
  - Serum M-component (minimum absolute increase of 0.5g/dl) or
  - Urine M-component (minimum absolute increase of 200mg/24h) or
  - Percentage of bone marrow plasma cells (minimum absolute percentage of 10%)
  - Size of bone lesions or soft tissue plasmacytoma
  - Only in patients without measurable serum and urine M-protein levels: The difference between involved and uninvolved FLC levels. The absolute increase must be >10 mg/dL. The FLC ratio must be abnormal.
- Definite development of new bone lesions or new plasmacytoma
- Development of hypercalcemia solely attributable to the disease (corrected serum calcium >11.5 mg/dL)

<sup>a</sup>All relapse categories require 2 consecutive assessments made at any time before classification as relapse or progression or institution of a new therapy.

#### 6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI

Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site ([http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/ctc.htm#ctc\\_40](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40)).

## **7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN**

### **7.1 DEFINITIONS**

#### **7.1.1 Adverse Event**

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form unless otherwise noted above in Section **6.1**.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections **7.2** , **7.3** and **7.4**.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

#### **7.1.2 Suspected adverse reaction**

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

#### **7.1.3 Unexpected adverse reaction**

An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

“Unexpected” also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

#### 7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

#### 7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

#### 7.1.6 Disability

A substantial disruption of a person’s ability to conduct normal life functions.

#### 7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

#### 7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

#### 7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

#### 7.1.10 Unanticipated Problem

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Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
  - (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  - (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

## 7.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

### 7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

### 7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
  - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
  - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
  - All Grade 5 events regardless of attribution;
  - All Serious Events regardless of attribution.

**NOTE:** Grade 1 events are not required to be reported.

### 7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

### 7.3 IND SPONSOR REPORTING CRITERIA

An investigator must **immediately** report to the sponsor, using the mandatory MedWatch form 3500a or equivalent, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

- All Grade 5 (fatal) events (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- All other serious adverse events including deaths due to progressive disease must be reported within one business day

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

Events will be submitted to the Center for Cancer Research (CCR) at: [CCRsafety@mail.nih.gov](mailto:CCRsafety@mail.nih.gov) and to the CCR PI and study coordinator.

#### 7.3.1 Reporting Pregnancy

##### 7.3.1.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form “Describe Event or Problem”.

Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as Grade 3 “Pregnancy, puerperium and perinatal conditions - Other (pregnancy)” under the Pregnancy, puerperium and perinatal conditions SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but no later than 24 hours of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

### 7.3.1.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study treatment, and for 4 months after finish the last cell infusion

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until (120 days) after the last dose after last cell infusion should, if possible, be followed up and documented.

## 7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

### 7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of anti-BCMA CAR T cells as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the anti-BCMA CAR-expressing T cells, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information.

Adverse events may be reported by using the FDA Form 3500a.

### 7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, the IRB continuing review report can be sent to IBC in lieu of a separate report. Please include the IBC protocol number on the report.

#### 7.4.2.1 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- the title and purpose of the trial
- clinical site
- the Principal Investigator
- clinical protocol identifiers/participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed,
- if the trial has been completed, a brief description of any study results.

#### 7.4.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

### 7.5 DATA AND SAFETY MONITORING PLAN

#### 7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular biweekly basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS and to the Sponsor.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

#### 7.5.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability

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- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by an NCI contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct

### 7.5.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

## 8 STATISTICAL CONSIDERATIONS

The primary endpoint of this trial is to determine the safety of administering anti-BCMA-CAR-expressing T cells to patients with relapsed or persistent B-cell malignancies. Secondary objectives of this trial are to measure any anti-malignancy effect that might occur, to assess the feasibility of administering anti-BCMA-CAR-expressing T cells, and to measure persistence and function of anti-BCMA-CAR-expressing T cells.

The trial was to be a dose-escalation with 5 dose levels. The number of anti-BCMA-CAR-expressing T cells transferred for each cohort was originally to be as follows:

Dose level 1	$0.3 \times 10^6$ CAR+ T cells per kg of recipient bodyweight
Dose level 2	$1.0 \times 10^6$ CAR+ T cells per kg of recipient bodyweight
Dose level 3	$3.0 \times 10^6$ CAR+ T cells per kg of recipient bodyweight
Dose level 4	$9.0 \times 10^6$ CAR+ T cells per kg of recipient bodyweight
Dose level 5	$15.0 \times 10^6$ CAR+ T cells per kg of recipient bodyweight

As of amendment E, we have completed accrual to the first 3 dose levels using the approach described in the next 3 paragraphs. Each dose level will include a minimum of 3 patients. There will be a minimum of 7 days between the start of the conditioning chemotherapy regimen for sequential patients. Patients will be enrolled sequentially; therefore, enrollment will not proceed to a higher dose level until all patients have been treated in the prior dose level. If sufficient cells cannot be grown to meet the criteria for the assigned cohort, the patient will receive the dose of

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cells called for by one dose level lower than the assigned cohort. If sufficient cells cannot be grown to meet the dose requirement called for one dose level lower than the assigned cohort, the treatment will be aborted. If a DLT occurs in an additional patient entered at a lower dose due to cell growth limitations, accrual will continue at this level as described in the dose-escalation scheme below. Accrual will be halted at the higher level until accrual at the lower level is complete as described above.

Should none of the first 3 patients treated on a dose level experience a DLT, accrual can start on the next higher dose level after a 14-day delay following treatment of the third patient. Should 1 of 3 patients experience a dose limiting toxicity at a particular dose level, three more patients would be treated at that dose level to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher level. If 1/6 patients have a DLT at a particular dose level, accrual can proceed to the next higher dose level. If a level with 2 or more DLTs in 3-6 patients has been identified, 3 additional patients will be accrued at the next-lowest dose for a total of 6, in order to further characterize the safety of the maximum tolerated dose. The maximum tolerated dose is the dose at which a maximum of 1 of 6 patients has a DLT. After a maximum tolerated dose is defined, additional patients can be treated on this trial: up to 8 total additional recipients can be treated after a MTD is established in order to better define safety and toxicity at that dose level. If cell growth limitations preclude administration of the maximum tolerated dose, the patient will receive as many cells as possible up to the maximum tolerated dose.

DLTs that occur in the cohort of patients receiving a repeat treatment will not affect the dose escalation of patients receiving an initial treatment. However, excessive DLTs among re-treatments will result in a discontinuation of this practice. Specifically, if 2-3 of the first 3 patients re-treated, 3 or more of the first 6 re-treated patients, 4 or more out of the first 9 re-treated patients, or greater than 1/3 of the total patients receiving a repeat treatment experiences a DLT during repeat treatment, then repeat treatments will be discontinued altogether.

As of Amendment E, we have decided to not enroll patients on dose level 5. The plan for the future of this protocol is to further explore dose level 4,  $9.0 \times 10^6$  CAR T cells/kg. We have already treated 4 patients on dose level 4 ( $9 \times 10^6$  CAR+ T cells/kg). One of 4 low-dose patients experienced a DLT on dose level 4. If 2 more patients can be treated on this dose level without a DLT, we will declare dose level 4 the maximum tolerated dose and proceed to treat 8 patients on an expansion cohort as described on page 35 of the protocol. If 2 more patients cannot be treated on Dose Level 4 without a DLT, we will hold discussions with the FDA and NCI IRB to ask to proceed with a lower dose to be determined at that time. Regarding the expansion cohort of 8 patients, if more than 1 patient on this expansion cohort has a DLT, enrollment on the expansion cohort will stop. We plan to treat a maximum of 10 more patients on this protocol (2 more on dose level 4 followed by an expansion cohort of 8 patients). We have decided to not explore dose level 5. As indicated in section 3.3.4, patients may be re-enrolled on the study as new patients to allow re-treatment, and these patients will be considered both times in the total sample size for the study. With 18 patients enrolled as of January 5, 2017, and an additional planned accrual of up to 10 patients, the trial will enroll no more than 30 patients including up to 2 inevaluable patients.

The degree of persistence of anti-BCMA-CAR-transduced T cells will be evaluated by a quantitative measure (flow cytometry or quantitative PCR) in all patients. Anti-malignancy effects will be measured by clinical response and categorized according to the International Uniform Response Criteria for Multiple Myeloma (Section 6.2). The clinical multiple myeloma responses will be interpreted cautiously in the context of a pilot study which may be used to guide parameters for study in future protocols if warranted.

All other evaluations of secondary objectives will be performed using exploratory techniques. No formal adjustment for multiple comparisons will be used since the evaluations are being done to generate hypotheses.

## 9 COLLABORATIVE AGREEMENTS

### 9.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

There is a CRADA for this protocol, with Bluebird Bio, Inc. for the development of lentiviral based chimeric antigen receptors (CAR) vectors that target the B cell maturation antigen (BCMA) for the treatment of Multiple Myeloma.

CRADA #2936 has been executed between Bluebird Bio, Inc. and the NCI.

## 10 HUMAN SUBJECTS PROTECTIONS

### 10.1 RATIONALE FOR SUBJECT SELECTION

- The patients to be entered in this protocol have multiple myeloma which is an almost always incurable disease; moreover, patients will have progressive or relapsed myeloma despite at least 3 prior therapies. These patients have limited life expectancies. Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.
- Multiple myeloma (MM) remains an incurable disease despite recent advances in therapy.
- Over the last 40 years and throughout the most recent era of improved therapy with novel agents, the depth of the disease response to therapy (including T- cells targeting B-cell maturation antigen) has remained the single most predictive factor for event free and overall survival in MM
- Therefore, improving the rate and depth of responses remains a high priority for clinical research in MM.

- This justifies the enrollment of all subjects with MM eligible for T- cells targeting B-cell maturation antigen in this phase I/II study in an attempt to improve the clinical outcome while feasibility and safety are being evaluated.
- Because patients on previous trials of CAR T cells have experienced hypotension, tachycardia, prolonged fevers, neurological toxicities, and depressed myocardial function. In many cases these toxicities were severe enough to require intensive care unit admission. We will limit enrollment to patients 70 years of age or less because based on our admittedly limited experience with prior CAR-T cell clinical trials, younger patients tolerate and recover from these toxicities better than elderly patients.

## 10.2 PARTICIPATION OF CHILDREN

Children will not be enrolled on this study. Multiple myeloma is extremely uncommon in children; moreover, since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit.

## 10.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section 10.4), all subjects  $\geq$  age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the “NIH Advance Directive for Health Care and Medical Research Participation” form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

## 10.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The experimental treatment has a chance to provide clinical benefit though this is unknown. A goal of this study is to improve upon the number of patients who may benefit from adoptive cell therapy by using genetically-modified T-cells. This specific protocol is being performed to evaluate a genetically modified T-cell therapy for multiple myeloma, which is an almost always incurable disease. Only patients with multiple myeloma who have progressive or relapsed myeloma despite at least 3 prior lines of therapy will be enrolled. The risks of the study fall into 3 general categories. First, chemotherapy that could cause cytopenias is part of the protocol. As with any chemotherapy that causes neutropenia and thrombocytopenia, this chemotherapy could cause toxicities such as infections and bleeding. The second category of toxicity is cytokine-release type toxicities such as high fevers, hypotension and neurological toxicities such as delirium, obtundation, myoclonus, seizures, headache, and transient focal neurological toxicities including aphasia and focal paresis. These cytokine-release-type toxicities have been detected in other clinical trials of CAR T cells during the first 2 weeks after anti-BCMA CAR T cells were infused.<sup>4,95</sup> The third main category of toxicity is direct damage to normal tissues by the CAR T

cells. This could happen because of unexpected expression of BCMA on normal cells or because of unexpected cross-reactivity of the anti-BCMA CAR with proteins other than BCMA in vivo. Another potential toxicity of anti-BCMA CAR T cells is hypogammaglobulinemia due to depletion of plasma cells and mature B cells. Hypogammaglobulinemia has been a complication of many patients on clinical trials of anti-BCMA CAR-expressing T cells.<sup>4,32</sup> Hypogammaglobulinemia in these patients was routinely treated with infusions of intravenous immunoglobulins.<sup>4</sup>

The gammaretroviral vector used in this trial inserts into the T-cell DNA of patients, so in theory, insertional mutagenesis could occur, but insertional mutagenesis has not occurred in any of the hundreds of patients treated with mature T cells that were genetically modified by gammaretroviral vectors.<sup>34-36</sup>

The success of this clinical trial cannot be predicted at this time. Because all patients in this protocol have advanced multiple myeloma and limited life expectancies the potential benefit is thought to outweigh the potential risks. It is also anticipated that this study will provide scientific information relevant to tumor immunotherapy.

## **10.5 CONSENT AND ASSENT PROCESS AND DOCUMENTATION**

The patient, along with family members or friends, will be presented with a detailed description of the protocol treatment. The specific requirements, objectives, and potential advantages and disadvantages will be presented. The Informed Consent document is given to the patient who is requested to review it and to ask questions prior to agreeing to participate in the treatment portion of this protocol. The patient will be reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The permission for additional biopsies for research will be obtained from patients at the time of the biopsy procedure. The research nurse, Principal Investigator or his designee is responsible for obtaining written informed consent from the patient.

### **10.5.1 Telephone re-consent procedure**

Reconsent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records. The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

### **10.5.2 Short form consent process for non-English speaking patients**

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS

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Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2) and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation. Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

## **11 PHARMACEUTICAL INFORMATION**

### **11.1 RETROVIRAL VECTOR CONTAINING THE ANTI-BCMA CAR GENE**

The retroviral vector supernatant (PG13-MSGV-11D5-3CD828Z B1) encoding a chimeric antigen receptor (CAR) directed against B-cell maturation antigen (BCMA) was prepared and preserved following cGMP conditions in the Surgery Branch Vector Production Facility (SBVPF). The retroviral vector utilizes the MSGV retroviral vector backbone and consists of 7007 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites, the anti-BCMA CAR protein containing a signal peptide from human CD8-alpha, 11D5-3 light chain variable region (11D5-3 VL), linker peptide, 11D5-3 heavy chain variable region (11D5-3 VH), CD8 (hinge and transmembrane), CD28 (cytoplasmic region), and TCR zeta (cytoplasmic region), followed by the murine stem cell virus 3'LTR. The physical titer was determined by RNA dot blot according to sponsor certificate.

The supernatant will be stored at -80 C or shipped on dry ice and stored in the Dept. of Transfusion Medicine, NIH or at Cryonix, Rockville, MD. Both storage facilities are equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in *in vitro* transductions of T cells. There will be no re-use of the same unit of supernatant for different patients. Retroviral titer has been shown to be stable after immediate thawing and immediate use. Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2). The specific guidelines for Biosafety Level-2 (BSL-2) can be viewed at <http://bmb1.od.nih.gov/sect3bsl2.htm>

### **11.2 CYCLOPHOSPHAMIDE**

#### **11.2.1 Source**

Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a lyophilized powder in various vial sizes.

#### **11.2.2 Toxicities**

- 1) Nausea and vomiting - variable; symptomatically improved with standard anti-emetics and/or benzodiazepines [e.g., lorazepam].
- 2) Water retention – cyclophosphamide may rarely provoke the syndrome of inappropriate antidiuretic hormone secretion and resultant hyponatremia, usually manifested 12-48 hours after IV administration, necessitating frequent accurate assessment [q 1-2 hours] of intake, urine output and urine specific gravity. This effect can be counteracted by furosemide. Fluid restriction is not feasible during administration of high dose cyclophosphamide.
- 3) Cardiomyopathy - cyclophosphamide may cause severe, sometimes lethal, hemorrhagic myocardial necrosis or congestive cardiomyopathy. Patients may present with congestive cardiomyopathy as late as 2 weeks after the last dose of cyclophosphamide. The clinical syndrome has been observed in patients receiving the dose of cyclophosphamide used in this protocol. In an attempt to minimize this complication, patients with significant cardiac dysfunction are excluded from this protocol [see patient eligibility]. Congestive failure is managed according to standard medical therapeutics.
- 4) Hemorrhagic cystitis – this is a serious, potentially life-threatening complication related to injury of the bladder epithelium by cyclophosphamide metabolites.
- 5) Although sub-clinical hematuria is not uncommon at this dose level, clinically significant hematuria or serious hemorrhage can usually be avoided by maintaining a high urine volume and frequent voidings and the administration of Mesna. Diuresis is maintained for 24 hours after completion of last dose by parenteral infusions of normal saline with potassium chloride. Careful monitoring of serum and urine electrolytes is mandated. Furosemide may be required to ensure this diuresis. Continuous bladder irrigation may be used for control of significant hematuria.
- 6) Sterility
- 7) Less common but serious complications include pulmonary fibrosis and secondary malignancies. Less common but reversible toxicities include alopecia and skin rash.

#### 11.2.3 Formulation and preparation

Reconstituted with sterile water for injection to yield a final concentration of 20 mg/ml as described in the package insert.

#### 11.2.4 Stability and Storage

Vials are stored at room temperature. Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8°C. Once diluted for administration, cyclophosphamide will be provided an expiration date based on the standards set by the NIH CC Pharmacy Department.

#### 11.2.5 Administration procedures

The cyclophosphamide used in this regimen will be given as Intravenous infusion over 30 minutes.

### 11.3 FLUDARABINE

#### 11.3.1 Source

Fludarabine monophosphate will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a white, lyophilized powder. Each vial contains 50

mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH. Fludarabine is stored at room temperature.

#### 11.3.2 Toxicity

Fludarabine toxicities include myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, auto-immune hemolytic anemia, auto-immune thrombocytopenia, paresthesias, peripheral neuropathy, renal, and pulmonary toxicity (interstitial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered almost exclusively after very high doses of fludarabine monophosphate. Such toxicity has only rarely been demonstrated at the 25-30 mg/m<sup>2</sup>/day dosage of fludarabine. Very rarely described complications include transfusion-associated graft-versus-host disease, thrombotic thrombocytopenic purpura, and liver failure. Tumor lysis syndrome following fludarabine administration has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed post-fludarabine, especially in heavily pre-treated individuals, and in individuals receiving fludarabine combined with other agent.

#### 11.3.3 Formulation and preparation

FLUDARA IV should be prepared for parenteral use by aseptically adding Sterile Water for Injection, USP. When reconstituted with 2 ml of Sterile Water for Injection, each ml of the resulting solution will contain 25 mg of Fludarabine Phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7–8.5. Fludarabine will be diluted in 100 to 125ml of either 5% dextrose in water or 0.9% sodium chloride, and infused IV over 30 minutes.

#### 11.3.4 Stability and Storage

Reconstituted FLUDARA IV should be stored in the refrigerator between 36 and 46 degrees F. Because reconstituted FLUDARA IV contains no antimicrobial preservative, care must be taken to assure the sterility of the prepared solution. Once diluted for administration, fludarabine will be provided an expiration date based on the standards set by the NIH CC Pharmacy DepartmentAdministration procedures

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Fludarabine is administered as an IV infusion in 100 ml 0.9% sodium chloride, USP over 15 to 30 minutes. Dose reduction for renal impairment

For Creatinine Clearance of 30-70 mL/min/1.73m<sup>2</sup> there will be a 20% dose reduction. If the Creatinine Clearance is less than 30mL/min/1.73m<sup>2</sup>, Fludarabine will not be administered.

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*Abbreviated Title: Anti-BCMA-CAR T cells*

*Version Date: September 21, 2017*

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## 13 APPENDICES

### 13.1 APPENDIX A-PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale*	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

\* As published in Am. J. Clin. Oncol.: Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

## 13.2 APPENDIX B: DATA COLLECTION ELEMENTS REQUIRED BY PROTOCOL

All of the following elements will be recorded in the C3D database:

### A. Patient Enrollment

- Date of birth, age, gender, race, ethnicity
- Height
- Weight
- Performance Status
- Date of original diagnosis
- Stage at diagnosis
- Plasmacytoma present: Yes or No
- Tumor Histology and date of confirmation
- BCMA expression by tumor type of tissue studied and date of confirmation
- Date of Informed Consent signature, consent version and date of registration
- Baseline History/Physical
- Baseline Symptoms
- Prior therapy
- Prior radiation
- Prior Bone Marrow Biopsy that includes the percent of plasma cells and the percent of BCMA cells
- Findings of consultations done at screening

### B. Study Drug administration and response for each course of therapy given

- Dates anti-BCMA-CAR-transduced T cells given
- Dose level, actual dose, schedule and route given
- Height, weight, and body surface area at start of each course
- Response assessment for each restaging performed
- Concomitant medications will not be collected in C3D

### C. Laboratory and Diagnostic Test Data

1. All Clinical laboratory and diagnostic test results done at screening and until day 30 post infusion with the following exceptions:  
Diagnostic tests which are not specified in the protocol, and if the results are not needed to document the start or end of an adverse event that requires reporting.  
Serologies-CMV, HSV, EBV, toxoplasmosis, adenovirus (patient and donor)  
TTV data
2. All staging studies including serum protein electrophoresis, urine protein electrophoresis, serum free light chains, bone marrow biopsy reports, flow cytometry reports, serum immunoglobulin reports including beta-2 microglobulin,

complete blood count and differential reports, serum creatinine reports, TBNK results, serum protein, urine albumin, serum calcium (including ionized), ESR and CRP results. MRI, X-ray (including skeletal survey), and CT scan results will only be reported if they were used for staging.

**D. Adverse Events**

Please see section **6.1.1** Adverse Event Recording

**E. Tumor response and measurements**

- Restaging studies performed at protocol specified time points and as clinically indicated.
- Any physical exam findings will be collected as Adverse Events and labs results.
- Years 5-15 follow-up is only for survival.

**F. Off study**

- Date and reason for off study
- Date and cause of death
- Autopsy findings
- PI decision to end this study

### **13.3 APPENDIX C: GUIDELINES FOR MANAGEMENT OF COMMON TOXICITIES THAT OCCUR AFTER CAR T-CELL INFUSIONS**

Infusions of CAR T cells are often complicated by significant acute toxicities in the first 2 to 3 weeks after the infusion. In many cases the toxicities correlate with serum inflammatory cytokine levels.<sup>4</sup>

The toxicities most often experienced by patients receiving infusions of anti-BCMA CAR T cells include, but are not limited to, tumor lysis syndrome, fever, fatigue, hypotension, tachycardia, acute renal failure, and neurological toxicities such as aphasia, ataxia, headache, somnolence, and coma. Fever is usually the first toxicity to occur. CARs targeting different antigens than BCMA might have different toxicities.

#### **Suggested Guidelines for common acute CAR T-cell toxicities**

Note these are guidelines that might require modification based on clinical circumstances of each patient, and failure to exactly follow these guidelines is not a protocol deviation.

Administration of corticosteroids should be avoided if at all possible to avoid killing or impairing the function of the CAR T cells.

1. All patients with significant malignancy burdens and without a contradiction such as allergy should be started on allopurinol at the time of the start of the chemotherapy conditioning regimen or 1 day before the CAR T cell infusion. The suggested allopurinol dose is 200 to 300 mg/day with a possible loading dose of 300 to 400 mg.
2. Vital signs should be checked a minimum of every 4 hours during hospitalization. Increasing the time interval between vital sign checks for patient convenience or other reasons should be avoided.
3. Strict ins and outs should be recorded on all patients.
4. Fevers should be treated with acetaminophen and comfort measures. NSAIDs and corticosteroids should be avoided.
5. Administer fresh frozen plasma (FFP) for a PTT 1.5-fold or more above the upper limit of normal.
6. For patients with an increased PTT, check the fibrinogen level and keep the fibrinogen level above 100 mg/dL with cryoprecipitate.
7. Patients with a heart rate persistently higher than 115/minute and fever should have vital signs checked every 2 hours.
8. Patients who are neutropenic and febrile should be receiving broad-spectrum antibiotics.
9. Patients at risk of syncope

Patients on this protocol will be placed on strict fall precautions including instructions to get out of bed only with assistance under the following conditions:

1. Any history of syncope or near-syncope within 1 month before Anti BCMA CAR T-cell infusion or any time after CAR T-cell infusion.
2. Any blood pressure reading of less than 90 mm Hg systolic blood pressure after anti-BCMA CAR T-cell infusion.
3. Heart rate greater than 100 beats per minute.

Any patient with syncope, near-syncope, or light-headedness will have orthostatic blood pressure and heart rate checked and receive intravenous fluids as appropriate. These patients will also receive an ECG.

Hypotension is a common toxicity requiring intensive care unit (ICU) admission. In general patients should be kept well-hydrated. Maintenance I.V. fluids (normal saline (NS) should be started on most patients with high fevers especially if oral intake is poor or the patient has tachycardia. I.V. fluids are not necessary for patients with good oral intake and mild fevers. For patients who are not having hypotension or tumor lysis syndrome, a generally even fluid balance should be strived for after allowing for insensible fluid losses in patients with high fevers. The baseline systolic blood pressure is defined for this protocol as the average of all systolic blood pressure readings obtained during the 24 hours prior to the CAR T-cell infusion. The first treatment for hypotension is administration of IV NS boluses.

- Patients with a systolic blood pressure that is 80% or less of their baseline blood pressure and less than 100 mm Hg should receive a 1 L NS bolus.
- Patients with a systolic blood pressure less than 85 mm Hg should receive a 1 L NS bolus regardless of baseline blood pressure.

These I.V. fluid management suggestions may need to be modified based on the clinical characteristics of individual patients such as pulmonary status, cardiac function, edema and other factors.

10. Patients receiving fluid boluses for hypotension should have a stat EKG and troponin, and a cardiac echocardiogram within 24 hours.
11. Patients should be transferred to the ICU under these circumstances. Patients not meeting these criteria could also require ICU admission at the discretion of the clinical team caring for the patient.
  - Systolic blood pressure 75% or less than the patient's baseline blood pressure and less than 100 mm Hg after administration of a 1L NS bolus.
  - Anytime the systolic blood pressure is less than 85 mm Hg after a 1L NS bolus.
  - Continuous tachycardia with a heart rate higher than 125 beats per minute on 2 occasions separated by at least 4 hours.
  - Oxygen requirement of more than a 4L standard nasal cannula
  - Greater than grade 2 neurological toxicity

12. All patients transferred to the ICU for hypotension or tachycardia should have a stat EKG and a cardiac echocardiogram at the time of transfer.
13. Patients with hypotension not responding to IV fluid resuscitation should be started on norepinephrine at doses called for by standard ICU guidelines.
14. Patients should have a cardiac echocardiogram and an EKG within 24 hours of starting norepinephrine.
15. Patients in the ICU should get twice-daily labs (CBC with differential, acute care panel, mineral panel, hepatic panel, uric acid, LDH. Patients in the ICU should also get a daily troponin level).
16. Anecdotal evidence suggests that the IL-6 receptor blocker tocilizumab can be an effective treatment for cytokine-release syndrome toxicities after CAR T-cell infusions. Tocilizumab should be administered under the following circumstances if the listed disorders are thought to be due to cytokine release from CAR T cells. Tocilizumab is administered at a dose 8 mg/kg infused IV over 1 hour (dose should not exceed 800 mg).
  - Left ventricular ejection fraction 40% or less by echocardiogram
  - Creatinine greater than 2.5-fold higher than the most recent level prior to CAR T-cell infusion
  - Norepinephrine requirement of 3  $\mu$ g/min or more for 36 hours since the first administration of norepinephrine even if norepinephrine administration was not continuous.
  - Systolic blood pressure of 90 mm Hg cannot be maintained with norepinephrine.
  - Oxygen requirement 55% or greater fraction of inspired oxygen (FIO2) for more than 2 continuous hours.
  - Dyspnea that is severe enough to potentially require mechanical ventilation.
  - PTT or INR>2x upper limit of normal
  - Clinically-significant bleeding
  - Creatine kinase greater than 5x upper limit of normal for greater than 2 days
17. THERE IS NO EVIDENCE THAT TOCILIZUMAB HELPS NEUROLOGICAL TOXICITY, SO IT SHOULD NOT BE ADMINISTERED FOR THIS PURPOSE.
18. If no improvement in hypotension or tachycardia occurs within 6 hours of tocilizumab infusion, consider other agents such as methylprednisolone 1 mg/kg every 12 hours or etanercept.
19. Avoid meperidine due to seizure risk.
20. In the setting of cytokine-release syndrome, patients with a left ventricular ejection fraction of 30% or less and patients who in the opinion of an ICU physician is at significant risk of

needing intubation in the next 4 hours should be given methylprednisolone 100 mg every 6 hours.

21. Some patients may experience decreases in serum immunoglobulins. Replacement of serum IgG with intravenous IgG is recommended for serum IgG levels below 400 mg/dL.
22. In general, follow standard vaccination practices including an annual influenza vaccination in the absence of data to suggest otherwise.
23. All patients with grade 2 or greater neurological toxicities should get a neurology consult.
24. The following patients should receive dexamethasone 10 mg intravenously every 6 hours until the toxicities improve to Grade 1 or resolve or until at least 8 doses of dexamethasone have been given.
  1. Patients with Grade 3 or 4 neurological toxicities except that dexamethasone is not recommended for isolated Grade 3.
  2. Any generalized seizure

**13.4 APPENDIX D: INFUSION INSTRUCTIONS****Equipment:**

Primary IV tubing (2)

Secondary IV tubing (1)

NS (sodium chloride 0.9%) 250cc bags (2)

IV infusion pump

Gloves

Steps:	Key Points:
1. On the morning of infusion the primary RN will be notified of the approximate time TIL cells will be ready (usually between 11AM and noon).	
2. Verify the physician orders: - to administer the cells - for the date of administration - for premedication orders - protocol number	<ul style="list-style-type: none"> <li>Premeds are acetaminophen 650 mg PO and diphenhydramine 12.5 mg IV.</li> </ul>
3. Verify that the protocol consent and DPA are signed.	
4. Ensure that emergency and monitoring equipment are available in the patient's room: - oxygen - suction - vital sign monitor with pulse oximeter and thermometer	

5. Provide patient education covering infusion procedure, potential complications and associated symptoms to report.	
7. Measure and record baseline vital signs, respiratory and circulatory assessments.	
8. Verify the patency of the patient's IV access.	A central venous access device such as a non-valved PICC line should be used.
9. Hang a primary line of 250cc NS at a kvo rate - <b>NEW</b> bag and <b>NEW</b> tubing.  This <b>MUST</b> be ready and infusing <b>prior</b> to the cells being delivered to the unit.  The patient's primary IV hydration can infuse via a separate lumen while the cells are infusing, but <b>NO MEDs</b> should be administered during this time.	This will be the dedicated NS line for infusing the cells. Under no circumstances are any other substances to be infused into the line.  Cell death occurs quickly – the infusion must be initiated immediately.  Do not infuse medication during the cell infusion. If emergency meds must be administered, use the hydration or emergency NS IV line.
Have a second bag of 250cc NS and tubing ready as an emergency line.	This will be the emergency IV solution and can be used for medication administration.  <b>Do not use an inline filter for cells.</b>
10. The primary RN will be notified approximately 10 minutes before the cells arrive on the unit. The cells will be hand delivered to the bedside.  It is critical to be at the bedside awaiting the arrival of the cells for infusion.	It is critical to be at the bedside awaiting the arrival of the cells for infusion; have baseline VS, assessment, and IV lines hooked up when the cells arrive. <b>Cell death occurs as soon as the cells are removed from the laboratory.</b> Initiate the infusion as quickly as possible.
12. Prior to spiking the cell bag, two RNs will perform the identification procedure.	

<b>Both</b> RNs must sign the tag on the cell bag.	
<p>13. Infuse the cells by infusion pump over 20 minutes.</p> <p>a. Piggyback the cells into the dedicated NS line; use the backflush technique to prime the line.</p> <p>b. While the cells are infusing, <b>gently</b> agitate the bag of cells <b>every few minutes to prevent clumping in the bag</b>. When the cell bag is empty, backflush NS to rinse the bag and infuse this at the same rate as the cells; rinse bag until NS runs clear.</p> <p>Note, when very small cell doses are given, the cells will arrive at the bedside in a syringe. In these cases, the cells should be manually infused over 20 minutes via the syringe.</p>	
<p>14. Measure and record VS before and after the cell infusion, q1h x 4and then q4h after completion of the infusion.</p> <p>a. Assess and document the patient's respiratory and circulatory status post cell infusion.</p>	
<p>15. Documentation:</p> <p>a. After the cells have infused, remove the adhesive backed "cell therapy product" tag from the cell bag and place it on a progress note in the patient's chart.</p> <p>b. Document the cell infusion in CRIS using the appropriate screens.</p>	