**Abbreviated Title:** Anti-SLAMF7 CAR T cells for MM

**CC Protocol #:** *19-C-0102 C*

IBC#: RD-19-III-02

NCT#: NCT03958656

**Version Date: April 14, 2020**

**Title: A Phase I Clinical Trial of T-cells Expressing an anti-SLAMF7 CAR for Treating Multiple Myeloma**

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| Agent Name: | Anti-SLAMF7 CAR- transduced autologous T cells | Rimiducid |
| IND Number: | 18793 | 18793 |
| Sponsor: | Center for Cancer Research | Center for Cancer Research |
| Manufacturer | NIH DTM | Bellicum Inc. |

Commercial Agents: Cyclophosphamide, fludarabine

## PRÉCIS

### Background:

- Multiple myeloma (MM) is a nearly always incurable malignancy of plasma cells.
- T cells can be genetically modified to express chimeric antigen receptors (CARs) that target malignancy-associated antigens.
- SLAMF7 is highly and uniformly expressed on MM cells but is absent on normal tissues except for some leukocytes, including a subset of $CD8^+$ T cells, natural killer (NK) cells, B cells, plasma cells and monocytes.
- We have constructed a novel anti-SLAMF7 CAR that can specifically recognize SLAMF7-expressing target cells and eradicate SLAMF7-expressing tumors in mice.
- This protocol will test genetic modification of autologous T cells with genes encoding an inducible caspase 9 (IC9) cell-suicide system plus the anti-SLAMF7 CAR.
- Administration of the dimerizer drug Rimiducid (AP1903) is necessary to activate the IC9 suicide gene and eliminate CAR T cells.
- In this protocol, the suicide gene system will be used to eliminate CAR-expressing T cells in case of severe toxicities caused by the CAR T cells.
- Possible toxicities include cytokine-associated toxicities such as hypotension, and neurological toxicities. Elimination of NK cells and normal plasma cells could make patients more susceptible to infections. Unknown toxicities are also possible.

### Objectives:

### Primary

- Determine the safety, feasibility of administering T cells expressing an anti-SLAMF7 CAR plus IC9 cell-suicide system to patients with MM.

### Eligibility:

- Greater than or equal to 18 years of age and less than or equal to age 73.
- Patients must have measurable MM defined as a serum M-protein $\geq$0.6 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 of 1.5 cm or more in largest dimension, or greater than or equal to 30% bone marrow plasma cells.
- Patients must have previously received at least 3 different treatment regimens for MM.
- Patients must have prior exposure to an immunomodulatory drug (IMiD) such as lenalidomide, and a proteasome inhibitor
- Patients must have a creatinine level of $\leq$ 1.5 mg/dL
- Patients must have a cardiac ejection fraction $\geq$ 50%.
- An ECOG performance status of 0-2 is required.

- Patients on any anticoagulant medications except aspirin are not eligible.
- No active infections are allowed.
- Absolute neutrophil count ≥ 1000/μL, platelet count ≥ 55,000/μL, hemoglobin ≥ 8g/dL
- ALT and AST ≤ 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 and initiation of protocol treatment. Systemic therapy includes corticosteroids at a dose equivalent to more than 5 mg of prednisone.
- Bone marrow plasma cells must make up less than or equal to 50% of total bone marrow cells ≤ 24 days prior to the start of protocol treatment.
- The patient's MM will need to be assessed for SLAMF7 expression by flow cytometry or immunohistochemistry performed at the NIH. The myeloma must express SLAMF7. If unstained, paraffin-embedded bone marrow or plasmacytoma sections are available from prior biopsies, these can be used to determine SLAMF7 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 SLAMF7 expression. The sample for SLAMF7 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, and T cells will be modified to express the IC9-anti-SLAMF7 CAR construct.
- The chemotherapy conditioning regimen is cyclophosphamide 300 mg/m$^2$ daily for 3 days and fludarabine 30 mg/m$^2$ daily for 3 days. The intent of chemotherapy is to enhance CAR T-cell activity.
- After the chemotherapy ends, the patients will have two days with no treatments and then receive an infusion of CAR T cells.
- The initial dose level will be 0.66x10$^6$ Anti-SLAMF7-CAR + T cells/kg of recipient bodyweight.
- The cell dose administered will be escalated for up to 4 doses until a maximum tolerated dose is determined.
- Following the T-cell infusion, there will be a mandatory 9-day minimum inpatient hospitalization to monitor for toxicity.
- Outpatient follow-up is planned for 2 weeks, and 1, 2, 3, 4, 6, 9, and 12 months after the CAR T-cell infusion. Afterwards, follow-up will be every six months up to at least 3 years after infusion.

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# 1 INTRODUCTION

## 1.1 STUDY OBJECTIVES

### 1.1.1 Primary Objective

- Determine the safety and feasibility of administering T cells genetically modified to express a novel chimeric antigen receptor (CAR) targeting signaling lymphocyte activation molecule family member 7 (SLAMF7) and an inactivated caspase 9 cell-suicide system to patients with multiple myeloma (MM).

### 1.1.2 Exploratory Objectives

- Evaluate the *in vivo* persistence and phenotype of the CAR T cells.
- Assess for associations between toxicity and immunologic parameters including blood and bone marrow CAR T-cell and cytokine levels.
- Assess CAR T cells for anti-MM activity post infusion by evaluating MM responses by standard methods.
- Assess efficacy of Rimiducid to eliminate CAR T cells in patients with severe infections and other toxicities

## 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.(1, 2) Many 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.(3, 4) Anti-CD19 CARs are now FDA-approved treatments. Recently, three phase 1 trials have shown that CAR T cells targeting B-cell maturation antigen (BCMA) are effective and have a tolerable safety profile in relapsed multiple myeloma.(5-8) The lasting complete remissions of MM that sometimes occur in the setting of alloHSCT and the encouraging results obtained treating patients with anti-BCMA and anti-CD19-CAR-expressing T cells are rationales for attempting to develop CAR T-cell therapies for MM.

Signaling lymphocyte activation molecule 7 (SLAMF7), also known as CRACC, CS1 or CD319, is a homophilic, glycosylated cell surface protein that is uniformly expressed at high levels in MM as shown by gene expression studies and immunohistochemical analysis of whole blood, plasmacytomas and bone marrow tissue.(6, 9-13) Although SLAMF7 is expressed on a proportion of various lymphocyte subsets including NK cells, NKT cells, some $CD8^+$ T cells, some B cells, some monocytes/macrophages and some dendritic cells, it is not expressed on normal nonhematopoietic tissues.(9) The limited expression of SLAMF7 in normal tissues makes it a

promising target for CAR T-cell therapies. However, to mitigate potential toxicities that may occur with the depletion of endogenous NK, $CD8^+$ T cells, and other SLAMF7-expressing leukocytes, we have engineered a DNA construct that includes CAR and a suicide gene that can be activated if prolonged or severe toxicities occur.

This protocol describes a novel T-cell therapy for patients with relapsed/refractory MM. This new CAR differs from prior anti-myeloma CAR therapies by targeting an antigen that is uniformly and highly expressed on MM cells as well as incorporating a suicide gene to limit toxicity of therapy. The anti-SLAMF7 CAR will incorporate a CD28 costimulatory domain. This trial will enroll patients with advanced MM persisting or progressing 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 demonstrates that depleting endogenous lymphocytes, and possibly other leukocytes, with chemotherapy or total body irradiation dramatically increases the anti-tumor activity of adoptively transferred T cells.(14-16) After the lymphocyte-depleting chemotherapy, patients will receive an infusion of autologous anti-SLAMF7-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**

Multiple myeloma (MM) is a neoplasm of monoclonal plasma cells.(17, 18) 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.(18, 19) MM is the second most frequently occurring hematologic cancer in the United States (U.S.).(18) An estimated 22,350 new cases of MM were diagnosed in the U.S. in 2013, and an estimated 10,710 patients died from MM in the U.S. in 2013.(20) Recent improvements in the therapy of MM have occurred.(17, 18, 21) 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.(18, 21)

Current standard therapies for MM include various combinations of dexamethasone, bortezomib and its analogs, lenalidomide and its analogs, prednisone, melphalan, and cyclophosphamide.(17, 18, 21, 22) Myeloablative doses of chemotherapy followed by autologous stem cell transplantation (ASCT) is a standard therapy for MM patients with good performance status, and adequate bone marrow stem cells.(18, 21) 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.(18, 21) Myeloablative chemotherapy followed by autologous transplantation can also be used as a treatment for relapsed MM in some cases.(18, 21, 23) Use of the International Uniform Response Criteria for Multiple Myeloma is the most common approach for assessing clinical outcomes in MM.(24) Despite the recent improvements in treatment, MM remains an almost always incurable disease.(21, 25, 26) Patients obtaining remissions of MM almost always relapse.(21) The median overall survival of patients with relapsed MM is 3 years or less.(25) 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.(27) 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.(26) Utilizing the immune system to treat MM is one possible way to improve therapy of MM. Recently published data from two phase 1 clinical trials utilizing anti-BCMA CAR T cells to treat relapsed/refractory MM have produced promising results. An NCI trial demonstrated an ORR of was 81% with 63% very good partial remissions or complete remission; a multi-center clinical trial of a different anti-BCMA CAR demonstrated ORR of 94% in 18 patients at 40 weeks.(5, 6)

### 1.2.3 **Allogeneic transplantation for MM**

Allogeneic stem cell transplantation (alloHSCT) can cure a fraction of patients with MM.(28, 29) Myeloablative alloHSCT can induce long-term complete remissions of MM, but also has a transplant-related mortality rate (TRM) of 20% to 50%.(28, 29) The high TRM of myeloablative alloHSCT led investigators to test nonmyeloablative alloHSCT for MM.(28-30) 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.(1, 28, 29) 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.(1, 30) 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.(1) 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.(1, 28, 29) Direct evidence that lymphocytes can eliminate MM comes from donor lymphocyte infusions (DLIs).(2, 29) Twenty-two to twenty-eight percent of patients receiving DLIs to treat persisting MM after alloHSCT have achieved complete remissions (CRs).(2, 28, 29) Of note, some of these patients obtaining CRs after DLIs did not receive any other therapies around the time of their DLI.(2) 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(2, 28, 29) 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.(31-38) 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.(3-6, 35, 39, 40) Genetically modified antigen-specific T cells can be generated from peripheral blood mononuclear cells (PBMC) in sufficient numbers for clinical

treatment within <10 days.(3) Genetically modifying T cells with gammaretroviruses consistently causes high and sustained levels of expression of introduced genes without in vitro selection(4, 39, 41, 42), and genetic modification of mature T cells with gammaretroviruses has a long history of safety in humans.(43-45) There are two general approaches for generating antigen-specific T cells by genetic modification: introducing genes encoding natural αβ T cell receptors (TCRs) or introducing genes encoding chimeric antigen receptors (CARs).(32, 34, 35, 37) CARs are fusion proteins incorporating antigen recognition moieties and T cell activation domains.(36, 46-48) The antigen-binding domains of most CARs currently undergoing clinical and preclinical development are antibody variable regions.(32, 36, 46, 48) 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(32, 34, 35, 46), 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.(32, 35, 49-51)

### 1.2.5 **Chimeric antigen receptors**

Preclinical experiments evaluating CAR-expressing T cells as cancer therapy were initiated in 1993.(52, 53) These experiments led to a clinical trial of CAR-transduced T cells targeting the α-folate receptor on ovarian cancer cells; no tumor regressions were observed during this clinical trial.(54) CARs that were capable of recognizing a variety of tumor-associated antigens have been evaluated in many centers.(32, 46) 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(4, 39, 41-43, 55-57), lentiviruses(3, 58-61), or transposon systems(62, 63) 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.(3, 55, 64) 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 CD3ζ 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.(46, 55, 59, 65-67) Much of the preclinical work evaluating CARs has been performed with CARs targeting the B-cell antigen CD19.(15, 55-57, 62, 68, 69) 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.(57, 66) These studies showed that adding CD28 moieties to CARs enhanced antigen-specific cytokine production and proliferation by anti-CD19 CAR T cells.(66, 70, 71) T cells expressing CARs with CD28 signaling moieties and CD3ζ signaling domains were more effective than T cells expressing CARs without CD28 moieties at eradicating human leukemia cells from mice.(70, 71) Subsequently, CARs incorporating other signaling domains from costimulatory molecules such as 4-1BB (CD137) were developed.(58) Anti-CD19 CARs containing the signaling domains of both 4-1BB and CD3ζ were superior to CARs containing the signaling domains of CD3ζ without any costimulatory domains at eradicating human malignant cells from mice.(59, 65) Similar to CD28, including 4-1BB signaling moieties in CARs led to increased CD19-specific proliferation and enhanced in vivo persistence.(59) 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.(59, 65)

Results from several clinical trials of anti-CD19 CAR T cells have been reported to date in peer-reviewed papers.(3, 4, 39, 41, 42, 72-75) 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.(41) This clinical trial utilized a gammaretroviral vector to introduce an anti-CD19 CAR containing the signaling domains of the CD28 and CD3ζ molecules.(41) Subsequent development of this CAR was carried out, and it was found that anti-CD19 CAR T cells were an effective therapy for a variety of B-cell malignancies.(74, 76) Subsequent studies with this same CAR demonstrated the effectiveness of anti-CD19 CAR T cells against diffuse large B-cell lymphoma and led to multicenter phase II trials and FDA approval.(74)

### 1.2.6 **Chimeric antigen receptor therapy in Multiple Myeloma**

Success of CAR T-cells against leukemia and lymphoma has encouraged development of CAR T-cell therapies for MM. A critical factor in determining the success or failure of a CAR-T therapy is the choice of target antigen. It is important that a selected antigen is uniformly expressed on the malignancy targeted by CAR T cells.(77-80) MM is a clonal malignancy, but over time, multiple sub-clones of MM evolve.(81-85) This leads to genetic and phenotypic heterogeneity of the MM cells within the same patient.(83, 84, 86) This phenotypic heterogeneity includes differences in cell surface antigen expression.(85) To avoid toxicity, the targeted antigen should be absent on essential normal tissues because CAR T cells might destroy cells expressing the target antigen whether the antigen-expressing cells are normal or malignant. Many potential CAR targets in MM have been the subject of pre-clinical testing including CD44 variant 6, CD70 and CD56.(87-90) CARs with CD19, immunoglobin kappa light chain and BCMA moieties have been tested both in the preclinical and clinical trial setting:

CD19:

Several investigators have reported a population of cells that are distinct from the bulk population of malignant MM plasma cells; these cells phenotypically resemble B-cells and their role might be that of a cancer stem cell.(82, 86, 91-93) Circulating B-cells that are clonally related to malignant plasma cells have been characterized in patients with MM; in-vitro studies suggest that CD138-negative cells that express B-cell surface antigens including CD45, CD20 and CD19 can give rise to myeloma colonies.(82, 86, 91, 92, 94) Several studies suggest that the postulated myeloma stem cells have intrinsic drug-resistant properties that may promote relapse.(82, 92, 94) Garfall et al. hypothesized that CAR-Ts targeting CD19 would deplete myeloma cells with stem cell properties after myeloablative chemotherapy and standard ASCT.(95, 96) Ten myeloma patients who had a history of progressive MM after a prior ASCT received a second ASCT followed by an infusion of $5x10^7$ anti-CD19 CAR T cells 12-14 days after infusion of stem cells.(95, 96) The ASCT chemotherapy conditioning regimen was 140-200 mg/m$^2$ of melphalan.(96) CD19$^+$ cells were detected at low levels among the MM plasma cells of seven of the nine patients evaluated by flow cytometry of pre-ASCT bone marrow; in these 7 patients 0.5-1.5% of MM cells were CD19$^+$.(96) This finding emphasizes that anti-CD19 CAR-Ts are intended to target MM stem cells, not the bulk

population of MM cells. All 10 treated patients have progressed; the median PFS was 185 days (range 42-479).(96)

Immunoglobulin Kappa Light Chain:
Plasma cells do not generally express cell-surface immunoglobulin, but Ramos et al have employed a strategy to target a postulated multiple myeloma stem cell population that expresses surface immunoglobulins by generating a CAR targeting kappa light chain.(97) Targeting kappa-expressing cells spares lambda-expressing B-lymphocytes.(97) In a phase I study, seven patients with multiple myeloma were treated with kappa-targeted CAR-T cells.(97) All seven MM patients had active disease at the time of CAR-T infusion and had one or more lines of prior chemotherapy; six out of seven patients had an ASCT in the past.(97) Three patients received salvage chemotherapy within four weeks prior to infusion of CAR-T.(97) Those who did not have prior chemotherapy within four weeks of CAR-T infusion or who did not have an ALC <500 received 12.5 mg/kg cyclophosphamide prior to infusion of CAR-T cells.(97) Four patients had SD lasting six weeks to 24 months, and three patients had no response to kappa CAR-T infusion.(97)

BCMA:
B-cell maturation antigen (BCMA) is expressed by normal and malignant plasma cells but not normal essential cells. BCMA is not expressed by hematopoietic stem cells or nonhematologic cells.(5, 98, 99) It is uniformly expressed on most cases of MM by IHC.(5) By flow-cytometry, cell-surface BCMA was expressed at varying levels by almost all cases of MM.(5) In pre-clinical work, T-cells expressing anti-BCMA CARs could specifically recognize BCMA, kill primary myeloma cells in vitro, and eradicate $BCMA^+$ tumors in mice.(98)

This pre-clinical work led to the first in-humans clinical trial of CAR T cells targeting BCMA at the National Cancer Institute (NCI).(5) An anti-BCMA CAR containing a murine scFv, a CD8 hinge and transmembrane region, a CD28 costimulatory domain, and a CD3ζ T-cell activation domain was created by synthesizing the appropriate DNA and ligating it into a gamma-retroviral backbone.(5) Patients who had MM with uniform BCMA expression by either immunohistochemistry (IHC) or flow cytometry were enrolled.(5) All participants received conditioning chemotherapy prior to infusion of CAR T cells with goals of depleting endogenous leukocytes, including depletion of regulatory T cells, and increasing serum cytokine levels of IL-15 and IL-7 to increase CAR-T activity.(5, 74, 77, 100) The chemotherapy regimen used was cyclophosphamide 300 $mg/m^2$ and fludarabine 30 $mg/m^2$ given daily on the same 3 days followed by CAR T-cell infusion 2 days after the end of the chemotherapy.(5) Administration of chemotherapy or radiation therapy to deplete recipient leukocytes has been shown to enhance the activity of adoptively-transferred T cells.(15, 16, 101)

Twenty-six patients with MM were treated on this study at the NCI. Patients were treated at doses of 0.3-9x$10^6$ $CAR^+$ T cells/kg. Of the patients treated on 0.3- 3x$10^6$ $CAR^+$ T cells/kg, patients had a median of 7 prior lines of therapy; of the patients treated on the highest dose level of 9x$10^6$ $CAR^+$ T cells/kg, patients had a median of 9.5 prior lines of therapy with 63% refractory to their last treatment.(8) At doses of 0.3-3x$10^6$ $CAR^+$ T cells/kg, 2 of 10 patients experienced objective responses of PR or better.(8) Of the 16 patients who received BCMA CAR T cells at a dose of 9x$10^6$ $CAR^+$ T cells/kg, 13 patients had responses of PR or better.(5, 8) The overall response rate for these patients was 81%.(8) All but one patient on the highest dose level had a substantial decrease in their

serum MM markers.(8) The median event-free survival for patients treated at a cell dose of 9x10⁶/kg was 31 weeks with six patients having ongoing remissions.(8) To date, five responses have lasted more than 6 months.(8) In nine of nine patients that were evaluated with bone marrow biopsies and immunohistochemistry staining for CD138 before and 2 months after CAR-BCMA infusion, bone marrow plasma cells steeply decreased between these 2-time points.(8) Of the 16 patients receiving 9x10$^6$ CAR$^+$ T cells/kg, 11 patients were evaluable for minimal residual disease (MRD), and all achieved MRD negative status following CAR-BCMA T-cell infusion.(8) CAR-BCMA T-cell toxicity was mild at lower dose levels, with no CRS grades of 3 or 4 at cell doses of 0.3-3x10$^6$ CAR$^+$ T cells/kg.(5) At a cell dose of 9x10$^6$ CAR$^+$ T cells/kg, CRS related toxicities were substantial.(8, 102) As the dose of CAR T cells escalated, patients began to have more symptoms of cytokine release syndrome, including fever and tachycardia.(5) The first two patients treated with 9x10$^6$ CAR$^+$ T cells/kg experienced substantial cytokine release syndrome (CRS) toxicities. Because of the toxicities experienced by these two patients, the latter 14 patients treated with 9x10$^6$ CAR-BCMA T cells/kg were required to have lower MM burdens with BM involvement by myeloma of less than 30% prior to cell infusion.(8) Six of sixteen patients receiving 9x10$^6$ CAR-BCMA T cells/kg (38%) required vasopressor support for hypotension for CRS following CAR T-cell infusion, and one patient required mechanical ventilation.(8) Five patients (31%) received tocilizumab and 3 patients (19%) received corticosteroids for CRS-related toxicities.(8) Two patients received stress-dose hydrocortisone for evidence of adrenal insufficiency before or during CRS.(8) Patients with CRS of Grade 3 or 4 had higher levels of bone marrow plasma cells compared with patients who had less than Grade 3 CRS, which indicates an increased chance of severe CRS in patients with high MM burdens(8).

Neurologic toxicities were limited to confusion or delirium in the setting of high fevers during CRS except for one patient who experienced encephalopathy and grade 3 muscle weakness of all extremities that was thought due to critical illness polyneuropathy.(8)

Most cytopenias occurred early after CAR-BCMA T-cell infusion and were attributable to chemotherapy, but two cases of cytopenias occurred after recovery of blood counts from chemotherapy-related cytopenias. Neutropenia rapidly resolved after filgrastim support in both of these cases.(8) Both patients received the thrombopoietin receptor agonist eltrombopag and prednisone. Platelets recovered to greater than 50,000/μL without platelet transfusions by 63 and 53 days after CAR-BCMA T-cell infusion in the two patients. Toxicities were managed without long-lasting complications.(5, 8)

A second anti-BCMA CAR T-cell clinical trial was conducted by bluebird bio and Celgene Inc. and was a multicenter trial.(6) Twenty-one patients with relapsed/refractory MM were treated with bb2121, an anti-BCMA CAR-T that expresses a CAR with the same scFv as the anti-BCMA CAR used at the NCI.(5, 6) In contrast to the NCI anti-BCMA CAR, bb2121 had a 4-1BB costimulatory motif, and was encoded by a lentivirus.(6) Bb2121 was administered after cyclophosphamide plus fludarabine lymphocyte-depleting chemotherapy and at planned dose levels of 50, 150, 450, 800, and 1,200 x 10$^6$ CAR+ T cells.(6) As of May 2017, 21 heavily-pretreated patients were evaluable for clinical response.(6) Of these, three patients were treated on the lowest dose level. The lowest dose level had minimal anti-myeloma activity and minimal toxicity. Patients had a median of 7 prior lines of therapy and all patients had a history of an autologous transplant.(6) Median follow-up after bb2121 infusion was 15.4 weeks.(6) There were no reported grade 3 or higher neurotoxicity events on the dose-escalation phase of this trial, but one patient did experience Grade 4 neurologic toxicity

on the expansion phase of this trial. CRS was primarily Grade 1 or 2 in 15 of 21 patients (71%); 2 patients had Grade 3 CRS that resolved in 24 hours.(6) Four patients received tocilizumab and one patient received steroids to manage CRS.(6) The ORR was 89% and increased to 100% for patients treated with doses of 150 x $10^6$ $CAR^+$ T cells or higher.(6) No patients treated with doses of 150 x $10^6$ CAR+ T cells or higher had disease progression with time of follow up between 8 and 54 weeks.(6) MRD negative results were obtained in all 4 patients evaluable for analysis.(6) Of the 18 patients treated at doses above the first dose level, the overall response rate (PR or better) was 94% and the CR rate was 56%.

Cohen et. al have published their initial findings from a phase I trial of anti-BCMA CAR-T without conditioning chemotherapy in patients with relapsed or refractory MM.(7) One patient was treated with 1.8x$10^8$ CAR-Ts, two with 2x$10^8$ CAR-Ts and three patients at the highest dose level of 5x$10^8$ CAR-Ts.(7) Toxicities were similar to those published by Ali et. al, except for an episode of grade 4 posterior reversible encephalopathy syndrome (PRES) that manifested as severe delirium, seizures, obtundation and cerebral edema.(7) This was treated with steroids, anti-epileptics, and interestingly, cyclophosphamide; there was no long-term neurological dysfunction.(7) Reported results show one patient has a stringent CR at seven months with an MRD-negative bone marrow by flow cytometry.(7) One patient who had pleural and dural MM involvement was found to have CAR-Ts cells in pleural fluid and CSF and achieved VGPR with resolution of extramedullary disease; this patient's VGPR lasted 5 months, and progression was associated with loss of BCMA expression on MM cells.(7)

Currently, a new clinical trial has opened targeting CD38 (clinicaltrials.gov: NCT03464916). CD38 is a transmembrane glycoprotein involved in cell-adhesion, signal transduction and calcium regulation.(103, 104) It is normally expressed on precursor B-cells, plasma cells, T-cells, NK-cells, and myeloid precursors.(104-106) Among non-hematologic organs, CD38 is expressed on prostate cells, in the nervous system, gut, muscle cells, and on osteoclasts.(104, 105) Two monoclonal antibodies against CD38 have been tested clinically, Daratumumab and Isatuximab.(107, 108) Although at an early stage of development, CAR T cells have great promise to improve MM treatment.

### 1.2.7 **SLAMF7**

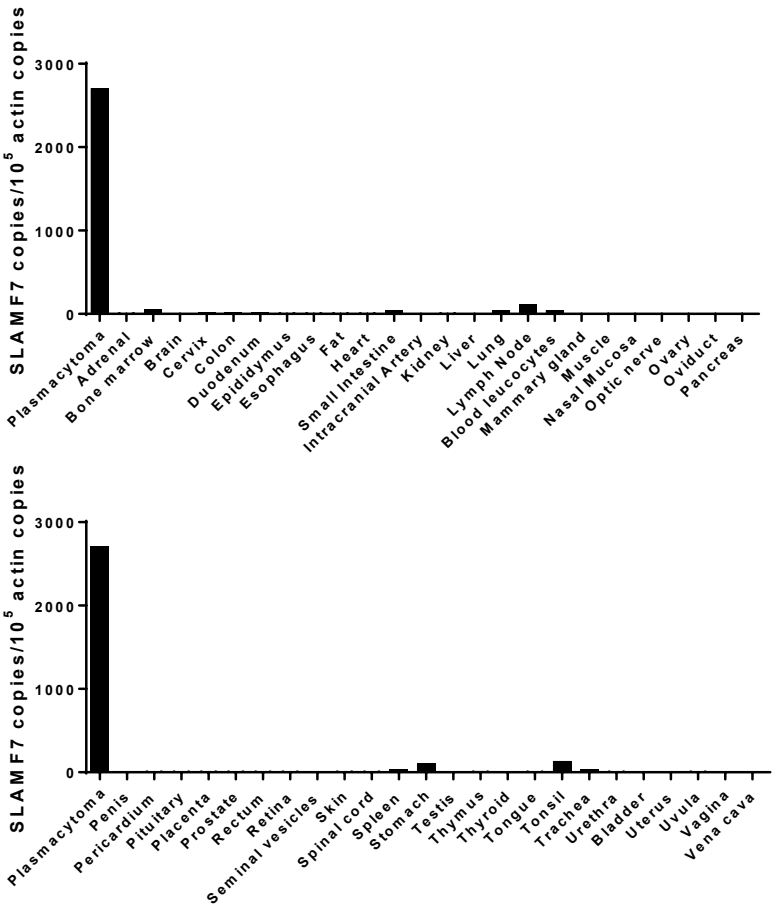
Signaling lymphocyte activation molecule 7 (SLAMF7), also known as CRACC, CS1 or CD319, is a homophilic, glycosylated cell surface protein and member of a larger signaling family of receptors involved in the effector functions of hematopoietic cells. (109-112) Receptors in this family modulate function through tyrosine switch motifs and intracellular adaptor proteins.(109, 112-114) The SLAMF7 structure is comprised of an extracellular region containing a single Ig variable-like domain and a single C2 domain, a transmembrane domain and a cytoplasmic domain with two tyrosine-based switch motifs.(111, 115)

A critical factor for any antigen to be considered as a target for immunotherapy is the antigen's expression pattern in normal tissues. Although SLAMF7 is highly expressed in almost all cases of MM it has very limited or absent expression on normal tissues aside from leukocytes such as $CD8^+$ T cells, natural killer (NK) cells, B cells, plasma cells, some monocytes, NKT cells, and some dendritic cells. (9, 10, 110, 112, 113) Notably, hematopoietic stem cells do not express SLAMF7.(111) SLAMF7 is not expressed on the surface of normal organ epithelial tissue such as

lung, kidney, stomach, brain, breast, spleen, prostate, skeletal muscle, testis, liver, ovary, heart, thymus and small intestine epithelial tissue.(9, 10, 12) In one study by Hsi et al, organs staining positive for SLAMF7 were evaluated further by peri-cellular staining to detect infiltrating leukocytes and double staining with CD138 to detect plasma cells. Results showed that organs staining positive for SLAMF7 were due to the presence of infiltrating leukocytes and plasma cells, consistent with the published expression profile of SLAMF7.(9) Plasma cells and MM cells express higher levels of SLAMF7 mRNA and protein than on any other lymphocyte subset.(9)

In Figure **1**, actin cDNA copies and SLAMF7 cDNA copies were measured by qPCR in all of the samples, and the results were expressed as the number of SLAMF7 cDNA copies per $10^5$ actin cDNA copies.

**Figure 1**




We went on to evaluate SLAMF7 protein expression in normal human organs. Except for plasma cells and rare lymphocytes, we did not detect SLAMF7 protein expression by the cells of any of the organs that we stained. We detected plasma cells expressing cell-surface SLAMF7 in gastrointestinal organs. SLAMF7 expression by normal plasma cells probably accounts for the low levels of SLAMF7 RNA detected in these organs because we did not detect SLAMF7 expression by

any of the other cells except plasma cells and some lymphocytes and rare macrophages in these organs.

**Table 1**

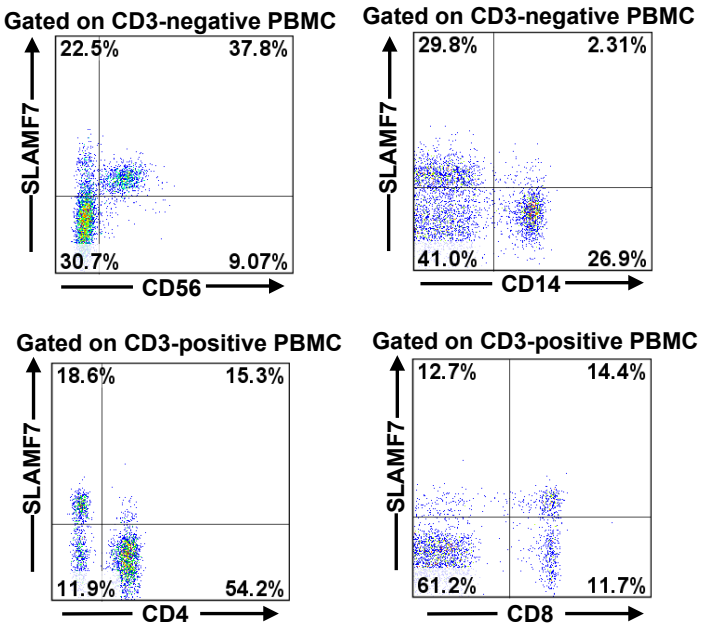
**Organs stained for SLAMF7 by immunohistochemistry and found to lack SLAMF7 expression except on plasma cells, some macrophages and some lymphocytes**

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

SLAMF7 is highly and uniformly expressed on MM tumor cells in whole blood and in the bone marrow detected by both gene expression and IHC; according to studies, 80-95% of primary myeloma cells collected from MM patients express SLAMF7.(9-13) It is not as strongly or as uniformly expressed on NK cells, $CD8^+$ T cells, B cells, monocytes or dendritic cells.(9, 10)

SLAMF7 is expressed on normal leukocytes, including NK cells and $CD8^+$ T cells. A small fraction of monocytes and $CD4^+$ T cells also express SLAMF7 (Figure 2). Figure 2 shows the results of flow cytometry analysis on unmanipulated peripheral blood mononuclear cells (PBMC). The results show that CD3-negative, $CD56^+$ NK cells almost all express SLAMF7. Also, a large fraction of $CD3^+CD8^+$ cells express SLAMF7.

**Figure 2**




SLAMF7 is expressed on MM cells independent of cytogenetic abnormalities.(116-118) Amplification of chromosome 1q23, the location of SLAMF7 gene family, is associated with more aggressive myeloma and unfavorable prognosis.(111) SLAMF7 is strongly and uniformly expressed in extramedullary plasmacytomas, lymphoplasmacytic lymphoma and bone marrow core samples of MM patients. Expression in MM persists after disease relapse and after proteasome inhibitor treatment.(111) Serum levels of soluble SLAMF7 correlate with stage and activity of MM in patients.(119) Downregulation of SLAMF7 in MM cells has been associated with reduced MM cell survival.(118) SLAMF7 may play a role in MM cell-bone marrow interactions.(111),(114)

Under normal conditions, SLAMF7 receptors play a role in regulating immune cell functions, such as cell adhesion, growth and survival; however, the receptor's full role is incompletely understood.(12, 112, 114) SLAMF7 receptor signaling may exert activating or inhibitory actions on cells depending on which lymphocyte subset is engaged and which adaptor protein is present. (9, 10, 120) For example, SLAMF7 is thought to be important for NK cell-mediated anti-viral activities and general tumor surveillance when the EAT-2 adaptor protein is present; however, SLAMF7 signaling that occurs in the absence of EAT-2 signaling in CD4 T cells, CD8 T cells and NKT cells leads to decreased proliferation and cytokine secretion.(9, 10, 120) In MM, SLAMF7 signaling lacks EAT-2 downstream activation, thus MM cells do not proliferate with receptor engagement.(117)

### 1.2.8 **Suicide Gene**

The high expression of SLAMF7 on MM cells makes it an attractive target for CARs aimed at MM, but because of the expression of SLAMF7 on normal leukocytes, a mechanism for eliminating anti-SLAMF7 CAR T cells after infusion into patients is necessary. In one pre-clinical study, cell line experiments from MM patients and healthy donors showed that on average 17.3% of NK cells,

35.4% of $CD8^+$ T cells, 66.5% of B cells and 85% of $CD4^+$ T cells were viable at the end of the fratricide assay with an anti-SLAMF7 CAR T cells.(10). Thus, to mitigate potential toxicity caused by lymphocyte depletion, the inducible caspase 9 system iCasp9 (IC9) is the preferred safety switch for this project because of its proven clinical effectiveness.(121) The IC9 system has been found to be safe and well tolerated and is the most clinically-tested of the various suicide gene systems.(121, 122)

The IC9 suicide gene system requires that T cells are genetically modified to express the IC9 gene.(121) The IC9 gene contains part of the apoptotic protein human caspase 9 protein fused to a drug binding domain derived from human FK-506 binding protein-12 (FKBP12).(123) Activation of the caspase 9 domain of the iCasp9 protein is dependent on dimerization with a small molecule drug, Rimiducid (AP1903) which cross-links the FKBP12 domains, initiating the apoptotic pathway of the T cell(122). T lymphocytes transduced with the IC9 system were shown in-vitro to retain their immune potential and can be eliminated with exposure to the dimerizing drug with high specificity and potency(122). The dimerizer drug, Rimiducid, has no reported effects on cells other than those engineered with the suicide gene.(124) The transgene is made from human products thus conferring low immunogenicity potential.(121, 124) Maximal killing occurred at about 0.3 ng/ml.(122) Pre-clinical studies with CAR T cells show that T cells engineered with the IC9 transgene were able to be eliminated within 3 days or less of administration; however, in the clinical setting, GVHD was controlled much faster than 3 days in some patients. (121, 125) An advantage of this system is that it utilizes endogenous apoptotic pathways, resulting in rapid elimination of the transduced cells.(121, 122)

In the clinical setting, the IC9 system has been shown to be safe and efficacious in a trial to rapidly control graft-versus-host-disease in patients who underwent stem cell transplantation for leukemia.(121) Five patients who had undergone haploidentical allogeneic stem cell transplantation for relapsed acute leukemia were treated with T cells engineered with the IC9 system. (121) These T cells propagated in vivo showing that the expression of IC9 did not inhibit survival or in vivo expansion of the transduced cells. Skin GVHD (four patients) and liver GVHD (one patient) developed in four patients; a single dose of dimerizing drug eliminated more than 90% of the modified T cells within 30 minutes after administration, which resulted in the end of GVHD in those patients without recurrence. (121) The dimerizing drug had no effect on the endogenous $CD3^+CD19^-$ negative untransduced T-cell population or on other blood counts. (121) Within 24 hours of infusion, GVHD abnormalities began to resolve and normalized within 24-48 hours after infusion.(121) No evidence of an immune response to the transgenic T cells was found.(121)

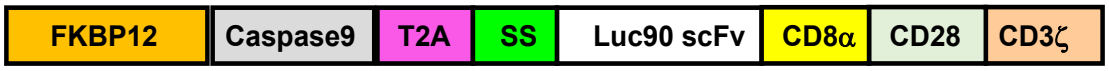
The four patients who developed GVHD in the clinical trial received 0.4 mg/kg of the dimerizing agent Rimiducid as a 2-hour infusion. (121) In a different study, pharmocokinetic data showed that the plasma concentration following administration of Rimiducid doses ranging from 0.01 to 1.0 mg/kg resulted in plasma concentrations of 10 to 1275 ng/ml with plasma levels falling to 18% of the maximum half an hour after infusion and 7% of the maximum 2 hours after the infusion.(121, 122) At these concentrations, pre-clinical studies showed little variation in the induction of apoptosis among patients with consistent elimination of more than 90% of IC9-expressing cells.[3] The terminal half life of Rimiducid is 5 hours (in vivo). (121)

### 1.2.9 **Anti-SLAMF7 CAR development and preclinical testing**

The suicide gene plus anti-SLAMF7 CAR construct to be assessed in this protocol contains the IC9 suicide gene, which is made up the of an FKBP12 dimerization domain and a caspase 9 domain, as

described in section 1.2.8, followed by a thosa asigna virus 2A (T2A) ribosomal skip domain followed by an anti-SLAM F7 CAR. The antigen recognition domain of the CAR is a single chain variable fragment from the murine Luc90 antibody. The anti-SLAMF7 CAR contains the hinge and transmembrane regions of the human CD8α molecule, the signaling moiety of the CD28 costimulatory molecule, and the signaling domains of the CD3ζ molecule. The CAR is designated Luc90-CD828Z (Figure 3). The clinical gene therapy vector encoding IC9-Luc90-CD828Z is MSGV1, which has been used in many prior clinical trials at the NCI.(126)

**Figure 3**




After transductions, we found high levels of cell surface expression of the Luc90-CD828Z anti-SLAMF7 CAR on the transduced T cells as shown in the representative example in Figure 4.

**Figure 4**

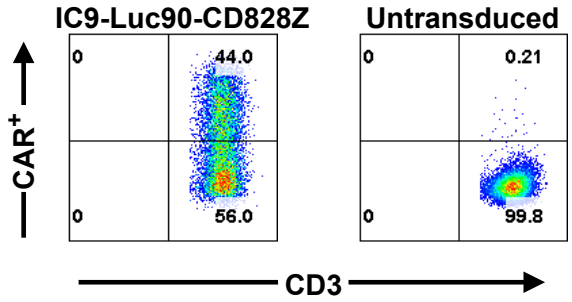



Figure 4 shows Luc90-CD828Z CAR expression on T cells from a multiple myeloma patient 5 days after transduction with gammaretroviruses encoding the Luc90-CD828Z. Transductions were carried out 2 days after the cultures were started, so the T cells had been in culture for a total of 7 days at the time of this analysis. Staining of untransduced T cells is also shown. The plots are gated on live, $CD3^+$ lymphocytes.

We also performed a series of in vitro assays to assess the function of anti-SLAMF7-CAR-expressing T cells, and we found that Luc90-CD828Z-CAR-expressing T cells exhibited SLAMF7-specific activities including CD107a upregulation and cytokine production in vitro. These experiments showed that anti-SLAMF7-CAR-expressing T cells are activated in a SLAMF7-specific manner.

## Figure 5

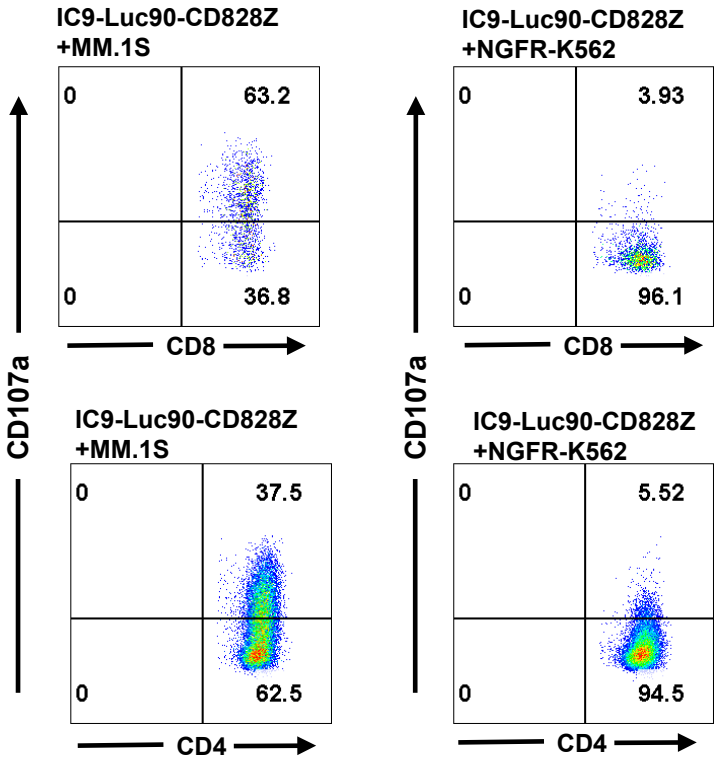



Figure 5 shows upregulation of CD107a, which indicates degranulation and correlates with cytotoxicity when Luc90-CD828Z CAR-expressing $CD8^+$ T cells (top row) or $CD4^+$ T cells (bottom row) were cultured with the SLAMF7-expressing cell line MM.1S. CD107a was not upregulated when Luc90-CD828Z-CAR-expressing T cells were cultured for 4 hours with the SLAMF7-negative control cell line NGFR-K562. Untransduced T cells did not upregulate CD107a when cultured with either MM.1S or NGFR-K562 (not shown). The plots are gated on live $CD3^+$ lymphocytes.

T cells transduced with IC9-Luc90CD828Z suicide gene plus CAR construct and T cells transduced with the Luc90CD828Z CAR alone both produced large amounts of IFNγ when they were cultured overnight with the SLAMF7-expressing cell lines SLAMF7-K562 and MM.1S (Table 2

). In contrast, the anti-SLAMF7 CAR-expressing T cells produced only background levels of IFNγ when they were cultured with the SLAMF7-negative target cell lines NGFR-K562 and CCRF-CEM. The anti-SLAMF7-CAR-transduced T cells also made minimal IFNγ when cultured without any target cell. As expected, untransduced T cells and T cells expressing the anti-CD19 CAR Hu19-CD828Z released only low levels of IFNγ in response to all target cells. The experiment depicted in Table 2

consisted of culturing the T cells with the indicated target cell lines overnight and then performing a standard IFNγ enzyme-linked immunosorbant assay (ELISA) to detect IFNγ in the culture supernatant.

**Table 2**

| T cells | SLAMF7-K562 | MM.1S | NGFR-K562 | CCRF-CEM | T cells alone | %CAR+ |
|---|---|---|---|---|---|---|
| Untransduced | 231.4 | 170.5 | 138.2 | 64.7 | 4.9 | |
| Hu19-CD828Z | 57.0 | 68.6 | 48.1 | 39.0 | 38.6 | 58.2 |
| Luc90-CD828Z | 90780.1 | 22519.4 | 22.9 | 19.2 | 18.6 | 83.0 |
| IC9-Luc90-CD828Z | 76955.3 | 30188.4 | 32.9 | 14.2 | 21.1 | 50.2 |

A second ELISA experiment performed in a similar manner utilizing T cells from a different patient is shown in Table 3

. In the experimental results shown in Table 3

, four different types of effector T cells from the same multiple myeloma patient were evaluated, untransduced T cells, T cells expressing the Hu19-CD828Z anti-CD19 CAR, T cells expressing the Luc90-CD828Z anti SLAMF7 CAR, and T cells expressing the IC9 suicide gene plus Luc90-CD828Z plus. The untransduced T cells and T cells expressing the anti-CD19 CAR expressed only background levels of IFNγ against all target cells. T cells expressing either Luc90-CD828Z or IC9-Luc90-CD828Z specifically recognized SLAMF7 as shown by the high levels of IFNγ released when the CAR-expressing T cells were cultured with the SLAMF7$^{+}$ target cells SLAMF7-K562; in contrast, the CAR-expressing T cells produced much lower levels of IFNγ when cultured with the SLAMF7-negative target cell lines A431-H9, Panc10.05, U251, COLO205, HEPG2, A549, TC71, and 624. T cells alone made low levels of IFNγ for all T cell types evaluated.

**Table 3**

| | | | | | Target cells | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T cells | SLAMF7-K562 | A431-H9 | Panc 10.05 | U251 | COLO205 | HEPG2 | A549 | TC71 | 624 | T cells | %CAR+ |
| Untransduced | 598.0 | 48.4 | 395.2 | 165.4 | 48.4 | 41.2 | 17.9 | 28.8 | 31.0 | 44.4 | 0.3 |
| Hu19-CD828Z | 521.8 | 129.5 | 367.3 | 173.3 | 74.2 | 398.4 | 87.5 | 247.1 | 144.6 | 89.4 | 29.9 |
| IC9-Luc90-CD828Z | 77961.3 | 423.8 | 390.6 | 217.1 | 496.6 | 212.2 | 349.0 | 722.1 | 662.7 | 215.7 | 42.0 |
| Luc90-CD828Z | 104728.5 | 509.7 | 526.3 | 227.5 | 749.0 | 196.8 | 447.9 | 522.9 | 823.2 | 247.5 | 68.1 |

Table 4

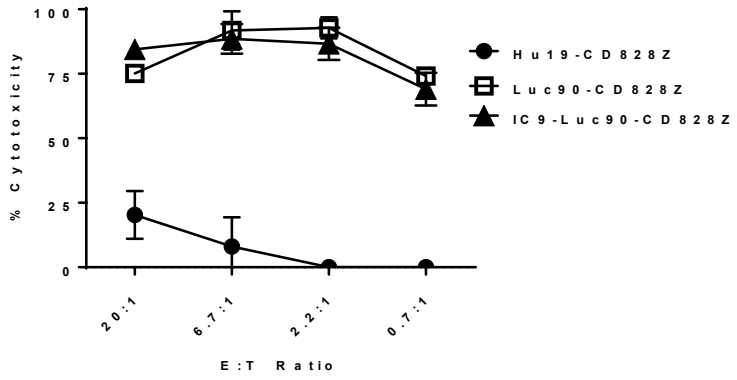
shows ELISA results from an experiment in which effector T cells were cultured in the presence of soluble SLAMF7 protein at concentrations ranging from 0 ng/mL to 200 ng/mL. Effector T cells used in this experiment were T cells from the same patient transduced with one of 2 anti-SLAMF7 CARs, either Luc90-CD828Z or Luc63-CD828Z. Untransduced T cells were also assessed. Target cells used in this experiment were either SLAMF7-RPMI (RPMI8226 cells transduced with the SLAMF7 gene) or NGFR-K562 cells, which are SLAMF7-negative. The T cells and target cells were cultured together overnight, and IFNγ was measured in the culture supernatant by a standard ELISA assay. The presence of soluble SLAMF7 protein did not block recognition of the RPMI8226 SLAMF$^{+}$ target cells. SLAMF7 protein also did not cause nonspecific activation of the T cells. All numbers are pg/mL IFNγ. The concentration of soluble SLAMF7 added is shown in ng/mL.

## Table 4

| | Target cells | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| T-cells | SLAMF7 RPMI 0 ng/ml | SLAMF7 RPMI 50 ng/mL | SLAMF7 RPMI 200 ng/mL | NGFR K562 0 ng/mL | NGFR K562 50 ng/mL | NGFR K562 200 ng/mL | T cells 0 ng/mL | T cells 50 ng/mL | T cells 200 ng/mL |
| Luc90-CD828Z | 23538 | 23315 | 21301 | 267 | 223 | 224 | 172 | 160 | 41 |
| Luc63-CD828Z | 3495 | 2873 | 3083 | 191 | 98 | 83 | 20 | 22 | 30 |
| Untransduced | 504 | 451 | 501 | 564 | 433 | 507 | 23 | 22 | 25 |
| Targets alone | 5 | | 5 | 5 | | 5 | | | |
| | | | | | | | | | |
| | 200 ng/mL soluble SLAMF7 alone: 6 | | | | | | | | |

Figure 4 presents the results of a 4-hour cytotoxicity assay in which MM.1S cells were used as target cells for either Hu19-CD828Z anti-CD19 negative-control T cells, or T cells transduced with the anti-SLAMF7 CAR Luc90-CD828Z, or T cells transduced with the IC9 suicide gene and Luc90-CD828Z CAR (IC9-Luc90-CD828Z). MM.1S is SLAMF7$^+$ and CD19-negative. The graph gives specific cytotoxicity of MM.1S cells relative to the negative-control SLAMF7-negative CCRF-CEM cell line. T cells expressing either Luc90-CD828Z or IC9 plus Luc90-CD828Z were able to cause specific cytotoxicity of the SLAMF7$^+$ cell line.

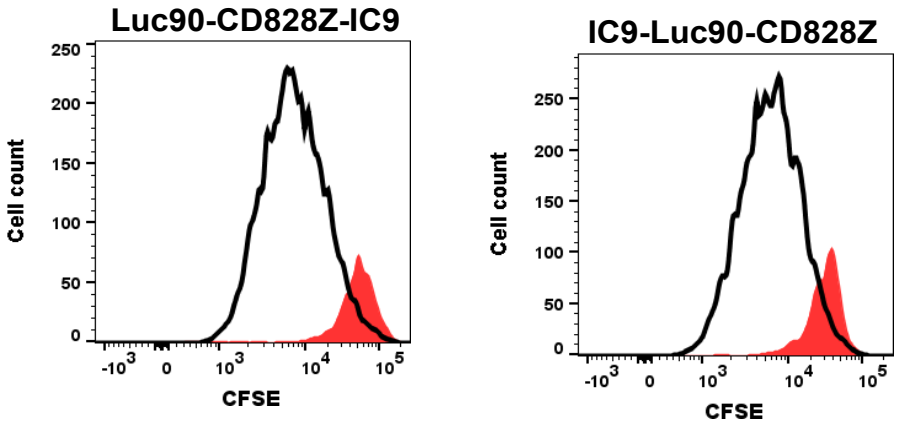
## Figure 6




Anti-SLAMF7-CAR-transduced T cells also proliferated in a SLAMF7-specific manner. Figure 7 shows a carboxyfluorescein diacetate,succinimidyl ester (CFSE) proliferation assay in which anti-SLAMF7-CAR-transduced T cells were cultured for 4 days with either SLAMF7-K562 cells or SLAMF7-negative NGFR-K562 cells. In a series of experiments, we compared 2 CAR plus suicide gene constructs. In Luc90-CD828Z-IC9, the sequence of the CAR is followed by the IC9 suicide gene. In IC9-Luc90-CD828Z, the suicide gene sequence comes first and is followed by the CAR gene. CFSE was diluted to a greater degree, indicating more proliferation, when the T cells transduced with either of the CAR plus suicide gene constructs were cultured with SLAMF7-K562 target cells (open histogram in Figure 7) compared to when T cells were cultured with SLAMF7-negative NGFR-K562 target cells (solid red histogram in Figure 7). The solid red histogram representing culture of anti-SLAMF7 CAR T cells with NGFR-K562 cells is smaller than the open

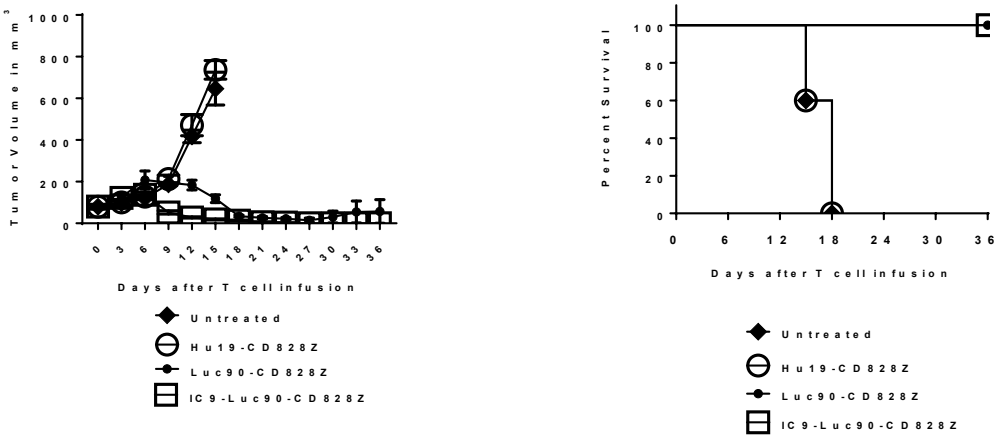
histogram representing culture of anti-SLAMF7 T cells with SLAMF7-K562 cells because fewer T cells that were cultured with NGFR-K562 were left alive at the end of the assay. The assay was conducted as described previously.(127) In multiple experiments, we consistently found equivalent in vitro proliferation of T cells expressing Luc90-CD828Z-IC9 compared with T cells expressing IC9-Luc90-CD828Z.

## Figure 7



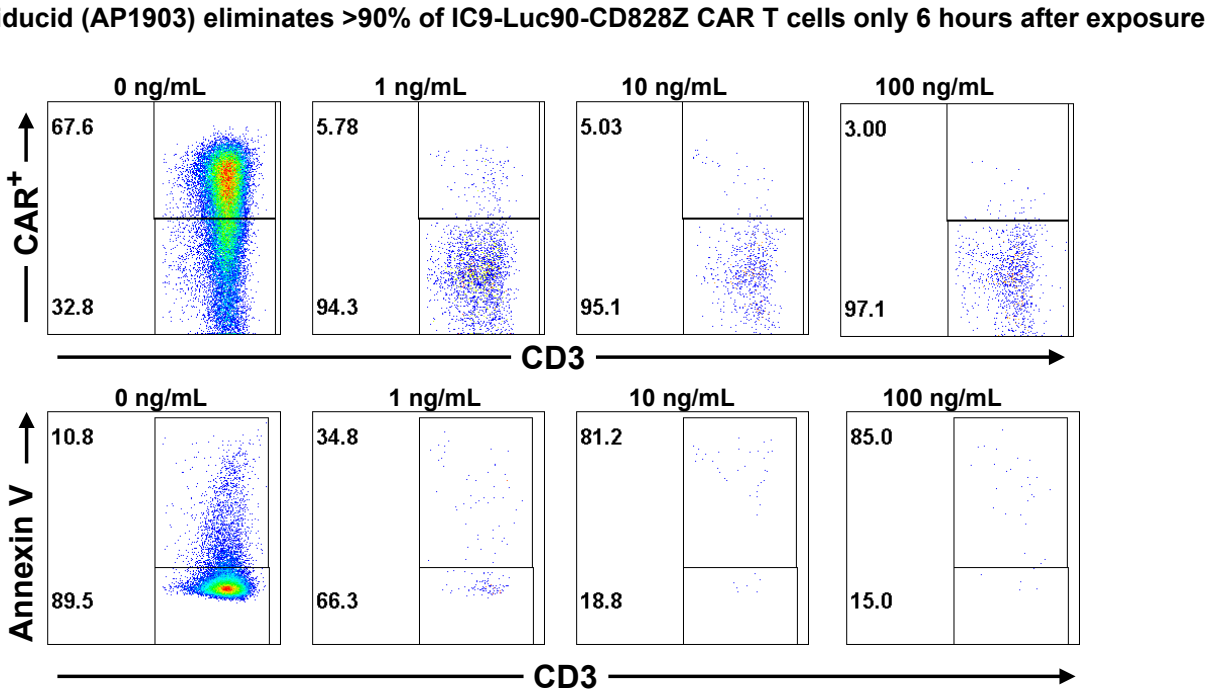

We established MM.1S human multiple myeloma cell line tumors in immunodeficient mice. We allowed sizable tumors to develop over 7 days, and then we treated the mice with a single intravenous infusion of T cells transduced with either the anti-SLAMF7 CAR Luc90-CD828Z or T cells transduced with Luc90-CD828Z plus the IC9 suicide gene. Control groups included mice treated with untransduced T cells or T cells transduced with the anti-CD19 CAR Hu19-CD828Z. After infusion of anti-SLAMF7-CAR T cells, dramatic regressions of all tumors occurred between day 6 and day 18 after the T cell infusion. In contrast, tumors continued to increase in size in all untreated mice and mice treated with the T cells expressing the Hu19-CD828Z anti-CD19 CAR. Mice receiving anti-SLAMF7-CAR T cells survived while untreated mice and mice receiving T cells expressing the anti-CD19 CAR Hu19-CD828Z developed large tumors that required that the mice be euthanized (Figure 8 ). In the experiments depicted in Figure 8, anti-SLAMF7-CAR T cells were infused on Day 0, and no other treatments were administered, and there were 5 mice in all groups.

## Figure 8



We conducted in vitro experiments to demonstrate the activity of AP1903 (Rimiducid) against T cells expressing both the Luc90-CD828Z CAR and the inactivated caspase 9 (IC9) suicide gene. We transduced T cells with MSGV-IC9-Luc90-CD828Z and then exposed them to graded concentrations of AP1903 in vitro for 6 hours prior to performing flow cytometry (Figure 9). Application of AP1903 caused a rapid disappearance of $CAR^+$ T cells, and most of the residual T cells were apoptotic as indicated by their annexin $V^+$ status (Figure 9). In a human clinical trial, the mean peak serum concentrations of AP1903 achieved with a single dose of 0.5 mg/kg of AP1903 was 626 ng/mL(122), so the in vitro concentrations that we assessed were substantially lower than clinically-achievable concentrations.

**Figure 9**




### 1.2.10 **Anti-SLAMF7 Therapy in MM:**

Elotuzumab, a humanized IgG1 kappa monoclonal antibody, in combination with lenalidomide and dexamethasone is an FDA approved therapy shown to have activity in MM in clinical trials.(117, 118, 128) Elotuzumab exerts it's effect through several mechanisms: elotuzumab impairs MM cell adhesion and exerts direct effects on primary MM cell survival in vitro; it mediates antibody-dependent cellular cytotoxicity mediated through NK cell interaction; and it directly activates NK cells through SLAMF7 receptor ligation.(9, 10, 129-131) In pre-clinical studies, elotuzumab did not exhibit cytotoxicity against NK cells.(9, 10, 129-131)

As a single agent, elotuzumab did not result in objective responses in a phase 1 trial in heavily pretreated MM.(132) At doses of 10 mg/kg and 20 mg/kg elotuzumab, SLAMF7 receptors on bone marrow-derived myeloma cells were consistently saturated.(132) Lower dose groups exhibited more variation of target cell saturation.(132) No opportunistic viral or fungal infections were seen on any dose of elotuzumab used in this phase 1 trial.(132) Transient decreases in absolute lymphocyte counts were observed after the first elotuzumab infusion only and resolved by day 7 of follow-up.(132) The transient decrease in lymphocytes (both SLAMF7 positive and negative NK cells, $CD8^+$ T cells, $CD4^+$ T cells and B cells) coincided with increase in IP-10 a chemokine that stimulates migration of activated T cells and NK cells.(132) There was no evidence of lymphocyte depletion associated with repeated dosing of elotuzumab and cytotoxicity was observed against MM cells but not against autologous NK cells.(132)

In a phase 3 trial, the combination of elotuzumab, lenalidomide and dexamethasone versus lenalidomide and dexamethasone in patients with relapsed refractory MM, resulted in a 30% decrease in the risk of disease progression or death.(117, 118, 131-133) The 1-year PFS rate in the elotuzumab cohort was 68% versus 57% in the control group.(117, 118, 131-133) The PFS was maintained at the 3 year extended follow up with a trend toward improved overall survival.(117, 118, 131-133)

In a phase 2 trial comparing bortezomib plus dexamethasone with the combination plus elotuzumab, PFS was statistically significantly longer in the elotuzumab group with a 28% reduction in the risk of progression of death.(118)

Results from pre-clinical work and clinical trials involving elotuzumab offer proof of principle that SLAMF7 may be a suitable target for future targeted T-cell therapy.

### 1.2.11 **Pre-clinical studies utilizing anti-SLAMF7 CAR T cells**

Several pre-clinical studies utilizing an anti-SLAMF7 CAR T-cell have been carried out.(10, 11, 13, 116) These studies show that T cells engineered to target SLAMF7 can be expanded and have activity in eradicating MM cells.

Several groups shown that MM cells from patients with untreated MM as well as relapsed/refractory MM express high, uniform levels of SLAMF7 on their surface despite prior proteasome inhibitor or immunomodulatory therapy.(9-11, 13, 116) Mathur et al and Wang et al report that >90% of MM cases they tested uniformly express SLAMF7.(11, 13)

Chu et al engineered an anti-SLAMF7 CAR with a CD28 costimulatory domain that was able to kill SLAMF7+ cells in MM cell lines, primary myeloma cells and mouse models.(116) The antigen density of SLAMF7+ determined the rate of killing in their experiments; in cell lines where SLAMF7 was expressed at low levels (RPMI8226), the anti-SLAMF7 CARs were not as effective at killing.(116) However, in cell lines with high expression of SLAMF7 (NCI-H929 and IM9) and in primary MM cells from patients with relapsed disease, the anti-SLAMF7 CAR was able to kill SLAMF7+ cells with high efficiency.(116) Furthermore, in a mouse model, anti-SLAMF7 CAR T cells suppressed growth and prolonged mouse survival; 44 days after treatment, their group observed a 100% survival rate for IM9 bearing mice receiving anti-SLAMF7 CAR T-cell infusion as compared with 28.6% of the control mice receiving mock T cells.(116)

Gogishvili et al created and tested anti-SLAMF7 CAR T cells with a CD28 costimulatory domain that was able to exert rapid and specific high level cytolysis of all SLAMF7+ myeloma targets in

vitro with cell lines and primary MM cells from patients with either untreated or relapsed/refractory disease.(10) The anti-SLAMF7 CAR was able to eradicate systemic myeloma in a xenograft mouse model that was sustained until the end of the 8-week observation period.(10)

Hypothesizing that T cells collected from MM patients are associated with anergy, Mathur et al have devised a system to engineer allogeneic anti-SLAMF7 CD4+ and CD8+ CAR T cells.(11) In order to decrease the potential for GVHD potentially associated with allogeneic CAR-T cells, Mathur et al have used gene editing technology to knock out endogenous TCR genes.(11) To further limit toxicity, they have included an elimination gene for safety that is activated by rituximab administration.(11) In pre-clinical studies, anti-SLAMF7 allogeneic T cells proliferate when co-cultured with myeloma cell lines and primary myeloma tumors but not in co-culture with control cells.(11) The anti-SLAMF7 allogeneic T cells eradicated established MM and induced durable remissions in mouse models.(11)

Finally, Wang et al sought to enhance the immune functions of anti-SLAMF7 CAR T cells by expanding the CAR T cells in the presence of lenalidomide in vitro.(13) Their pre-clinical results demonstrate that lenalidomide enhanced antitumor activity and persistence of the adoptively transferred anti-SLAMF7 CAR T cells in vivo mouse model including improving cytotoxicity, memory maintenance, Th1 cytokine production and immune synapse formation.(13)

Due to the expression of SLAMF7 on lymphocyte subsets and the toxicity potential with eradicating these lymphocytes with anti-SLAMF7 therapy, Gogishvili et al conducted a series of experiments aimed at determining the fratricide potential that could occur with anti-SLAMF7 CAR-T therapy.(10) They first demonstrated expression of the SLAMF7 protein on a fraction of naive and memory $CD4^+$ and $CD8^+$ T cells, NK and NKT cells, monocytes, and B cells that were obtained from the peripheral blood of MM patients.(10) After co-culture of autologous anti-SLAMF7 CAR T cells, they used flow-cytometry cytotoxicity assays to assess the fratricide potential.(10) They found that in each lymphocyte subset, a population of $SLAM^{+high}$ and $SLAM^{-/low}$ cells existed. Furthermore, engineered T cells reverted to a $SLAMF7^{-/low}$ state. $CD8^+$ SLAMF7-negative CAR T cells induced fratricide of $SLAMF7^{+high}$ cells but spared the $SLAMF7^{-/low}$ fraction of cells.(10) These $SLAMF7^{-/low}$ CD4 and CD8 lymphocytes were viable and functional based on IFN-gamma secretion after stimulation.(10) Their experiments demonstrated that on average 17.3% of NK cells, 35.4% of $CD8^+$ T cells, 66.5% of B cells, and 85% of $CD4^+$ T cells were viable at the end of the fratricide assay with SLAMF7-CAR T cells incubated with cell lines and primary MM cells.(10) Furthermore, they found that the $SLAMF7^{-/low}$ T cells were able to respond to specific antigens including CMV after incubation with anti-SLAMF7 CAR T cells.(10) Their data suggests that although anti-SLAMF7 CAR T-cell therapy may deplete lymphocytes that express high levels of SLAMF7, a subset of effector immune cells might be preserved that will provide protection against common pathogens including cytomegalovirus (CMV).(10) Similarly, Chu et al demonstrated that although their engineered anti-SLAMF7 CAR T cells were able to kill high SLAMF7 expressing MM cell lines, the CAR-T cells were unable to effectively kill cell lines that expressed low levels of SLAMF7.(116)

### 1.2.12 **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. Only patients with multiple myeloma who

have persisting or relapsed myeloma despite at least 3 prior lines of therapy will be enrolled. The risks of the study fall into six categories.

The first category is chemotherapy-related toxicity that could cause cytopenias. As with any chemotherapy that causes neutropenia and thrombocytopenia, this chemotherapy could cause toxicities such as infections and bleeding. CAR T cells might also cause cytopenias.

The second category of toxicity is cytokine-release-type toxicities such as high fevers, tachycardia, transient abnormalities of liver function and hypotension. These cytokine-release-type toxicities have been detected in other clinical trials of CAR T cells during the first two weeks after anti-CD19 CAR T cells and anti-BCMA CAR T cells were infused.(4, 5, 134)

A third category of potential toxicities are neurological toxicities such as delirium, obtundation, myoclonus, seizures, headache and transient focal neurological toxicities including aphasia and focal paresis. In previous anti-CD19 CAR trials, cytokine-release toxicities and neurological toxicities have been limited in duration with toxicities generally resolving within 2 days to 3 weeks.(5, 41, 74, 135, 136) In the NCI anti-BCMA trial, neurologic toxicities were limited to confusion or delirium in the setting of high fevers and one case of encephalopathy and muscle weakness of all extremities consistent with critical illness polyneuropathy/polymyopathy.(5) In most cases, these toxicities have been transient with toxicities generally resolving within 2 days to 2 weeks.

The fourth category of toxicity is direct damage to normal tissues by the CAR T cells. This could happen because of unexpected cross-reactivity of the CAR T cells with proteins other than SLAMF7 in vivo. This trial will be one of the first in human trials testing anti-SLAMF7 CAR T cells in patients. Although SLAMF7 is highly expressed in almost all cases of MM it has very limited or absent expression on normal tissues aside from leukocytes.(9, 10, 110, 112, 113) Hematopoietic stem cells do not express SLAMF7.(111) SLAMF7 is not expressed on the surface of normal organ epithelial tissue such as lung, kidney, stomach, brain, breast, spleen, prostate, skeletal muscle, testis, liver, ovary, heart, thymus and small intestine epithelial tissue.(9) We have performed extensive testing of the MSGV-IC9-Luc90CD828Z CAR T cells by culturing them with a variety of human cell lines and have not seen recognition of cell lines that did not express SLAMF7. In addition, we did not find any evidence of cross-reactivity in our cell line data or immunohistochemistry data from the NIH Department of Pathology.

A fifth category of toxicity that may be caused by anti-SLAMF7 T cells is impairment of normal immunity because SLAMF7 is expressed on leukocytes such as $CD8^+$ T cells, natural killer (NK) cells, B cells, plasma cells, some monocytes, NKT cells, and some dendritic cells.(5, 9, 10, 110, 112, 113) NK cell deficiencies are associated with severe, potentially life-threatening viral infections caused typically by members of the Herpesviridae (VZV, HSV, CMV, EBV) and Papillomaviridae (HPV) families.(137) Additionally, NK cells have potent cytotoxic activity against malignant cells and NK cell defects are linked to ineffective tumor surveillance and clearance. We have included a suicide gene in this trial in large part to allow elimination of anti-SLAMF7 CAR T cells in case depletion of recipient immune cells leads to infections.

Finally, genotoxicity is a theoretical risk of any type of integrating gene therapy. To our knowledge, genotoxicity, such as occurrence of a replication-competent retroviruses or transformation of T cells caused by insertional mutagenesis has never occurred in a clinical trials of T-cell gene therapies.(43,

44) The specific gene therapy vector backbone, MSGV1, proposed for use in this clinical trial has been used in hundreds of patients over the past 13+ years by our group and others.(4, 33, 138, 139)

The potential benefits to subjects enrolling on this trial include the possibility that the anti-SLAMF7-CAR T cells can cause a significant anti-myeloma effect. Many patients enrolled on trials of anti-CD19 CAR T cells and anti-BCMA CAR T cells obtained complete remissions of their advanced malignancies; there is a chance that recipients of the CAR-SLAMF7 T cells that are being evaluated in this protocol could 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 that might help future patients.

### 1.2.13 **Toxicity**

**Cytokine Release Syndrome:**

The primary acute toxicity observed to date with B cell malignancy-targeted and BCMA-targeted CAR T cells has been CRS, and this protocol will grade CRS as defined by Lee et al. (2019).(102) For this protocol, a CRS is defined as a constellation of symptoms which may include (but are not limited to) fever, chills, hypotension, dyspnea, hypoxia, confusion, mental status changes, seizures, myalgias, nausea and vomiting, and laboratory abnormalities including elevated AST, ALT, bilirubin, D-dimers, ferritin, urea and/or creatinine. Treatment of a CRS will follow the guidelines in Appendix C of this protocol.(102) Any subject with a fever ≥100.0°F should have a work up for CRS. The work-up of a CRS should include hospitalization, evaluation for an infectious etiology (e.g., blood cultures, urine culture, chest X-ray, as required), and have blood drawn for $CAR^+$ T cells and cytokine assessments along with study-outlined clinical laboratory tests.

**Immune cell depletion:**

Potential toxicities may arise from the impairment of normal immunity with anti-SLAMF7 CAR T-cell therapy because SLAMF7 is expressed on leukocytes such as $CD8^+$ T cells, natural killer (NK) cells, B cells, plasma cells, some monocytes, NKT cells, and some dendritic cells.(5, 9, 10, 110, 112, 113)

NK Cell Depletion: NK cells are lymphocytes of the innate immune system that mediate cytotoxic killing via germline encoded activation receptors.(140) SLAMF7 is expressed on all NK cells; however, it is expressed on $CD56^{dim}$NK cells uniformly and at a higher level than on $56^{bright}$NK cells.(9, 112, 114, 120, 141) $CD56^{dim}$NK cells comprise about 90% of circulating NK cells and are thought to be the mature subset of NK cells; they have the most potent cytotoxic effects.(140, 142) $CD56^{bright}$NK cells are thought to represent immature NK cells that are precursors of the $CD56^{dim}$ subset; $CD56^{bright}CD16^-$ NK cells cultured in the presence of skin fibroblasts differentiate into and have the characteristic phenotype and functional features of peripheral blood $CD56^{dim}$NK cells.(140, 142) Gogishvili et al observed that 17% of NK cells were still viable after incubation with anti-SLAMF7 CAR T cells.(10) Hematopoietic stem cells do not express SLAMF7. Thus, in the presence of peripheral destruction of NK cells by anti-SLAMF7 CAR T cells, the bone marrow should be able to produce new NK cells. In addition, $CD56^{bright}$NK cells may differentiate into cytotoxic NK cells with appropriate stimulation.(140, 142)

Although it is unclear to what level NK cell depletion will occur with anti-SLAMF7 CAR T-cell therapy, there is a risk of clinically relevant NK cell deficiency occurring with this therapy. NK cells

are important for protection against viral infections and in tumor cell surveillance; recently, their importance in immunoregulation and coordination of immunity has been elucidated.(140) NK cell deficiencies are associated with severe, potentially life-threatening viral infections caused by typically benign members of the Herpesviridae (VZV, HSV, CMV, EBV) and Papillomaviridae (HPV) families.(137) Additionally, NK cells have potent cytotoxic activity against malignant cells and NK cell defects are linked to ineffective tumor surveillance and clearance.(143) During malignant transformation of cells, host-stress molecules are upregulated; NK cells can recognize these antigens, leading to destruction of the transformed cell. (143)

Therapeutic approaches to NK cell depletion focus on monitoring for viral infections and malignant transformation as well as prophylactic treatment with antiviral drugs that target the Herpesviruses such as acyclovir, ganciclovir and related agents.(137) Prior to enrollment, all patients will be screened for current HSV/CMV/EBV infections by history and through laboratory work. Once enrolled, patients will have CMV PCR checked prior to treatment initiation, once a week during required hospitalization and at each follow-up appointment. Patients will be treated with standard anti-viral agents. If a patient were to have a severe infection from a member of either the Herpesviridae or Papillomaviridae families associated with NK cell deficiency the patient will be treated with Rimiducid, the dimerizer drug, that will signal apoptosis of the active anti-SLAMF7 CAR T cells.

CD8+ T-cell depletion: Depletion of CD8+ T cells may occur with anti-SLAMF7 therapy. Gogishvili et al observed that 35.4% of CD8+ T cells were still viable after incubation with anti-SLAMF7 CAR T cells.(10) In addition, the viable T cells were found to have specificity and able to exact killing of viral pathogens such as CMV.(10) Depletion of $CD8^+$ T cells may reduce the capacity for cytotoxic killing of pathogens.

Monocyte and dendritic cell depletion: A small subset of monocytes and some dendritic cells express SLAMF7. We do not anticipate a complete depletion of monocytes from anti-SLAMF7 CAR T cell therapy; however, patients will be carefully monitored for signs of infections. Peripheral monocytes will be measured daily during hospitalization and at each follow-up visit.

B cell depletion: A potential toxicity caused by anti-SLAMF7 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 and anti-BCMA T cells.(4, 41) We do not expect the same level of B cell depletion as prior anti-CD19 CAR T-cell trials as only a fraction of B-cells express SLAMF7. Hypogammaglobulinemia in patients treated with anti-CD19 CAR T-cell trials were routinely infused with intravenous immunoglobulins.(4)

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

We plan to administer a conditioning chemotherapy regimen of cyclophosphamide and fludarabine before infusions of CAR-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.(14, 16, 144) 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.(14, 16, 34, 35, 144, 145) 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.(14, 16, 145, 146) 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.(14) Experiments in a murine xenograft model showed that regulatory T cells could impair the anti-tumor efficacy of anti-CD19 CAR T cells.(102) Myeloid suppressor cells have been shown to inhibit anti-tumor responses.(146) 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.(15) 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.(15)

Strong suggestive evidence of enhancement of the activity of adoptively-transferred T cells has been generated in humans.(20, 39, 147) 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.(39, 42) 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.(3, 4, 41, 60) 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.(3, 4, 40, 41, 147, 148) Both cyclophosphamide and fludarabine are highly effective at depleting lymphocytes.(147, 148) One well-characterized and commonly used regimen is the combination of 300 mg/m$^2$ of cyclophosphamide administered daily for 3 days and fludarabine 30 mg/m$^2$ administered daily for three days on the same days as the cyclophosphamide.(149) Multiple cycles of this regimen can be tolerated by heavily pretreated leukemia patients.(74, 149)

The chemotherapy regimen that will be used in this protocol is cyclophosphamide 300 mg/m$^2$ and fludarabine 30 mg/m$^2$ given daily on days -5 to -3 followed by an infusion of CAR T cells on day 0.(5) This same chemotherapy regimen has been used in previous clinical trials.

### 1.2.15 **Rationale for dose-escalation and starting dose**

The clinical trial described in this protocol is planned as a dose escalation in which the number of anti-SLAMF7 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.(150-152) Second, in the setting of allogeneic transplantation, relapsed malignancy is often treated with infusions of allogeneic donor lymphocytes (DLIs).(153, 154) 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'.(153, 154)

The starting CAR T-cell dose of $0.66x10^6$ $CAR^+$ T cells/kg is based on experience in past CAR clinical trials. The $0.75x10^6$ $CAR^+$ T cells/kg dose is considered a dose lower than that at which substantial toxicity has been observed. The following clinical trials were used to guide the starting dose of this trial. Note that it was our goal to use a dose lower than the MTD of prior trials. NCI protocol 09-C-0082 (Kochenderfer et al Journal of Clinical Oncology, 2017), a trial of anti-CD19 CAR T cells had an MTD of $2x10^6$ $CAR^+$ T cells/kg.(74) NCI protocol 14-C-0168, a trial of anti-BCMA CAR T cells had an MTD of $9x10^6$ $CAR^+$ T cells/kg.(5) NCI protocol number 16-c-0054, a trial of anti-CD19 CAR T cells started at a dose of 0.66x106 CAR T cells/kg, and the maximum dose administered on this trial was $6x10^6$ CAR T cells/kg. A multicenter trial of anti-BCMA CAR T cells conducted by bluebird bio and Celgene Inc. determined that a flat dose of 150-450 $x10^6$ $CAR^+$ T cells was the optimal dose for the expansion phase of the study (unpublished results). The 450 x $10^6$ flat dose of CAR T cells would be $4.5x10^6$ $CAR^+$ T cells/kg for a 100 kg patient.

# 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 canceled or delayed until eligibility criteria are met.

### 2.1.1 Inclusion Criteria

#### 2.1.1.1 Multiple Myeloma criteria

- SLAMF7 expression must be detected on malignant plasma cells from either bone marrow or a plasmacytoma by flow cytometry or immunohistochemistry. A specific quantitative level of SLAMF7 expression for eligibility is not specified, but patients with multiple myeloma cells that are negative for SLAMF7 by flow cytometry and immunohistochemistry will not be enrolled. These assays must be performed at the National Institutes of Health (NIH). It is not required that the specimen used for SLAMF7 determination comes from a sample that was obtained after the patient's most recent treatment. If paraffin embedded unstained samples of bone marrow involved with MM or a plasmacytoma are available, these can be shipped to the NIH for SLAMF7 staining, otherwise new biopsies will need to be performed for determination of SLAMF7 expression.
- SLAMF7 expression will need to be documented on the majority of malignant plasma cells by flow cytometry at the NIH at some time after the original CAR-SLAMF7 T-cell infusion in all patients undergoing a second CAR-SLAMF7 T-cell infusion on this clinical trial.
- Bone marrow plasma cells must make up less than or equal to 50% of total bone marrow cells based on a bone marrow biopsy performed within 24 days of the start of protocol treatment.
- Patients must have received at least 3 different prior treatment regimens for multiple myeloma
- Must have prior exposure to an immunomodulatory drug (IMiD) such as lenalidomide and a proteasome inhibitor

- Patients must have measurable MM as defined by at least one of the criteria below.
  - Serum M-protein greater or equal to 0.6 g/dL.
  - 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 at least 1.5 cm in largest dimension
  - Bone marrow core biopsy with 30% or more plasma cells

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 sexes must be willing to practice birth control from the time of enrollment on this study and for four months after last day of receiving protocol treatment.
- 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.)
- A patient with a negative blood PCR test for hepatitis B DNA test can be enrolled. If hepatitis B DNA (PCR) testing is not available, patients with a negative hepatitis B surface antigen and negative hepatitis B core antibody can be enrolled.
- Patients must be tested for the presence of Hepatitis C antigen by PCR and be HCV RNA negative in order to be eligible. Only if Hepatitis C PCR testing is not available in a timely manner, patients who are Hepatitis C antibody-negative can be enrolled.
- Absolute neutrophil count greater than or equal to 1000/$mm^3$ without the support of filgrastim or other growth factors within the previous 10 days.
- Platelet count greater than or equal to 55,000/$mm^3$ without transfusion support in the past 14 days.
- Hemoglobin greater than or equal to 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 1.5 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 or cytopenias).

- Because this protocol requires collection of autologous blood cells by leukapheresis in order to prepare CAR-SLAMF7 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 14 days 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.
- For patients with past participation in gene-therapy, cryopreserved PBMC that have not been genetically-engineered must be available.
- Patients receiving prior gene therapy outside of NIH will not be eligible. Patients who previously received CAR T-cell therapy at the NCI will be potentially eligible.

2.1.2 **Exclusion criteria:**

2.1.2.1 Patients who are receiving any other investigational agents.

2.1.2.2 Patients on any anticoagulants except aspirin

2.1.2.3 Patients that require urgent therapy due to tumor mass effects or spinal cord compression.

2.1.2.4 Patients that have active hemolytic anemia.

2.1.2.5 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.

2.1.2.6 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. Women of child bearing potential cannot have a positive pregnancy test. Women of child-bearing potential are defined as all women except women who are post-menopausal or who have had a hysterectomy. Postmenopausal will be defined as women over the age of 55 who have not had a menstrual period in at least 1 year.

2.1.2.7 Active systemic infections (defined as infections causing fevers or requiring anti-microbial treatment), active coagulation disorders or other major uncontrolled illnesses of the cardiovascular, respiratory, endocrine, renal, gastrointestinal, genitourinary, neurologic, psychiatric, or immune system, history of myocardial infarction, active cardiac arrhythmias, active obstructive or restrictive pulmonary disease.

2.1.2.8 Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).

2.1.2.9 Systemic corticosteroid steroid therapy of greater than 5 mg/day of prednisone or equivalent dose of another corticosteroid (prednisone, dexamethasone, etc.) is not allowed within 14 days prior to either the required leukapheresis or within 14 days prior to CAR T-

cell infusion (and at any time after the CAR T cell infusion unless approved by the Principal Investigator or an Associate Investigator).

2.1.2.10 History of severe immediate hypersensitivity reaction to any of the agents used in this study.

2.1.2.11 Patient unwilling to undergo intensive care unit treatment including mechanical ventilation, cardiopulmonary resuscitation, vasoactive drugs, and hemodialysis.

2.1.2.12 History of allogeneic stem cell transplantation

2.1.2.13 Patients with currentspinal cord compression (without intradural myeloma involvement.

2.1.2.14 Patients who have a history (or current evidence) of cerebrospinal fluid multiple myeloma, or intra-dural central nervous system masses.

2.1.2.15 Patients with active autoimmune skin diseases such as psoriasis or other active autoimmune diseases such as rheumatoid arthritis.

2.1.2.16 Patients must not have required supplemental oxygen within the past month unless it was for a resolved infection.

### 2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on the NIH websites and on NIH social media forums, or from other patients through social media platforms. Participants will be recruited from the current patient population at NIH, and local community physicians.

## 2.2 SCREENING EVALUATION

### 2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes.

### 2.2.2 Screening activities performed after a consent for screening has been signed

Note: Screening evaluation testing/procedures are conducted under the separate screening protocol, 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols).

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, vital signs, ECOG, details about the exact size and location of any evaluable lesions that exist.
- Confirmation of diagnosis of MM by the NCI Laboratory of Pathology and confirmation of SLAMF7 expression on malignant plasma cells from either bone marrow or a plasmacytoma by flow cytometry or immunohistochemistry. The sample used for this SLAMF7 expression analysis can come from any time prior to enrollment on the protocol.
- Bone marrow aspirate and biopsy; specifically ask for SLAMF7 immunohistochemistry staining of the bone marrow biopsy. Order cytogenetics with interphase FISH (pretreatment aspirate only) and flow cytometry on the bone marrow aspirate. Specifically request 2 separate bone holes for bone marrow aspiration. The aspirate for hemepath (0.5 mL) and flow (2-2.5 mL) should be done from 1 bone hole and the aspirate for research (2-2.5 mL) should be done from a second bone hole followed by an aspirate for cytogenetics (2-4 mL) from the second bone hole. Note: Only bone marrow aspirates done before CAR T-cell treatment and 2 weeks after CAR T-cell infusion require aspirates through 2 bone holes. All other aspirates only need to be done through 1 bone hole.

  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 the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10, 3W-3808, Phone: 240-858-3755 Bone marrow aspirate cells and supernatant will be cryopreserved. As many vials as possible with 10 million cells or less will be cryopreserved, and 2 vials of supernatant with 0.5 to 1 mL each will be cryopreserved. A bone marrow biopsy must be performed within 24 days of the start of the protocol-required conditioning chemotherapy. If this screening bone marrow aspirate and biopsy is performed within 24 days of the start of the conditioning chemotherapy, a second baseline bone marrow biopsy does not need to be performed.
- Flow cytometry staining of bone marrow or plasmacytoma cells for SLAMF7 should be performed by Dr. Stetler-Stevenson's lab (NCI Laboratory of Pathology).
- 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)
- MRI of the brain
- CT scans of areas with possible lesions/plasmacytomas. This may include areas such as the head, neck, chest, abdomen and pelvis. (performed only if necessary, to document measurable malignancy in the event of no other miserable disease in blood, urine and bone marrow).
- Venous assessment for apheresis (Valid for up to 6 months before apheresis collection, does not need to be performed if cells have been previously collected)
- DNA (PCR) for Hepatitis B and C; antibody testing for HIV, HTLV-I/II, T. cruzi (Chagas agent), West Nile, and syphilis (RPR), only if DNA testing is not available, order Hepatitis C antibody, order Hepatitis B core antibody, and hepatitis B surface antigen.
- EKG
- Echocardiogram

- (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), 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
- 24-hour urine collection with immunofixation electrophoresis as clinically indicated
- Glucose-6-phosphate dehydrogenase (G6PD) deficiency screening
- TSH, T4, and T3
- Serum Cortisol
- PT/PTT
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential

### 2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

### 2.4 TREATMENT ASSIGNMENT PROCEDURES FOR THE REGISTRATION PURPOSES ONLY:

**Cohorts:**

| Number | Name | Description |
|---|---|---|
| 1 | Cohort 1 | Subjects enrolled to determine the MTD |
| 2 | Cohort 2 | Subjects enrolled after the MTD has been identified |

**Arms:**

| Number | Name | Description |
|---|---|---|
| 1 | Conditioning chemotherapy plus CAR T-cells dose escalation | Patients will receive escalating doses (up to 4 planned) of Anti-SLAMF7-CAR+ T cells infused on day 0 + Cyclophosphamide: 300 mg/m$^2$ IV infusion over 30 minutes on days -5, -4 and -3 + Fludarabine: 30 mg/m$^2$ IV infusion over 30 minutes administered immediately following the cyclophosphamide on days -5, -4, and -3 |

| Number | Name | Description |
|---|---|---|
| 2 | Conditioning chemotherapy plus CAR T-cells expansion phase | MTD dose of Anti-SLAMF7- CAR T Cells + Cyclophosphamide: 300 mg/m$^2$ IV infusion over 30 minutes on days -5, -4 and -3 + Fludarabine: 30 mg/m$^2$ IV infusion over 30 minutes administered immediately following the cyclophosphamide on days -5, -4, and -3 |

**Arm Assignment:**

Patients in cohort 1 will be directly assigned to arm 1

Patients in cohort 2 will be directly assigned to arm 2

# 3 STUDY IMPLEMENTATION

## 3.1 STUDY DESIGN

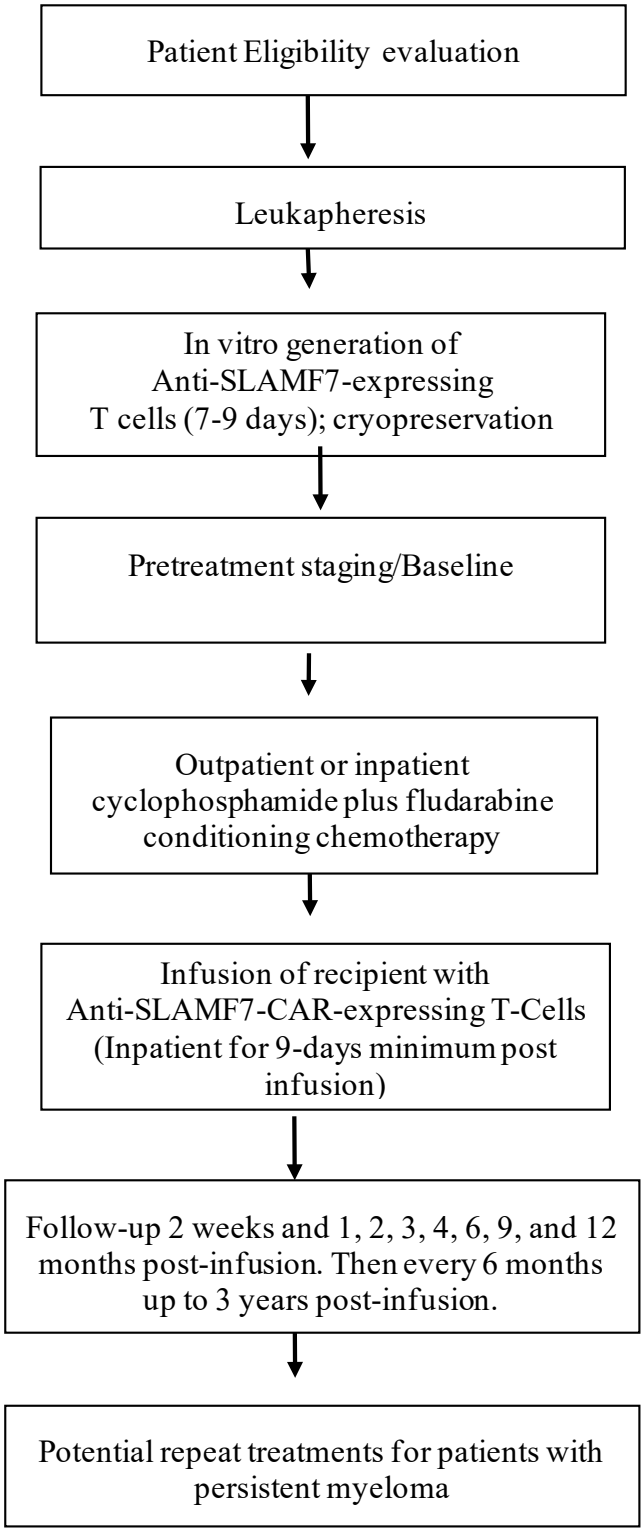
### 3.1.1 General study plan

This protocol is a phase I dose-escalation study of autologous T cells that are genetically modified to express an anti-SLAMF7 CAR.

The protocol will enroll patients with multiple myeloma previously treated with 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 SLAMF7 expression will be an important part of the eligibility screening. Patients enrolled on the study will undergo leukapheresis, and anti-SLAMF7-CAR-expressing T cells will be generated by transducing the patient's T cells with a gamma retrovirus encoding the anti-SLAMF7 CAR. Patients will receive a conditioning chemotherapy regimen of cyclophosphamide 300 mg/m$^2$ daily for 3 days and fludarabine 30 mg/m$^2$ 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-SLAMF7-CAR-expressing T cells. A minimum 9-day hospitalization will be required after the cell infusion to monitor closely for acute toxicities. It is expected that some patients will need to be hospitalized for up to 30 days to allow all toxicities to resolve to the point that discharge from the hospital is prudent. 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. They will be followed every 6 months afterwards for at least 3 years.

A small number of subjects may be eligible for re-enrollment if a patient is removed from the protocol before completing protocol treatment; or the patient is going to receive a second treatment. These patients 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 reenrollment study period. Any cryopreserved anti-SLAMF7 CAR T 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.

### 3.1.2 **Protocol schema**



### 3.1.3 **Dose Limiting Toxicity-the dose limiting toxicity assessment period is 28 days.**

DLTs are defined as follows (Note that DLTs will not occur in patients who do not receive CAR T-cell infusions but do receive the conditioning chemotherapy):

- Non-cardiac and non-neurologic **Grade 3 toxicities** possibly or probably or definitely related to the CAR T cells and **lasting more than 9 days** with exceptions below.
- Grade 4 toxicities possibly or probably or definitely related to the CAR T cells.
- Grade 5 toxicities possibly or probably or definitely related to CAR T cells.
- Grade 3 hypotension or hypoxia (Appendix F) occurring in the setting of grade 3 CRS (as graded according to Appendix F) and lasting more than 72 hours will be a DLT for the first 2 subjects on study.
- **Grade 3 cardiac and neurologic adverse** events lasting more than 72 hours will be DLTs except for those included in the list below.

The following grade 3 and 4 toxicities will not be DLTs:

- Neutropenia (ANC≤500/μL) lasting 10 days or less is not a DLT
- Neutropenia with an ANC>500/mL is not a DLT
- Anemia (Hgb<8 g/dL) lasting 10 days or less is not a DLT
- Anemia with Hgb greater than or equal to 8 g/dL is not a DLT even if blood transfusions are required
- Transfusion-dependent thrombocytopenia lasting 28 days or less is not a DLT
- Thrombocytopenia that is not transfusion-dependent is not a DLT
- Hypotension requiring continuous treatment with 3 μg/minute or less of norepinephrine for 72 hours or less is not a DLT.
- Grade 3 hypotension or hypoxia occurring in the setting of CRS (Appendix F) lasting more than 72 hours in patients treated after the first two patients on study will not be a DLT.
- Fever is not a DLT.
- All cytopenias except neutropenia, anemia, and thrombocytopenia are not DLTs
- Asymptomatic electrolyte disturbances regardless of grade are not DLTs
- Prolonged QT interval as long as ventricular arrhythmias do not occur is not a DLT
- Grade 3 creatine kinase elevation is not a DLT

- Infections controlled by antibiotics are not DLTs
- Grade 3 concentration impairment is not a DLT
- Grade 3 dysesthesia is not a DLT
- Grade 3 dysphasia is not a DLT
- Grade 3 headache is not a DLT
- Grade 3 memory impairment is not a DLT
- Grade 3 paresthesia is not a DLT
- Grade 3 syncope (only 1 uncomplicated resolved event) is not a DLT
- Grade 3 atrial fibrillation is not a DLT
- Grade 3 sinus bradycardia is not a DLT
- Grade 3 sinus tachycardia is not a DLT

### 3.1.4 **Dose Escalation**

The trial will be a dose-escalation with 4 dose levels based on the patient's **actual** bodyweight. **Each dose will be limited to the number of cells that would be administered up to a 120 kg patient.**

| **Dose Escalation Schedule** | |
|---|---|
| **Dose Level** | Dose of IND Agent |
| Level -1 | $0.3x10^6$ Anti-SLAMF7-CAR + T cells per kg of recipient bodyweight |
| Level 1 | $0.66x10^6$ Anti-SLAMF7-CAR + T cells per kg of recipient bodyweight |
| Level 2 | $2.0x10^6$ Anti-SLAMF7-CAR + T cells per kg of recipient bodyweight |
| Level 3 | $6.0x10^6$ Anti-SLAMF7-CAR + T cells per kg of recipient bodyweight |
| Level 4 | $12.0x10^6$ CAR+ T cells per kg of recipient bodyweight |

A minimum of 3 patients will be enrolled at each dose level. 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.

There will be a 28-day interval between the first and second patient treated on study. A minimum of 14 days will elapse between the second and third patient on the first dose level. After completing the 1st dose level, we will contact the FDA about potentially having a 14-day interval between the first and second patient on subsequent dose levels on a case by case basis.

If sufficient cells cannot be grown to meet the criteria for the assigned dose level, the treatment will be cancelled. 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. If insufficient cells are left over from the first cell production attempt, a second apheresis will be needed.

Should none of the first 3 patients treated on a dose level experience a DLT, the first patient can be infused on the next higher dose level after a 28-day delay following CAR T-cell infusion 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. If 1/6 patients have a DLT at a particular dose level, the first patient can be infused on the next higher dose level after a 28-day delay following CAR T-cell infusion of the 6th 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 MTD.

Any dose level with 3 treated patients and no DLTs can be expanded to 6 patients at the PI's discretion to better understand toxicity of this dose level prior to proceeding to the next higher dose level. After the 6 patients are treated, the dose can be escalated to the next higher dose level if 0/6 or 1/6 DLTs occur. If 2 or more DLTs occur, the cell dose must be reduced to the next lower dose level.

If 2 of 3 patients on Dose level 1 experience DLTs, accrual will proceed on dose level -1.

The MTD is the dose at which a maximum of 1 of 6 patients has a DLT. After an MTD is defined, additional patients can be treated at the MTD. Up to 12 total additional recipients can be treated after an MTD is established to more completely characterize toxicity of the MTD. If cell growth limitations preclude administration of the MTD, the patient will receive as many cells as possible up to the MTD. If it proves to be technically impossible or impractical to achieve the higher dose levels due to cell production constraints and an MTD 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 |

| Number of Patients with DLT<br><br>at a Given Dose Level | Escalation Decision Rule |
|---|---|
| | 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 | Enter up to 3 more patients at this dose level. If 0 of these 3 patients experience DLT, proceed to the next dose level. 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. |
| ≤1 out of 6 at the highest dose or 1 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 have a delay in planned treatment if they have active infections defined as infections causing fevers or infections requiring intravenous 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. Exceptions to this would be if the grade 3 toxicity was present at baseline or related to progressing disease.

## 3.3 DRUG ADMINISTRATION

### 3.3.1 Leukapheresis

The patient will undergo a 15 to 20 - liter leukapheresis will be processed 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-SLAMF7-CAR-expressing T-cell preparation

After cells are obtained by apheresis, further cell processing to generate 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 a complete cell production will be retained in the Department of Transfusion Medicine; the excess cells will be sent to the Surgery Branch Cell Production Facility (SB-CPF), Bldg. 10, Room: 3W-3808, Phone: 240-858-3755 for cryopreservation at 100 to 300 million PBMC per vial. These cells will be used in research. The anti-CD3 monoclonal antibody OKT3 will be used to stimulate T-cell proliferation. Two days after the start of the T-cell cultures, the cells will be transduced by exposing them to a supernatant containing replication-incompetent gamma retroviruses encoding the CAR. The cells will continue to proliferate in culture. The total cell production process will take 7 to 9 days before cells are cryopreserved. Ten vials of the infused cells will be cryopreserved and stored in the Surgery Branch Cell Production Facility SB-CPF. Each vial will contain 10- 20 million cells.

Before infusion, the percentage of T cells expressing the CAR will be determined by flow cytometry, and this percentage of CAR 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 either be disposed of or transferred from the Department of Transfusion Medicine to the Principal Investigator of this protocol for storage in the Surgery Branch Cell Production Facility (SB-CPF) and possible use in research.

### 3.3.3 Conditioning chemotherapy and CAR-SLAMF7 T-cell administration-chemotherapy administration can be either inpatient or outpatient

#### 3.3.3.1 Overall summary of the treatment plan

| Drug | Dose | Days |
|---|---|---|
| Cyclophosphamide | 300 mg/m$^2$ IV infusion over 30 minutes | Daily x 3 doses on days -5, -4, -3 |
| Fludarabine | 30 mg/m$^2$ 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-SLAMF7-CAR T cells | Variable. | One-time dose on day 0 |

3.3.3.2 Detailed treatment plan

**Note that patients must meet still meet protocol enrollment criteria with the exception that a platelet count as low as 50,000/µL is allowed based on laboratory results from 7 days or less and physical examination within 3 days or less before chemotherapy starts.**

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.

On days -5, -4, and -3, cyclophosphamide at a dose of 300 mg/m$^2$ 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$^2$ I.V. fludarabine in 100 mL 0.9% sodium chloride over 30 minutes. **Note: in patients with an estimated creatinine clearance of 30-70 mL/minute/1.73m$^2$, the daily dose of fludarabine will be reduced by 20% (see 11.3.6).** Creatinine clearance will be estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.

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: 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 using an **infusion pump**, 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 Appendix D.

Days 1 to 9: Mandatory minimal hospitalization for observation and treatment as necessary. Note: hospitalization for CAR T-cell patients is routinely extended; extension of hospitalization beyond the required 9 days is anticipated for CAR T-cell toxicity management. 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 C.

A CBC with differential 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 **Rimiducid administration**

Rimiducid may be administered a under the following conditions at the discretion of the Principal Investigator:

- Significant herpes virus infections such as cytomegalovirus infections, herpes zoster, or other significant viral infections in the setting of a peripheral blood NK cell count less than the lower limit of normal as determined on lymphocyte phenotyping (TBNK) performed at the NIH Department of Laboratory Medicine. "Significant" will be determined by the Principal Investigator.
- Significant organ damage possibly, probably or definitely attributable to CAR T cells. "Significant" will be determined by the Principal Investigator.
- Severe cytokine-release syndrome not responsive to high-dose corticosteroids of 200 mg of methylprednisolone within 1 hour. If a participant has Cytokine Release Syndrome that improves after the Rimiducid is given, methylprednisolone will be discontinued.
- Neutrophil count <500/μL lasting 7 to 9 days (Rimiducid can be given anywhere from 7 to 9 days of neutrophil count<500/μL)
- Transfusion-dependent thrombocytopenia lasting 21 to 23 days (Rimiducid administered anytime between 21 and 23 days of transfusion dependence)
- Bleeding due to thrombocytopenia or life-threatening infections in the setting of neutropenia.

**Infusion of Rimiducid:** 0.4 mg/kg of Rimiducid is infused in 100 mL of normal saline over 2 hours. Patients <50 kg or >150 kg, may have different volume than 100 mL but will still be infused over 2 hours.

A second dose of Rimiducid will be given when 2 criteria are met: **1.** An indication for a 1st dose of Rimiducid persists at least 48 hours after the first dose. **2.** CAR T cells are still detected in the patient's blood 1 day after the first dose of Rimiducid.

**Premedication for Rimiducid Infusion:** Patients will receive acetaminophen 650 mg orally and diphenhydramine 12.5 mg IV approximately 30-60 minutes prior to start of Rimiducid infusion.

### 3.3.5 **Potential repeat treatment**

Retreatment will only be considered on a case by case basis after consultation with the IRB and FDA and after the maximum tolerated or maximum administered dose is determined.

## 3.4 PROTOCOL EVALUATION

### 3.4.1 Baseline evaluations and interventions

Bone marrow aspirate and biopsy must be performed within 24 days prior to the start of the conditioning chemotherapy. If the screening bone marrow aspirate and biopsy were done within 24 days of the start of conditioning chemotherapy, the baseline bone marrow aspirate and biopsy should not be done unless clinically indicated. Specifically ask for SLAMF7, BCMA, CD3, CD8, and CD4 immunohistochemistry staining of the bone marrow biopsy. Order cytogenetics with interphase FISH (pretreatment only) and flow cytometry on the bone marrow aspirate. Specifically request 2 separate bone holes for bone marrow aspiration. The aspirate for hemepath (0.5 mL) and flow (2-2.5 mL) should be done from 1 bone hole and the aspirate for research (2-2.5 mL) should be done from a second bone hole followed by an aspirate for cytogenetics (1-2 mL) from the second bone hole. Note: Only bone marrow aspirates done before CAR T-cell treatment and 2 weeks after infusion require aspirates through 2 bone holes. All other bone marrow aspirates only need to be done through 1 bone hole. For all bone marrow aspirates, bone marrow supernatant must be collected and frozen in the same manner as serum is collected and frozen.

SLAMF7 immunohistochemical staining is performed in the NIH Dept. of Laboratory Medicine with the anti-SLAMF7 antibody clone 3B3, LSBio part number LS-C340266.

**The following tests must be completed within 14 days prior to 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 another device.

- Physical exam with vital signs and oxygen saturation
- PET scan in all patients to measure boney disease and soft tissue plasmacytomas for safety reason.
- CT scans of neck, chest, abdomen and pelvis in all patients. CT scans of other locations will be performed clinically to image only areas of previously known plasmacytomas. If area of known plasmacytoma was imaged at the NIH within 14 days, CT scan of that area will not be repeated.
- MRI only if needed to define measurable multiple myeloma plasmacytomas staging or spine lesions that not evaluable completely by CT)
- 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 low serum cortisol or other clinical indications (This may be performed after start of chemotherapy as long it is performed before the cell infusion)
- 24-hour urine collection with immunofixation electrophoresis
- 24-hour urine collection for urine protein
- Serum immunofixation electrophoresis

- Blood CMV PCR
- 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)
- 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 the Surgery Branch Cell Production Facility (SB-CPF), Bldg. 10, Room: 3W-3808, Phone: 240-858-3755.
- 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 the Figg lab; For sample pick-up, page 102-11964.

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 CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK (creatine kinase), Uric Acid (to be repeated on the first day of the chemotherapy at the discretion of the PI)
- β2-microglobulin
- ABO typing
- Ionized calcium
- CBC with differential and platelet count
- PT/PTT
- Fibrinogen
- Urinalysis; if results are abnormal, send for urine culture
- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential
- C-reactive protein (CRP)

3.4.2 **Studies to be performed on Day 0 and 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 9 with differential every 24 hours. After day 9 do a CBC with differential daily until discharge. (In the case of a later day infusion or early discharge, this may be only once a day.)
- TBNK on the day of CAR T-cell infusion (day 0), day 7 and day 14 after infusion
- Immunoglobulin free light chains day 9 (+/- 1 day) after infusion
- Serum immunofixation electrophoresis (if patient had a measurable M-protein prior to treatment) day 9 (+/- 1 day) after infusion
- Chemistries twice a day starting from day 0 to day 9. After day 9 do chemistries once a day until discharge: (Sodium (Na), Potassium (K), Chloride (Cl), Total $CO^2$ (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Uric Acid, creatine kinase. (In the case of a later day infusion or early discharge, this may be only once a day.) All twice daily labs can be reduced to once daily after 9 days following CAR T-cell infusion.
- PT/PTT and fibrinogen daily while hospitalized
- **Day 0 Research Blood:** 1 SST tube will be drawn on the morning of CAR cell infusion prior to infusion of CAR T cells. Send to the Figg lab; for sample pick-up, page 102-11964.
- **Post-infusion 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 CAR-SLAMF7 -transduced T cells, 56 mL of patient peripheral blood will be obtained (6 CPT tubes 8 mL each and 1 SST tube 8 mL). Send CPT tubes to the Surgery Branch Cell Production Facility SB-CPF. Bldg. 10 3W-3808. Phone: 240-858-3755. Send SST tubes to the Figg lab; for sample pick-up, page 102-11964
- **Additional Post-infusion research blood:** 1 SST tube will be drawn on the first Sunday after CAR T-cell infusion. This tube may be stored refrigerated on the nursing unit and processed first thing Monday morning at the latest. Send to the Figg lab; For sample pick-up, page 102-11964.
- **Rimiducid Administration Research Blood:** Draw the below samples immediately before Rimiducid administration, 1 day post Rimiducid , and 3 days post Rimiducid
    - 6 CPT tubes of Research Blood (48 mL) will be collected to obtain blood for immunological testing. Send to the Surgery Branch Cell Production Facility (SB-CPF), Bldg. 10, Room: 3W-3808, Phone: 240-858-3755

- 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. Send to the Figg lab; For sample pick-up, page 102-11964.

### 3.4.3 Post-infusion outpatient evaluation

After completion of therapy the patient will be followed for potential complications related to CAR-T-cell infusion. The patient will be seen at the NIH in follow-up to evaluate disease status and late problems related to a CART-cell infusion at days +14 (+/- 1 day), +30 (+/- 5 days), +60(+/- 7 days), +90(+/- 7 days), and +120(+/- 7 days); and at 180(+/- 14 days), 270 (+/- 14 days), and 365 (+/- 30 days) months after CART-cell infusion. After 12 months, the patient will be seen every 6 months (+/- 30 days) up to three years. After 3 years total follow-up, patients will be, transferred to the long-term follow-up protocol 15-C-0141. All of these time points have allowable ranges (see Table in section **3.5**). 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. Send to the Surgery Branch Cell Production Facility (SB-CPF), Bldg. 10, Room: 3W-3808, Phone: 240-858-3755

- 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. Send to the Figg lab; For sample pick-up, page 102-11964.Note: **after the first year of follow-up, research blood will be reduced to 4 CPT tubes (32 mL total) during required protocol visits**.
- **Rimiducid Administration Research Blood:** Draw the below samples immediately before Rimiducid administration, 1 day post Rimiducid, and 3 days post Rimiducid
    - 6 CPT tubes of Research Blood (48 mL) will be collected to obtain blood for immunological testing. Send to the Surgery Branch Cell Production Facility (SB-CPF), Bldg. 10, Room: 3W-3808, Phone: 240-858-3755.
    - 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. Send to the Figg lab; For sample pick-up, page 102-11964.
- PET scans will be performed at 3 and 12, 24 and 36 months after cell-infusion.
- CT scans of areas with tumors/plasmacytomas will be performed at 1, 2, 4, 6, 9, 12, 18, 24, 30 and 36 months after CAR T-cell infusion (only to evaluate known soft-tissue plasmacytomas ). This may include areas such as the head, neck, chest, abdomen and pelvis. CT scans will NOT be performed in areas where there are no known tumor plasmacytomas at baseline unless clinically indicated or if there is concern for a new plasmacytoma that would constitute progressive disease.
- MRIs will be performed to evaluate tumors not evaluable completely by CT, especially to follow measurable myeloma staging or spine lesions
- Physical exam with vital signs and oxygen saturation

- Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT, AST, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid
- PT/PTT, fibrinogen
- TBNK
- Ionized calcium
- CBC with differential
- C-reactive protein (CRP)
- Blood for serum β2-microglobulin
- Blood for immunoglobulin free light chains
- Blood for serum immunofixation electrophoresis
- 24-hour urine collection with immunofixation electrophoresis timed urine (can be omitted if not needed for staging after day 30).
- 24-hour urine collection with protein timed urine (can be omitted if not needed for staging after day 30).
- Urinalysis: if results are abnormal, send for urine culture
- At the 2-week follow-up, bone marrow aspirate and biopsy must be performed. Aspirate must be sent for flow cytometry to the lab of Dr. Maryalice Stetler-Stevenson. SLAMF7 and BCMA staining must be requested for the flow cytometry. Also, request flow cytometry for anti-SLAMF7 CAR T cells. SLAMF7, BCMA immunohistochemistry should also be requested on the bone marrow biopsy. Specifically ask for SLAMF7, BCMA, CD3, CD8, and CD4 immunohistochemistry staining of the bone marrow biopsy. Specifically request 2 separate bone holes for bone marrow aspiration. The aspirate for hemepath (0.5 mL) and flow (2-2.5 mL) should be done from 1 bone hole and the aspirate for research (2-2.5 mL) should be done from a second bone hole.
- 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. SLAMF7 and BCMA staining must be requested for the flow cytometry. Also, request flow cytometry for anti-SLAMF7 CAR T cells. SLAMF7 and BCMA immunohistochemistry should also be requested on the bone marrow biopsy. This may also be performed at day +30 if clinicallyindicated.Specifically ask for SLAMF7, BCMA, CD3, CD8, and CD4 immunohistochemistry staining of the bone marrow biopsy. The aspirate for hemepath (0.5 mL), flow (2-2.5 mL) and research (2-2.5 mL) can be done from 1 bone hole.
- At the 6-month follow-up appointment, a bone marrow aspirate and biopsy will be performed. Aspirate must be sent for flow cytometry to the lab of Dr. Maryalice Stetler-Stevenson. SLAMF7 and BCMA staining must be requested for the flow cytometry. Also, request flow cytometry for anti-SLAMF7 CAR T cells. SLAMF7 and BCMA immunohistochemistry should also be requested on the bone marrow biopsy. Specifically

ask for SLAMF7, BCMA, CD3, CD8, and CD4 immunohistochemistry staining of the bone marrow biopsy. The aspirate for hemepath (0.5 mL), flow (2-2.5 mL) and research (2-2.5 mL) can be done from 1 bone hole.

- At all time-points, bone marrow biopsies might be done to evaluate for MM progression or to investigate cytopenias.
- For each bone marrow aspirate performed, send one tube of bone marrow aspirate to the Surgery Branch Cell Production Facility (SB-CPF), Bldg. 10, Room:3W-3808, Phone: 240-858-3755. Bone marrow cells should be cryopreserved, and the liquid bone marrow supernatant should also be saved at -80 degrees. As many vials as possible with 10 million cells or less will be cryopreserved, and 2 vials of supernatant with 0.5 to 1 mL each will be cryopreserved.
- Gene-therapy-specific follow-up must be carried out as described in section **3.6**

## 3.5 STUDY CALENDAR

| | | | Inpatient | | Follow up | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Procedures[a] | Screening | Baseline | Day 0 | Post Cells while inpt[c] | Day+ 14 (+- 1 day) | Day+ 30 (+/- 5 days) | Day +60 (+/- 7 days) | Day +90 (+/- 7 days) | Day +120 (+/- 7 days) | Day +180 (+/- 14 days) | Day +270 (+/- 14 days) | Day +365 (+/- 30 days) | Every 6 months after day 365(+/- 30 days) up to 3 years post CAR cells. |
| Clinical Assessments | | | | | | | | | | | | | |
| *History and PE* | X | X[b] | X[c] | X[c] | X | X | X | X | X | X | X | X | X |
| *Vital signs, O2 saturation* | X | X | X[c] | X[c] | X | X | X | X | X | X | X | X | X |
| *Weight* | X | | | | | | | | | | | | |
| *Performance Score (ECOG)* | X | | | | | | | | | | | | |
| *Lesion Location (If applicable)* | X | | | | | | | | | | | | |
| *Pathology Confirmation* | X | | | | | | | | | | | | |
| Laboratory Assessments | | | | | | | | | | | | | |
| *CBC with differential* | X | X | X[c] | X[c] | X | X | X | X | X | X | X | X | X |
| *Sodium, Potassium Chloride, CO2, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT,* | X | X | X[c] | X[c] | XX | X | X | X | X | X | X | X | X |

| | | | Inpatient | | Follow up | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Procedures[a] | Screening | Baseline | Day 0 | Post Cells while inpt[c] | Day+ 14 (+- 1 day) | Day+ 30 (+/- 5 days) | Day +60 (+/- 7 days) | Day +90 (+/- 7 days) | Day +120 (+/- 7 days) | Day +180 (+/- 14 days) | Day +270 (+/- 14 days) | Day +365 (+/- 30 days) | Every 6 months after day 365(+/- 30 days) up to 3 years post CAR cells. |
| *AST, T. Bilirubin, D. Bilirubin, LDH, Total Protein, CK, Uric Acid* | | | | | | | | | | | | | |
| *Serum C-reactive protein* | | X | X[c] | X[c] | X | X | X | X | X | X | X | X | X |
| *PT/PTT* | X | X | X[c] | X[c] | X | X | X | X | X | X | X | X | X |
| *Fibrinogen* | | X | X[c] | X[c] | X | X | X | X | X | X | X | X | X |
| *Ionized calcium* | | X | | | X | X | X | X | X | X | X | X | X |
| *TBNK* | | X | X | X[d] (D7) | X | X | X | X | X | X | X | X | X |
| *Urinalysis (culture prn)* | | X | | | X | X | X | X | X | X | X | X | X |
| *CMV PCR (Blood)* | | X | | | | | | | | | | | |
| *Anti-CMV, HSV, EBV, T.cruzi, toxoplasmosis* | X | X[e] | | | | | | | | | | | |
| *PCR for HCV and HBV; antibody test for HIV, HTLV-I/II, T.cruzi, West Nile, RPR* | X[f] | | | | | | | | | | | | |
| *TSH, T4, T3* | X | | | | | | | | | | | | |

| | | | Inpatient | | Follow up | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Procedures[a] | Screening | Baseline | Day 0 | Post Cells while inpt[c] | Day+ 14 (+- 1 day) | Day+ 30 (+/- 5 days) | Day +60 (+/- 7 days) | Day +90 (+/- 7 days) | Day +120 (+/- 7 days) | Day +180 (+/- 14 days) | Day +270 (+/- 14 days) | Day +365 (+/- 30 days) | Every 6 months after day 365(+/- 30 days) up to 3 years post CAR cells. |
| *G6PD* | X | | | | | | | | | | | | |
| *Serum Cortisol* | X | | | | | | | | | | | | |
| *ABO typing* | | X | | | | | | | | | | | |
| *B2-HCG (serum or urine in women of childbearing potential)* | X | X | | | | | | | | | | | |
| MM Lab Assessments | | | | | | | | | | | | | |
| *β2-microglobulin* | | X | | | X | X | X | X | X | X | X | X | X |
| *24 hr urine* with immunofixation *electrophoresis* and urine 24 hour protein | X | X | | | X | X | X[g] | X[g] | X[g] | X[g] | X[g] | X[g] | X[g] |
| *Serum immunoglobulin free light chains* | X | X | | X[h] (D9) | X | X | X | X | X | X | X | X | X |
| Serum immunofixation electrophoresis | X | X | | X[h] (D9) | X | X | X | X | X | X | X | X | X |
| Radiological Assessments | | | | | | | | | | | | | |
| CT scan of disease affected areas | X[z] | X[y] | | | | X[i] | X[i] | | X[i] | X[i] | X[i] | X[i] | X[i] |
| PET scan | | X[w], | | | | | | X[v] | | | | X [w] | |

| | | | Inpatient | | Follow up | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Procedures[a] | Screening | Baseline | Day 0 | Post Cells while inpt[c] | Day+ 14 (+- 1 day) | Day+ 30 (+/- 5 days) | Day +60 (+/- 7 days) | Day +90 (+/- 7 days) | Day +120 (+/- 7 days) | Day +180 (+/- 14 days) | Day +270 (+/- 14 days) | Day +365 (+/- 30 days) | Every 6 months after day 365(+/- 30 days) up to 3 years post CAR cells. |
| *Brain MRI* | X | | | | | | | | | | | | |
| *MRI* | X[i] | X[i] | | | | X[j] | X[j] | X[j] | X[j] | X[j] | X[j] | X[j] | X[j] |
| Other Specific Assessments | | | | | | | | | | | | | |
| *250 microgram cosyntropin test* | | X[k] | | | | | | | | | | | |
| *EKG, echocardiogram* | X | | | | | | | | | | | | |
| *RCR, Gene Therapy Follow Up* | | X | | | | | | X | | X | | X | X[l] |
| *Central Venous catheter placement* | | X | | | | | | | | | | | |
| *Venous Assessment for Apheresis* | X[m] | | | | | | | | | | | | |
| Response Evaluation | | | | | | | | | | | | | |
| *Bone marrow aspirate/biopsy*[n, o, p] | | X[q] | | | X | X[x] | X | | | X | | | |
| *Research Blood* | | X[r] | X[s,, t] | X[s, t] | X[t, u] | X[t, u] | X[t,, u] | X[t,, u] | X[t,, u] | X[t,, u] | X[t,, u] | X[t, u] | X[t,, u] |
| *Adverse Events* | | | X | X | X | X | X | X | X | X | X | X | X |
| *Concomitant Medications* | | X | X | X | X | X | X | X | X | X | X | X | X |

a. see section 2.2 and section 3.3.1 for details
b. Medical history not required at baseline
c. see section 3.4.2 for details of testing during hospitalization

d. TBNK is performed on D7
e. 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
f. only if DNA testing is not available, order Hepatitis C antibody, order Hepatitis B core antibody, and hepatitis B surface antigen.
g. Urine electrophoresis can be omitted after D30 if not needed for staging
h. Draw immunoglobulin free light chains and serum immunofixation electrophoresis on day 9 after CAR T-cell infusion (electrophoresis if M-protein at baseline).
i. if useful for response assessment to evaluate known multiple myeloma plasmacytomas that identified at screening or baseline clinically except for day 30 and 120.Note: only day 30 and day 120 CT scan will be done on patients who had known plasmacytoma 1.5 cm.
j. MRIs will be done only to evaluate tumors not evaluable completely by CT, especially to establish measurable myeloma
k. only if clinical suspicion of adrenal insufficiency
l. see section 3.4.3 for details
m. Venous assessment valid for 6 months before cell collection
n. bone marrow biopsy will include flow cytometry, SLAMF7 expression, cytogenetics (pretreatment bone marrow aspirate only), amyloid staining (pretreatment bone marrow biopsy only), research samples, and molecular studies of each sample. Note that 2 separate bone holes will be required for the pretreatment and 2-week follow-up bone marrow aspirates. All other bone marrow aspirates will only require 1 bone hole.
o. Additional bone marrow biopsies and aspirates might be needed if clinicallyindicated due to low blood counts or for myeloma response assessment.
p. A bone marrow aspirate and biopsy with flow cytometry will also be performed to confirm suspected complete remission.
q. Bone marrow biopsy does not need to be repeated for baseline if done previously within 24 days
r. Baseline Research Blood (See section 3.4.1)
s. Inpatient Research Blood (See section 3.4.2)
t. Rimiducid Administration Research Blood (See section 3.4.2 and 3.4.3)
u. Follow up Research Blood (See section 3.4.3)
v. PET research scan to evaluate MM in all patients
w. To measure boney disease and soft tissue plasmacytomas for safety reason
x. Only if clinicallyindicated
y. CT scan of neck, chest, abdomen and pelvis in all patients for to measure plasmacytoma staging will not be repeated if done within 14 days of protocol treatment start
z. CT scan of areas with possible lesions/plasmacytomas (this research scan only be done per if needed to establish measurable disease). If outside CT scans of plasmacytomas are available within 30 days, we can use these images to establish measurable disease.

## 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 treatment protocol. Physical examinations will be performed and documented annually for 5 years following cell infusion to evaluate long-term safety. After 3 years, patients who are still on this study will be transferred to the long-term gene therapy protocol NCI protocol 15-c-0141 to complete long-term gene-therapy follow-up.
- 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 or <0.1% of PBMC (whichever is higher). 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.
- S+L- culture-based replication-competent retrovirus testing will be conducted on the infusion CAR T cells of all patients

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

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days after 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. Off-treatment criteria applies to eligibility for potential repeat treatments; and also, cancellations of treatment for toxicity arising any time before the cell infusion.

Patients will be taken off treatment for the following:

- Any DLT makes patients ineligible for repeat treatments.
- The patient started chemotherapy but cannot complete the entire treatment (ending with completed cell infusion) for any reason specified in the protocol or PI discretion. If the reason that the patient is no longer eligible can be rapidly resolved within 1 day, the patient can proceed on treatment, otherwise the patient must come off treatment.
- The patient receives any other treatment for multiple myeloma except bisphosphonates and treatment on this protocol. Bisphosphonates are allowed when administered to prevent osteopenia more than 2 months after the CAR T-cell infusion.
- 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 (all patients coming off study must enroll on the long-term gene therapy follow-up protocol 15-c-0041):

- The patient completes the study upon reaching 3 years after CAR T-cell infusion.
- The patient completes a safety visit 30 day after last administration of study treatment (only for patients who have received chemotherapy but will not receive a cell infusion)
- The patient voluntarily withdraws
- There is significant patient noncompliance
- PI discretion
- Death
- Development of progressive or relapsed multiple myeloma after CAR T cell infusion.
- The patient receives any anti-myeloma therapy except bisphosphonates. Bisphosphonates are allowed 2 months or more after the CAR T-cell infusion.
- The patient no longer meets the eligibility criteria for the protocol after enrolling but before start of the chemotherapy conditioning regimen. In this case, treatment will be delayed until the patient meets eligibility criteria, or if the patient will never likely meet eligibility criteria, the patient will be removed from the study. An exception to this is that a platelet count of 50,000 or more is considered adequate to start chemotherapy. This platelet level is slightly lower than that required for initial protocol enrollment.

## 3.8 PROTOCOL STOPPING CRITERIA

- If no responses of PR or CR occur after 2 patients are treated on the highest dose level, the protocol will be stopped.
- Instructions for how to proceed when toxicity occurs will be as instructed by the dose escalation section of the protocol.
- A death on study not attributable to progressive malignancy within 30 days of a cell infusion for the initial 5 subjects will be a cause for a pause to accrual pending amendment of the protocol approved/reviewed by the FDA and NIH Intramural IRB.
- If 3 or more of the first 9 treated patients experience grade 4 toxicity possibly or probably attributable to CAR T cells within 30 days of cell infusion, this will be a cause for a pause to accrual to reassess the safety of the product pending amendment of the protocol approved/reviewed by the FDA and NIH Intramural IRB.

# 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 atovaquone or inhaled pentamidine. Patients with a CD4 T-cell count less than 200 will also be maintained on acyclovir or valacyclovir.

  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. 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.

- Neutropenic patients will start on broad spectrum antibiotics with a first fever of 38.3 C 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/mm3. 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 2000/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 Principal Investigator.

### 4.6 TOXICITY MANAGEMENT GUIDELINES

Guidelines for management of common CAR toxicities including cytokine release syndrome (CRS) and neurologic toxicity are in section Appendix C. Rimiducid will be given as described in Section 3.3.4.

## 5 BIOSPECIMEN COLLECTION

Biospecimen collection on this protocol will consist of blood draws and acquisition of bone marrow aspirates and possible biopsies of plasmacytomas for research purposes. Blood and bone marrow collection is described above in Sections 2.2, 3.4, and 3.5.

### 5.1 CORRELATIVE STUDIES FOR RESEARCH

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

- One heparinized syringe containing 2-2.5 mL of bone marrow aspirate to be sent to the Surgery Branch Cell Production Facility (SB-CPF), Bldg. 10, 3W-3808, Phone: 240-858-3755. It will be used in functional assays to see if CAR-SLAMF7 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. Surgery Branch Cell Production Facility (SB-CPF), Bldg. 10, 3W-3808, Phone: 240-858-3755
- 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 the SST tubes will be sent to Figg lab; For sample pick-up, page 102-11964An apheresis is required to obtain cells used to prepare the 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 Day 0 and after 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 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). Also, 1 SST tube (8 mL) will be drawn on the morning of CAR cell

infusion prior to infusion of CAR T cells. 1 SST tube (8 mL) will be drawn on the first Sunday after CAR T-cell infusion. The 1 SST tube will be sent tto the Figg lab; For sample pick-up, page 102-11964. The 6 CPT tubes sent to Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808, Phone: 240-858-3755.

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 CAR T-cell infusion. After the 12-month follow-up appointment patients will return for follow-up every 6 months up until 3 years after infusion. 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. Send CPT tubes to Surgery Branch Cell Production Facility SB-CPF, Bldg. 10 3W-3808, Phone: 240-858-3755.
  - 1 SST tube (8 mL) of Research Blood will be obtained for serum collection. The SST tubes will be sent to Figg lab. For sample pick-up, page 102-11964.

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

- At the 2-week, 2 months and 6- month follow-ups, bone marrow aspirate and biopsy will be performed 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 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 months after infusion, and CAR+ T cell analysis will continue until the CAR+ T cell level drops to undetectable levels or levels reach <0.1% of PBMC. 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 might be used to measure the SLAMF7-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 or similar assays.

- A human anti-mouse ELISA assay will be performed retrospectively on serum collected before administration of anti-SLAMF7 CAR T cells and 2 to 3 months after anti-SLAMF7 CAR T-cell administration for all patients who previously received treatment involving any CAR derived from a murine antibody including previous treatment with the IC9-Luc90-CD828Z CAR used in this study.
- 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 does not need to be banked for gene-therapy monitoring after the 12-month post-infusion time-point. 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 SB 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 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 **Biospecimen collection with Rimiducid administration:**

If Rimiducid is infused, we will collect research blood (6 CPT tubes and 1 SST tube) for monitoring CAR T-cell levels immediately before the infusion and 1 day and 3 days after the infusion.

### 5.1.6 **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 NCI Laboratory 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.

### 5.1.7 Future studies

Blood and tissue specimens collected during 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 Samples Sent to Figg Lab

- Venous blood samples will be collected in either a 4-mL or an 8-mL SST tube to be processed for serum and stored for future research.
    - Record the date and exact time of draw on the tube. Blood tubes may be kept in the refrigerator until pick-up.
    - For sample pick-up, page 102-11964.
    - For immediate help, call 240-760-6180 (main Blood Processing Core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).
    - For questions regarding sample processing, contact the Blood Processing Core (BPC) at NCIBloodcore@mail.nih.gov or 240-760-6180.
    - The samples will be processed, barcoded, and stored in the Figg lab until requested by the investigator.

After delivery to the SB-CPF, peripheral blood mononuclear cell samples will be sent to the Head, Clinical Support Laboratory Clinical Services Program, Applied/Developmental Directorate Frederick National Laboratory for Cancer Research for processing and cryopreservation. They will be stored long-term at the NCI Frederick Repository.

### 5.2.2 Sample Storage, Tracking and Disposition

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. All samples will be sent to Blood Processing Core (BPC) and/or SB-CPF for processing and/or and storage until they are distributed to the designated place of analysis as described in the protocol.

Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

#### 5.2.2.1 Samples Managed by Dr. Figg's Blood Processing Core (BPC)

##### 5.2.2.1.1 BPC Contact Information

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

##### 5.2.2.1.2 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) (Dr. Figg's lab) will be barcoded, with data entered and stored in the LABrador (aka Lab Samples) utilized by the BPC. This is a secure program, with access to LABrador limited to defined Figg lab personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All Figg lab personnel with access to patient information complete the NIH online Protection of Human Subjects course, in the required time frame.

LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

Sample bar-codes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the LABrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

##### 5.2.2.1.3 Sample Storage

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80□C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB-approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed.

### 5.2.3 Sample Storage, Tracking, and Disposition for Surgery Branch

Samples received by the Surgery Branch research lab will be tracked using password protected web-based NCI database Lab matrix. All specimens will be tracked for date of receipt in the Surgery Branch lab, date analyzed, date returned to the originating hospital and/or date destroyed. Specimens will be stored in a locked laboratory cabinet or refrigerators in a locked research lab. All specimens will be entered into Lab matrix with identification and storage location. Access to the stored specimens will be restricted. Access to Lab matrix will be granted upon PI approval only. It is the responsibility of the NCI PI to ensure that the specimens are being used and stored in a manner consistent with IRB approval. All samples are stored in monitored freezers/refrigerators in 3NW NCI-SB laboratories at specified temperatures with alarm systems in place.

### 5.2.4 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol.

If the patient withdraws consent, the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reports will be made per the requirements of section 7.2.

### 5.2.5 Samples for Genetic/Genomic Analysis

No genetic/genomic analysis will be performed.

# 6 DATA COLLECTION AND EVALUATION

## 6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system, C3D and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. 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.

All ongoing AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Document AEs from the first study intervention, Study Day 1, through the end of treatment. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

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.

**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, this will be reported expeditiously per requirements in section **7.2.1**.

### 6.1.1 Adverse event recording:

- Grade 1 adverse events will not be recorded.
- Grade 2 adverse events that will be recorded:
    a. Adverse events regardless of attribution will be recorded for the first year after the infusion.
    b. Unexpected events that are possibly, probably, or definitely related to the research will be recorded after the first year.
- All grade 3, 4, and 5 adverse events will be recorded regardless of attribution.
- All serious events regardless of attribution

## 6.2 DATA SHARING PLANS

### 6.2.1 Human Data Sharing Plan

**What data will be shared?**

I will share human data generated in this research for future research as follows:

_x__ Coded, linked data in an NIH-funded or approved public repository.

_x__ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)

_x__ Coded, linked or identified data with approved outside collaborators under appropriate agreements.

**How and where will the data be shared?**

Data will be shared through:

_x__ An NIH-funded or approved public repository. Insert name or names: ClinicalTrials.gov.

_x__ BTRIS (automatic for activities in the Clinical Center)

_x__ Approved outside collaborators under appropriate individual agreements.

_x__ Publication and/or public presentations.

**When will the data be shared**

_x__ Before publication.

_x__ At the time of publication or shortly thereafter.

## 6.3 GENOMIC DATA SHARING PLAN

Genomic data will not be generated.

## 6.4 RESPONSE CRITERIA

Responses will be categorized by using the International Uniform Response Criteria for Multiple myeloma 2016 updated version.(155) 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.

### 6.4.1 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.

#### 6.4.1.1 Definition of measurable disease(24)

Any one or combination of these abnormalities defines measurable disease:

- Serum M-protein greater or equal to 0.6 g/dL (6 g/L).
- Urine M-protein greater or equal to 200 mg/24 h.
- Serum FLC assay: involved FLC level greater or equal to10 mg/dL (100 mg/L) provided serum FLC ratio is abnormal.
- Presence of a biopsy-proven plasmacytoma

6.4.1.2 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 urbidometry 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)

6.4.1.3 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 at baseline and followed for response with CT scans or MRI scans by measuring target lesions with the sums of the products of the diameters method. For purposes of defining CR, masses greater than 1.5 cm will be considered abnormal.

6.4.2 **International Myeloma Working Group uniform response criteria:**

6.4.2.1 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 (only1 bone marrow evaluation is needed).

6.4.2.2 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

6.4.2.3 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)
- 90% decrease in the sum of the products of the diameters of soft tissue plasmacytomas is required.

6.4.2.4 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 6.4.1.1),

- 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, 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)

6.4.2.6 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)

- 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 κ/λ ratio. An abnormal κ/λ 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 κ/λ ration of greater than 4:1 or less than 1:2.

6.4.2.7 Progressive Disease (PD)[a]

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
  - Only in patients without measurable M-protein, percentage of bone marrow plasma cells (minimum absolute percentage of 10%)
  - 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 clear increase in size of one or more bone lesion(s) (must be 50% or greater increase of a lesion at least 1 cm in longest length at nadir) on CT scan or new lesion 1.5 cm or larger on CT scan.
- 50% or more increase in the sum of the products of the diameters of multiple soft tissue plasmacytomas or 50% or more increase in the size of a single soft tissue plasmacytoma.
- Development of hypercalcemia solely attributable to the disease (corrected serum calcium >11.5 mg/dL)

[a]All relapse categories require 2 consecutive assessments made at any time before classification as relapse or progression or institution of a new therapy.

### 6.4.3 **Minimal residual disease (MRD) criteria (requires complete response as defined above)**

Flow MRD-negative-Absence of aberrant clonal plasma cells in the bone marrow by flow cytometry using EuroFlow standard operating procedures or equivalent (which includes NCI Dept. of Pathology multicolor flow); must have sensitivity of one in $10^5$ nucleated cells.

Sequencing MRD-negative-absence of clonal plasma cells by next generation sequencing on bone marrow aspirate with a method with sensitivity of 1 in $10^5$ nucleated cells.

Imaging plus MRD-negative-MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less than mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue.

### 6.5 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 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

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

### 7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

### 7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

#### 7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

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

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

### 7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

### 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 CAR-SLAMF7 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 CAR-SLAMF7 -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 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

### 7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular weekly 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. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

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 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 NIH Intramural 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 SPONSOR SAFETY REPORTING

## 8.1 DEFINITIONS

### 8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

### 8.1.2 Serious Adverse Event (SAE)

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 event (see 8.1.3)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing

condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.

- A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
- Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

- 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.

### 8.1.3 **Life-threatening**

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

### 8.1.4 **Severity**

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

### 8.1.5 **Relationship to Study Product**

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

## 8.2 **ASSESSMENT OF SAFETY EVENTS**

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs

occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1.** All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

## 8.3 REPORTING OF SERIOUS ADVERSE EVENTS

Any AE that meets a protocol-defined serious criterion or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in section 8.2.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

## 8.4 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

### 8.4.1 Reporting by CCR to Bellicum Pharmaceuticals Inc.

All events listed below must be reported in the defined timelines to CCRsafety@mail.nih.gov.

The CCR Office of Regulatory Affairs will send all reports to the manufacturer as described below.

Rimiducid is provided under NCI CTA# 01096-18 with Bellicum Pharmaceuticals Inc. Data sharing with Bellicum Pharmaceuticals Inc. must be carried out as follows:

- An annual report of all patients receiving Rimiducid. This report will consist of a brief summary of each patient's Grade 2 or greater adverse events and multiple myeloma responses after the date of Rimiducid administration (report to Paul Woodard, MD of Bellicum, Inc. at pwoodard@bellicum.com).
- Communications with the FDA related to Rimiducid must be shared within 10 calendar days. Report to this email address: BellicumSafety@pharmalex.com. Also send to Paul Woodard, MD of Bellicum, Inc. at pwoodard@bellicum.com.

- Pregnancies must be reported to BellicumSafety@pharmalex.com. Also send a copy to Paul Woodard, MD of Bellicum, Inc. at pwoodard@bellicum.com.

### 8.4.2 Reporting by Study Team to Bellicum Pharmaceuticals Inc.

- SAEs possibly or definitely related to Rimiducid must be reported within 24 hours of the sponsor becoming aware of the SAE. Study team will report to this email address: BellicumSafety@pharmalex.com. Back-up reporting is to this phone number: 1-617-315-4825. Also, report to Paul Woodard, MD of Bellicum, Inc. at pwoodard@bellicum.com.
- Any use of Rimiducid must be reported within 3 calendar days and must include the following (report to Paul Woodard, MD of Bellicum, Inc. at pwoodard@bellicum.com.) by Principal Investigator:
    - Patient age, sex, malignancy, brief history including indication for Rimiducid
    - Dose of CAR T cells received by the patient and percentage of infused cells expressing the CAR
    - Blood CAR T-cell levels before and after Rimiducid administration (may take longer than 3 days to obtain these results).
    - Blood cytokine levels before and after Rimiducid administration (may take longer than 3 days to obtain these results).
    - Timing and nature of Grade 2 or greater AEs within the first 3 days after Rimiducid administration.

## 8.5 REPORTING PREGNANCY

### 8.5.1 Maternal exposure

If a patient becomes pregnant during the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section 8.1.2) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

### 8.5.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of receiving protocol treatment.

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 should, if possible, be followed up and documented.

### 8.6 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected in expedited manner to the FDA in accordance to 21 CFR 312.32. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

## 9 CLINICAL MONITORING

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 and secondary study endpoints; adherence to the protocol, regulations, ICH E6, 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
- 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 a CCR 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.

## 10 STATISTICAL CONSIDERATIONS

### 10.1 STATISTICAL HYPOTHESIS

The primary endpoint of this trial is to determine the safety of administering CAR-expressing T cells to patients with multiple myeloma. The primary approach for assessing this endpoint will be through a dose escalation.

Exploratory objectives of this trial are to measure any anti-malignancy effect that might occur, to assess the feasibility of administering CAR-expressing T cells, and to measure persistence, function, and gene expression of CAR-expressing T cells. In addition, the efficacy of Rimiducid at inducing apoptosis of the SLAMF7 expressing CAR T cells and mitigating toxicity including severe infections will be assessed.

## 10.2 SAMPLE SIZE DETERMINATION

The sample size of this clinical trial will be determined by the requirements of the dose-escalation scheme. A maximum of 24 treated patients will be needed to complete the dose escalation scheme, and an additional 12-patient expansion group will be treated at the MTD. The 12-patient expansion group to better characterize the MTD is needed because of the substantial variability between patients receiving infusions of CAR T cells. The total number of treated patients will be a maximum of 36. The trial uses a dose-escalation design, with 4 dose levels and a -1 dose level if needed. The number of $CAR^+$ T cells transferred for each dose level is as follows:

Dose level -1 $0.3x10^6$ CAR+ T cells per kg of recipient bodyweight

Dose level 1 $0.66x10^6$ CAR+ T cells per kg of recipient bodyweight

Dose level 2 $2.0x10^6$ CAR+ T cells per kg of recipient bodyweight

Dose level 3 $6.0x10^6$ CAR+ T cells per kg of recipient bodyweight

Dose level 4 $12.0x10^6$ CAR+ T cells per kg of recipient bodyweight

If sufficient cells cannot be grown to meet the criteria for the assigned dose level, the patient will receive the dose of cells called for by one dose level lower than the assigned dose level. If sufficient cells cannot be grown to meet the dose requirement called for one dose level lower than the assigned dose level, 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 in section 3.1.4. 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, the first patient can be infused on the next higher dose level after a 28-day delay following CAR T-cell infusion of the third patient. Should 1 of 3 patients experience a DLT at a particular dose level, three more patients would be treated at that dose level. If 1/6 patients have a DLT at a particular dose level, the first patient can be infused on the next higher dose level after a 28-day delay following CAR T-cell infusion of the $6^{th}$ 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 MTD. The MTD is the dose at which a maximum of 1 of 6 patients has a DLT. After a MTD is defined, additional patients can be treated on this trial: up to 12 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 to a patient in the expansion group, the patient will receive as many cells as possible up to the MTD.

DLTs that occur in patients receiving a repeat treatment will not affect the dose escalation because no retreatments will be allowed until the optimal CAR T-cell dose is established. Use of Rimiducid will be recorded and used in descriptive statistics.

## 10.3 POPULATIONS FOR ANALYSES

Toxicity Analysis: All patients will be evaluable for toxicity from the time of Anti-SLAMF7-CAR T cell infusion. Toxicity data will be collected for all patients.

Response Analysis - Intention to treat: any subjects who enroll onto the trial and provide consent and who receive at least one cycle of treatment will be included in analyses dataset.

## 10.4 STATISTICAL ANALYSES

### 10.4.1 General Approach

This is a standard 3+3 dose escalation study. Toxicity data will be obtained and used to determine the MTD of the treatment.

### 10.4.2 Analysis of the Primary Endpoints

The toxicity obtained on each patient will be determined and used to report the number of patients at each dose level who experience a DLT, following standard phase I procedures.

### 10.4.3 Analysis of the Secondary Endpoint(s)

There are no secondary endpoints.

### 10.4.4 Safety Analyses

The study will determine the toxicity experienced on each patient enrolled on the trial and report this by dose level.

### 10.4.5 Baseline Descriptive Statistics

None will be provided in a formal manner; brief descriptions may be incorporated in a final report.

### 10.4.6 Planned Interim Analyses

Toxicity will be evaluated at each dose level as the patients accrue to the trial. Stopping criteria are described in section 3.8.

### 10.4.7 Sub-Group Analyses

None

### 10.4.8 Tabulation of individual Participant Data

None

### 10.4.9 Exploratory Analyses

The degree of persistence of CAR-transduced T cells will be evaluated by a quantitative measure (flow cytometry and/or quantitative PCR) in all patients. Serum cytokine levels are other important exploratory assessments. Anti-malignancy effects will be measured by clinical response and categorized according to the International Uniform Response Criteria for Multiple Myeloma (Section 6.4). 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. Efficacy of Rimiducid will be assessed by resolution of clinical toxicity and by evaluating elimination of CAR+ T cells by monitoring blood CAR T-cell levels by qPCR and/or flow cytometry.

All other evaluations of exploratory 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. Exploratory assessments will include analysis for associations between clinical outcomes such as severity of toxicity and serum cytokine levels or severity of toxicity and blood CAR T-cell levels. Parametric and nonparametric tests will be used as appropriate.

# 11 COLLABORATIVE AGREEMENTS

## 11.1 CLINICAL TRIAL AGREEMENT

Rimiducid is provided under a Clinical Trial Agreement (CTA) NCI CTA# 01096-18 with Bellicum Pharmaceuticals Inc.

# 12 HUMAN SUBJECTS PROTECTIONS

## 12.1 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 sexes 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.
- 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.
- The upper age limit of this protocol will be 73 years of age. CAR T-cell therapies often result in patients being admitted to intensive care units due to cytokine-release syndrome. These critically ill patients often need vasopressor drugs and occasionally need mechanical

ventilation among other intensive care interventions. The mortality of critically-ill patients progressively increases with age above 20 years as shown in the Acute Physiology and Chronic Health Evaluation (APACHE) IV: Hospital mortality assessment for today's critically ill patients.(156) Because mortality of critically-ill patients reaches >10% at age 70 (Table 6 of the cited paper). Because CAR toxicity is very unpredictable and based on my extensive past experience treating patient with CAR T cells on phase I clinical trials, I choose to not enroll patients over the age of 73.

## 12.2 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.

## 12.3 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 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 (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E 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.

## 12.4 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 clinical trial is being performed to evaluate a genetically-modified T-cell therapy for multiple myeloma, which is an almost always incurable disease.(21) Only patients with multiple myeloma who have persisting or relapsed myeloma despite at least 3 prior lines of therapy will be enrolled. The risks of the study fall into six categories.

The first category is chemotherapy-related toxicity that could cause cytopenias. 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, tachycardia, transient abnormalities of liver function and hypotension. These cytokine-release-type toxicities

have been detected in other clinical trials of CAR T cells during the first two weeks after anti-CD19 CAR T cells and anti-BCMA CAR T cells were infused.(4, 5, 134)

A third category of potential toxicities are neurological toxicities such as delirium, obtundation, myoclonus, seizures, headache and transient focal neurological toxicities including aphasia and focal paresis. In previous anti-CD19 CAR trials, cytokine-release toxicities and neurological toxicities have been limited in duration with toxicities generally resolving within 2 days to 3 weeks.(5, 41, 74, 135, 136) In the NCI anti-BCMA trial, neurologic toxicities were limited to confusion or delirium in the setting of high fevers and one case of encephalopathy and muscle weakness of all extremities consistent with critical illness polyneuropathy/polymyopathy.(5) In most cases, these toxicities have been transient with toxicities generally resolving within 2 days to 2 weeks.

The fourth category of toxicity is direct damage to normal tissues by the CAR T cells. This could happen because of unexpected cross-reactivity of the anti-SLAMF7 CAR with proteins other than SLAMF7 in vivo. This trial will be one of the first in human trials testing anti-SLAMF7 CAR T cells in patients with multiple myeloma who have failed at least three previous lines of therapy. Although SLAMF7 is highly expressed in almost all cases of MM it has very limited or absent expression on normal tissues aside from leukocytes.(9, 10, 110, 112, 113) Hematopoietic stem cells do not express SLAMF7.(111) SLAMF7 is not expressed on the surface of normal organ epithelial tissue such as lung, kidney, stomach, brain, breast, spleen, prostate, skeletal muscle, testis, liver, ovary, heart, thymus and small intestine epithelial tissue.(9) We have performed extensive testing of the MSGV-iCasp9-Luc90CD828Z CAR T cells by culturing them with a variety of human cell lines and have not seen recognition of cell lines that did not express SLAMF7. In addition, we did not find any evidence of cross-reactivity in our cell line data or immunohistochemistry data from the NIH Department of Pathology.

A fifth category of toxicity that may be caused by anti-SLAMF7 CAR T cells is impairment of normal immunity because SLAMF7 is expressed on leukocytes such as $CD8^+$ T cells, natural killer (NK) cells, B cells, plasma cells, some monocytes, NKT cells, and some dendritic cells.(5, 9, 10, 110, 112, 113) NK cell deficiencies are associated with severe, potentially life-threatening viral infections caused typically by benign members of the Herpesviridae (VZV, HSV, CMV, EBV) and Papillomaviridae (HPV) families.(137) Additionally, NK cells have potent cytotoxic activity against malignant cells and NK cell defects are linked to ineffective tumor surveillance and clearance. We have included a suicide gene in this trial in large part to allow elimination of anti-SLAMF7 CAR T cells in case depletion of recipient immune cells leads to infections. Greater detail about the depletion of leukocyte subsets is provided below.

Finally, genotoxicity is a theoretical risk of any type of integrating gene therapy. To our knowledge, genotoxicity, such as occurrence of a replication-competent retroviruses or transformation of T cells caused by insertional mutagenesis has never occurred in a clinical trials of T-cell gene therapies.(43, 44) The specific gene therapy vector backbone, MSGV1, proposed for use in this clinical trial has been used in hundreds of patients over the past 13+ years by our group and others.(4, 33, 138, 139)

The potential benefits to subjects enrolling on this trial include the possibility that the CAR T cells can cause a significant anti-myeloma effect. Many patients enrolled on trials of anti-CD19 CAR T cells and anti-BCMA CAR T cells obtained complete remissions of their advanced malignancies;

there is a chance that recipients of the CAR T cells that are being evaluated in this protocol could 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 by aiding in the development of new therapies might help future patients.

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.

The risks associated with biopsies are pain and bleeding at the biopsy site. In order to minimize pain, local anesthesia will be used. Rarely, there is a risk of infection at the sampling site.

### 12.4.1 **Risks of exposure to Ionizing Radiation**

The procedures for performing the CT scans will follow clinical policies, no special procedures apply to these additional assessments for research purposes. In summary, subjects may receive additional radiation exposure from up to seven (7) CT neck +CAP, two (2) CT-Guided biopsies and three (3)18FDG-PET/CT scans in the first year of the study.

The total additional radiation dose for research purposes will be approximately 15.6 rem in the first year of the study. Being exposed to too much radiation can cause harmful side effects such as an increase in the risk of cancer.

Most patients will obtain much less than the maximum levels of radiation exposure since CT scans will only be carried out in patients who have measurable plasmacytomas after baseline assessment. In addition, CT imaging will be modified based on plasmacytoma locations. For example, many patients will not receive any post-infusion neck CTs since many patients do not have baseline neck involvement with myeloma. Most activity of CAR T cells occurs in the first 3 months after infusion, so we need to assess the onset of myeloma responses carefully during this time-period. Note that our imaging practices are similar to those in the largest multicenter anti-BCMA CAR study, the bb2121 trial .(157) The radiation exposure on this study is in fact, less than what is outlined in the Raje et al. study.

## 12.5 **CONSENT PROCESS AND DOCUMENTATION**

The informed consent document will be provided to the participant or consent designee(s) (e.g., the parent/guardian if participant is a minor, legally authorized representative [LAR] if participant is an adult unable to consent) for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person in a private area (e.g., clinic consult room) or remotely (e.g., via telephone or other NIH approved remote platforms) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s).

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.

# 13 PHARMACEUTICAL INFORMATION

Note: The commercial drugs used in this study will not alter labelling of the FDA approved drugs and nor does the investigation involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.

## 13.1 RETROVIRAL VECTOR CONTAINING THE CAR-SLAMF7 GENE

### 13.1.1 Cells manufacturing

The retroviral vector supernatant (MSGV1-IC9-Luc90CD828Z) (encoding a CAR directed against SLAMF7 with the iCasp9 suicide gene system was prepared and preserved following cGMP conditions in the University of Cincinnati Medical Center Vector Production Facility. The retroviral vector utilizes the MSGV retroviral vector backbone including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites, the anti-SLAMF7 CAR protein containing a signal peptide from human CD8-alpha signal sequence, a single-chain variable fragment antigen recognition domain derived from the murine Luc90 antibody, CD8-alpha (hinge and transmembrane domains), CD28 (cytoplasmic region), and CD3-zeta (cytoplasmic region), followed by the murine stem cell virus 3'LTR.

The supernatant will be stored at –80°C or shipped on dry ice and stored in the Dept. of Transfusion Medicine, NIH or at Fisher Biosciences, Rockville, MD. Both storage facilities are equipped with around-the-clock temperature monitoring. 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://bmbl.od.nih.gov/sect3bsl2.htm

### 13.1.2 Source

After cells are obtained by apheresis, further cell processing to generate 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.

### 13.1.3 Toxicities

Please refer to section 1.2.13.

### 13.1.4 Administration procedures:

Please see section 3.3 3.1.4.Potential Drug Interactions

There are no known drug interactions.

## 13.2 COMMERCIAL AGENTS:

Please refer to the US approved package insert for the full prescribing information here:
http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.DrugDetails

### 13.2.1 **Cyclophosphamide**

#### 13.2.1.1 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.

#### 13.2.1.2 Administration procedures

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

### 13.2.2 **Fludarabine**

#### 13.2.2.1 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.

#### 13.2.2.2 Administration procedures

Fludarabine is administered as an IV infusion in 100 mL 0.9% sodium chloride, USP over 30 minutes.

## 13.3 **RIMIDUCID**

### 13.3.1 **Source**

Rimiducid will be supplied by a CTA from Bellicum Inc.

### 13.3.2 **Toxicity**

No serious adverse events occurred during the Phase 1 study in volunteers.(122) The incidence of adverse events was very low following each treatment, with all adverse events being mild in severity. Only one adverse event was considered possibly related to AP1903. This was an episode of vasodilatation, described as "facial flushing" for 1 volunteer at the 1.0 mg/kg AP1903 level. This event occurred at 3 minutes after the start of infusion and resolved after 32 minutes' duration. All other adverse events reported during the study were considered by the investigator to be unrelated or to have improbable relationship to the study drug. These events included chest pain, flu syndrome, halitosis, headache, injection site pain, vasodilatation, increased cough, rhinitis, rash, gum hemorrhage, and ecchymosis.(122)

### 13.3.3 **Formulation and preparation**

This information is from the Rimiducid "Guidance for Investigators" from Bellicum, Inc. The Rimiducid for Injection is packaged in 3 ml Type 1 clear glass serum vials. The contents of each vial are composed of the labeled content 10 mg (2 ml) of Rimiducid drug substance dissolved in a sterile, endotoxin free, 25% Solutol HS 15/Water for Injection solution at an Rimiducid concentration of 5 mg/ml and at pH 5.0 – 7.5. Each vial is stoppered with a Teflon© coated serum stopper and a yellow flip-off seal.

The primary product label (applied directly to the vial) for Rimiducid for Injection will contain the following information: product name, Rimiducid for Injection; the manufacturer's lot number; product concentration, 5 mg/ml; volume of solution available in the vial; total Rimiducid contents of the vial (10 mg); a statement, "For IV Infusion Following Dilution, contains no preservatives" and the IND notation, "Caution: New Drug-Limited by Federal Law to Investigational Use". The product will be labeled according to the requirement of each competent authority.

Rimiducid is formulated in 25% Solutol HS 15, a non-ionic surfactant. Prior to administration, rimiducid is diluted in normal saline, which may lead to the formation of micro-micelles. Micelles of polyethoxylated pharmaceutical surfactants, such as Solutol, are known to activate complement, leading to anaphylactoid-like reactions or pseudoallergy. Such responses may be the result of the activation of mast cell and subsequent degranulation (Szebeni 2001).

Bellicum recommends that patients are pre-medicated using standard procedures for the prevention of infusion reactions prior to administration of rimiducid. Such pre-treatment regimens would typically be administered thirty minutes to one hour prior to the infusion of rimiducid.
Rimiducid for Injection must be warmed to room temperature prior to dilution.

Drug interactions: Rimiducid is an investigational new drug and while there are no known verified drug interactions, investigators should be aware that potential unknown interactions and adverse effects may occur. It should be noted that rimiducid is an inhibitor of CYP3A4 and CYP2C8 and therefore, co-administration of rimiducid with other substrates of CYP3A4 and CYP2C8 could lead to elevated concentrations of these concomitant medications. However, a drug/drug interaction study was conducted to evaluate the potential interaction of rimiducid with substrates of CYP3A4 using the probe midazolam. This study indicated that following a single IV dose of 0.5 mg/kg or less, rimiducid is unlikely to alter the clearance of co-administered CYP3A4 substrates.

Rimiducid was tested in normal, healthy people at doses 2.5-fold greater than those used in the clinical trials using gene modified cells (Covance Study No. 1711-1). A side effect noted was facial flushing (redness) in one person. Although not observed in patients to date, rimiducid was associated with modest elevations of hepatic transaminases, bilirubin and alkaline phosphatase in the acute and subacute non-human primate study. It is possible that other side effects may occur.

In the Bellicum BP-PC-001 trial, one patient exhibited an adverse event of cytokine mediated release or hypersensitivity like reaction upon infusion of rimiducid and one patient experienced urticaria and flushing, which resolved with antihistamine administration. None of these AEs were considered serious.

Rimiducid for Injection is incompatible with materials containing plasticizer or DEHP and materials sterilized with ethylene oxide.

**Rimiducid (AP1903) with iCasp9 Switch suspected unexpected serious adverse reaction (SUSARs):** Currently, no SUSARs have been reported under this program. For regulatory reporting purposes, there are currently no adverse events expected.

The Rimiducid for Injection vials must be stored at 5°C ± 3°C (41°F ± 5°F) in a limited access, qualified refrigerator, preferably without light (but not required to protect from light). Prior to administration, the calculated dose will be diluted to 100 mL in 0.9% normal saline for infusion.(121, 122)

13.3.4 **Administration procedures:**

For use, the Rimiducid will be diluted prior to administration. Rimiducid is administered via I.V. infusion at the target dose diluted in normal saline with volume as appropriate for age of patient to be administered over 2 hours, using a DEHP-free saline bag and solution set.

Rimiducid for Injection (0.4 mg/kg) in a total volume of 100 ml of Rimiducid plus normal saline will be administered via IV infusion over 2 hours, using a non-DEHP, non-ethylene oxide sterilized infusion set and an infusion pump. For patients <50 kg or >150 kg, a volume of administration of Rimiducid different than 100 mL is allowed (121, 122)

**Note:** Recommendations include premedication per institutional standards (acetaminophen and antihistamine) 30 minutes prior to infusion for prevention of potential hypersensitivity to the Solutol HS 15 or rimiducid.

Dose reduction for renal impairment: Rimiducid is thought to be cleared by non-renal means. Dose adjustments for hepatic or renal insufficiency have not been established.

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# 15 APPENDICES

## 15.1 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.

## 15.2 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
- ECOG
- Date of original diagnosis
- Plasmacytoma present: Yes or No
- Tumor Histology and date of confirmation
- SLAMF7 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 SLAMF7 results
- Findings of consultations done at screening

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

- Dates anti-SLAMF7-CAR-transduced T cells given
- Dose level, actual dose in CAR+ T cells/kg, 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 such as CMV, HSV, EBV, toxoplasmosis, adenovirus, TTV data that were not need for eligibility will not be collected.
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, 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.

**F. Off study**

- Date and reason for off study
- Date and cause of death
- Autopsy findings if available

## 15.3 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.

The toxicities most often experienced by patients receiving infusions of 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.

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 or violation.

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

### General supportive care guidelines

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. As a minimum, keep hemoglobin greater than 8.0 g/dL and platelets greater than 20K/microliter.
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. Fevers should be treated with acetaminophen and comfort measures. NSAIDs and corticosteroids should be avoided.
8. Patients with a heart rate persistently higher than 115/minute and fever should have vital signs checked every 2 hours.
9. Patients who are neutropenic and febrile should be receiving broad-spectrum antibiotics.
10. Avoid meperidine due to seizure risk.
11. Minimize benzodiazepine use to avoid aggravating delirium.

12. 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 CAR T-cell infusion or any time after CAR T-cell infusion.
    2. Any blood pressure reading of less than 100 mm Hg systolic blood pressure after anti-CD19 CAR T-cell infusion if 100 mm Hg is lower than the patients baseline systolic blood pressure.
    3. Heart rate greater than 100 beats per minute.

13. 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.

14. A CBC will be obtained twice 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 2000/microliter.

15. 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 less than 80% of their baseline blood pressure <u>and</u> less than 100 mm Hg should receive a 1 L NS bolus.**
    - **Patients with a systolic blood pressure less than 90 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.

16. Patients receiving more than 1 fluid bolus for hypotension should have a stat EKG and troponin, and a cardiac echocardiogram as soon as possible.

## ICU transfer

Patients should be transferred to the ICU after consultation with the ICU physicians 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 less than 75% 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 90 mm Hg after a 1L NS bolus.
- Continuous tachycardia with a heart rate higher than 125 beats per minute on at least 2 occasions separated by 2 hours.
- Oxygen requirement of more than a 4L standard nasal cannula

1. All patients transferred to the ICU for hypotension or tachycardia should have a stat EKG and a cardiac echocardiogram within 6 hours of the time of transfer.
2. Patients with hypotension not responding to IV fluid resuscitation should be started on norepinephrine at doses called for by standard ICU guidelines.
3. Patients should have a cardiac echocardiogram and an EKG within 6 hours of starting norepinephrine.
4. 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).
5. Patients receiving vasopressors should have a cardiac echocardiogram at least every other day.

## Immunosuppressive drug administration

In general, immunosuppressive drugs are administered in a stepwise escalation based on toxicity severity. The first immunosuppressive drug administered is usually tocilizumab. If toxicity does not improve after tocilizumab, treatment progresses to intermediate-dose or high-dose methylprednisolone. For certain severe toxicities listed below, high-dose methylprednisolone must be given immediately.

## Tocilizumab administration

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 of **8 mg/kg** in patients weighing 30 kg or more, infused IV over 1 hour (dose should not exceed 800 mg); it may be infused up to three additional times if there is no clinical improvement in signs and symptoms after the first dose. The interval between consecutive doses should be at least 8 hours.

- Left ventricular ejection fraction less than 45% by echocardiogram
- Creatinine greater than 2-fold higher than the most recent level prior to CAR T-cell infusion
- Norepinephrine requirement at a dose greater than 3 μg/minute for 36 hours since the first administration of norepinephrine.
- Requirement of 5 mcg/minute or more of norepinephrine to maintain systolic blood pressure greater than 90 mm Hg.
- Oxygen requirement of 40% or greater fraction of inspired oxygen ($FIO_2$) to maintain oxygen saturation of >92%.
- Subjective significant dyspnea and respiratory rate greater than 25 for 2 hours or more.
- PTT or INR>2x upper limit of normal
- Bleeding possibly related to cytokine-release syndrome
- Creatine kinase greater than 5x upper limit of normal

## Intermediate-dose methylprednisolone for toxicities not responsive to tocilizumab

1. Give methylprednisolone 50 mg every 6 hours for any of the toxicities under "Tocilizumab administration" above that don't improve after tocilizumab administration.

## High-dose methylprednisolone should be given <u>immediately</u> under these circumstances:

1. Give methylprednisolone 200 mg every 6 hours for systolic blood pressure that is less than 90 mm Hg while the patient is on 15 mcg/minute or higher doses of norepinephrine. If the patient has not had tocilizumab, give 8 mg/kg of tocilizumab along with the methylprednisolone.
2. Give methylprednisolone 200 mg every 6 hours for hypotension requiring 15 mcg/minute or more of norepinephrine continuously for 8 hours or more. If the patient has not already had tocilizumab, administer 8 mg/kg tocilizumab in addition to methylprednisolone.
3. Give methylprednisolone 200 mg every 6 hours for any left ventricular ejection fraction 30% or less. If the patient has not already had tocilizumab, administer 8 mg/kg tocilizumab in addition to methylprednisolone.
4. Give methylprednisolone 200 mg every 6 hours for any situation in which pulmonary toxicity makes mechanical ventilation likely to be required within 4 hours. If the patient has not had tocilizumab, administer 8 mg/kg tocilizumab in addition to methylprednisolone.
5. In life-threating toxicity not improving after 200 mg of methylprednisolone Rimiducid should be administered, 1000 mg of methylprednisolone can be considered.

**In general, stop corticosteroid use when toxicity improves to a tolerable level. For example, in patients with hypotension, stop methylprednisolone 6 to 12 hours after vasopressors are no longer needed.**

## Neurological toxicity

1. All patients with neurological toxicities other than somnolence and delirium should get a neurology consult.
2. All patients with significant neurological toxicity should get an MRI of the brain.
3. All patients with significant neurological toxicity should get a lumbar puncture after MRI if it is safe to perform a lumbar puncture.
4. CARTOX-10 scale (Appendix E) will be administered daily in patients and used to monitor for aphasia/dysphasia in patients.
5. The following patients should receive dexamethasone 10 mg intravenously every 6 hours until the toxicities improve. Note: for seizures administer standard seizure therapies in addition to dexamethasone. For patients already getting higher doses of corticosteroids for CAR-related toxicity, it is not necessary to add dexamethasone 10 mg every 6 hours. Stop dexamethasone as soon as toxicity improves to a tolerable level; the duration of dexamethasone use will need to be determined on a patient to patient basis. Tocilizumab is possibly not effective for neurological toxicity, so it should not be given when patients have isolated neurological toxicity. Tocilizumab may be concurrently given with steroids if the patient has evidence of CRS in addition to neurological toxicity
    1. Inability of patient to follow simple commands such as “squeeze my fingers”.
    2. Any generalized seizure
    3. Somnolence severe enough to potentially limit airway protection
    4. Obtundation and stupor
    5. Ataxia severe enough to preclude ambulation
    6. Disorientation to person or place that persists longer than 48 hours
    7. Neurologic toxicity lasting more than 2 hours that is severe enough to interfere with activities of daily living (ADLs)
    8. Cerebral edema

## 15.4 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. The RN will be informed of the approximate time of cell arrival at the bedside. | |
| 2. Verify the physician orders:<br>- to administer the cells<br>- for the date of administration<br>- for premedication orders<br>- protocol number | • Premeds are acetaminophen 650 mg PO and diphenhydramine 12.5 mg IV. |
| 3. Verify that the protocol consent has been signed | |
| 4. Ensure that emergency and monitoring equipment are available in the patient's room:<br>- oxygen<br>- suction<br>- 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 - **NEW** bag and **NEW** tubing.<br>This **MUST** be ready and infusing **prior** to the cells being delivered to the unit.<br>The patient's primary IV hydration can infuse via a separate lumen while the cells are infusing, but **NO MEDs** should be administered during this time.<br>Have a second bag of 250cc NS and tubing ready as an emergency line. | This will be the dedicated NS line for infusing the cells. Under no circumstances are any other substances to be infused into the line.<br>Cell death occurs quickly – the infusion must be initiated immediately.<br>Do not infuse medication during the cell infusion. If emergency meds must be administered, use the hydration or emergency NS IV line.<br>This will be the emergency IV solution and can be used for medication administration.<br>**Do not use an inline filter for cells.** |
| 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.<br>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. **Cell death occurs as soon as the cells are removed from the laboratory**. Initiate the infusion as quickly as possible. |
| 12. Prior to spiking the cell bag, two RNs will perform the identification procedure. **Both** RNs must have their names charted in the CRIS cellular therapy flow sheet | |
| 13. Infuse the cells by **INFUSION PUMP** or syringe over 20-30 minutes.<br>a. Piggyback the cells into the dedicated NS line; use the backflush technique to prime the line.<br>b. While the cells are infusing, **gently** agitate the bag of cells **every few minutes**. When the cell bag is empty, backflush NS to rinse the bag | This prevents the cells from clumping in the bag. |

| | |
|---|---|
| and infuse this at the same rate as the cells; rinse bag until NS runs clear.<br>c. Note: in some cases cells will arrive from DTM in a syringe. In this case infuse the cells via syringe over 20-30 minutes in the dedicated NS line proximal port, see nursing cellular infusion SOP for further details. | |
| 14. Measure and record VS before and after the cell infusion, q1h x 4, and then q4h after completion of the infusion.<br>a. Assess and document the patient's respiratory and circulatory status post cell infusion. | |
| 15. Documentation:<br>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.<br>b. Document the cell infusion in CRIS using the appropriate screens. | |

## 15.5 APPENDIX E: CARTOX (ADAPTED FROM NEELAPU ET AL, 2018):

- One point is assigned for each task. Normal function defined by overall score of 10.
- TASKS:
  - Orientation to year, month, city, hospital and President of country (maximum 5 points)
  - Name three objects (ex: point to clock, pen, button) (maximum of 3 points)
  - Write a standard sentence (1 point)
  - Count backwards from 100 in tens (1 point)

## 15.6 APPENDIX F: CYTOKINE RELEASE SYNDROME GRADING ASSESSMENT (ADAPTED FROM LEE ET AL, 2019).

Grade 1 CRS:

- Fever and:
- Constitutional symptoms

Grade 2 CRS:

- Fever and:
- Hypotension; responds to fluids.
- Hypoxia requiring low flow nasal cannula or blow-by.

Grade 3 CRS:

- Fever and;
- Hypotension: requires pressor +/- vasopressin)

Hypoxia: requiring high-flow nasal cannula, facemask, nonrebreather mask or venturi mask. Grade 4 CRS:

- Fever and;
- Positive pressure
-
- Multiple Pressors