FRED HUTCHINSON CANCER RESEARCH CENTER

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A phase I study of adoptive immunotherapy for advanced B-cell maturation antigen (BCMA)$^+$ multiple myeloma with autologous CD4$^+$ and CD8$^+$ T cells engineered to express a BCMA-specific chimeric antigen receptor

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SYNOPSIS

Protocol Number: FHCRC 9762	
Title of Study: Phase I study of adoptive immunotherapy adoptive immunotherapy for advanced B-cell maturation antigen (BCMA)+ multiple myeloma with autologous CD4+ and CD8+ T cells engineered to express a BCMA-specific chimeric antigen receptor.	
Sponsor: Damian Green, MD	**Phase of Development:** Phase 1

Study Objectives:

Primary:

- To evaluate the safety of adoptive T cell therapy using ex vivo expanded BCMA-specific chimeric antigen receptor (CAR)-transduced T cells for relapsed or treatment refractory multiple myeloma (MM) patients
- Autologous CD8+ and CD4+ T cells will be transduced and administered in a 1:1 ratio to patients 36 to 96 hours following lymphodepleting chemotherapy. A BCMA-targeting CAR construct containing a 4-1BB/CD3ζ costimulatory domain will be evaluated in patients with active multiple myeloma.

Secondary:

- Determine the peak concentration, in vivo persistence and phenotype of transferred CAR-T cells,
- Estimate the maximal tolerated dose (MTD) with a modified continuous reassessment method (CRM) and evaluate the toxicity profile,
- Estimate the anti-tumor activity of adoptively transferred BCMA CAR-T cells in patients with measurable tumor burden prior to T cell transfer.

Study Design: Eligible MM patients will be over 21 years of age with a confirmed diagnosis of BCMA$^+$ MM ($\geq$10% CD138+ malignant plasma cells (PCs) by IHC on BM core biopsy or by flow cytometry) and confirmation of BCMA expression on tumor cells (BCMA+ MM comprising $\geq$5% of CD138+ cells) by flow cytometry; and who either 1) have relapsed or persistent disease following autologous stem cell transplant (ASCT), or 2) are ASCT ineligible and demonstrate persistent disease after $\geq$4 cycles of induction therapy including a proteasome inhibitor and an immunomodulatory drug (IMiD) administered either concurrently or in sequence.

Patients will be stratified into 2 cohorts based on tumor burden (low: 10-30% and high: >30% marrow involvement by malignant plasma cells by IHC [using CD138+ staining]). Patients will then receive escalating doses of autologous CAR-T cells transduced with a BCMA/4-1BB/CD3ζ (BCMA4ζ) construct following lymphodepleting chemotherapy. Each patient's selected CD8+ and CD4+ T cells will be transduced, enriched and expanded separately to produce more consistent T cell products and thereby facilitate an evaluation of safety and efficacy. Then pooled transduced CD8+ and CD4+ T cells will be administered at approximately a 1:1 target ratio to achieve the specified cell dose for each cohort of patients. The primary endpoint of the trial will be to determine the safety of BCMA4ζ CAR-T cells, using a modification of the continual reassessment method (CRM) to estimate the MTD.

Based on pre-clinical and clinical findings, we hypothesize that CD8+ and CD4+ BCMA CAR-T cells will persist long-term, migrate to memory cell niches and establish functional memory T cell populations when administered 36 to 96 hours after cyclophosphamide/fludarabine (Cy/Flu) chemotherapy. Preclinical data suggest that CAR-T cells with different signaling domains may exhibit distinct in vivo behavior, toxicity and durable antitumor efficacy.

We expect that CAR-T cells produced with the BCMA construct will be safe and well-tolerated in the clinical setting defined in this protocol. Analysis of serum cytokines and acute phase reactants (IL-6, IFN-γ, CRP, ferritin) will elucidate potential differences in the incidence of cytokine release syndrome (CRS), which have been noted in other trials with relatively high doses of activated CAR-T cells; serious and/or progressive symptoms and signs of CRS will be treated by the administration of anti-IL6R antibody (tocilizumab) and/or corticosteroids. The long-term impact of BCMA CAR therapy on PC function will be monitored by serial quantitative immunoglobulin (Ig) measurements; patients can be supported with intravenous Ig (IVIG), as needed. In the event of prolonged depletion

of normal PC and Ig deficiency or other serious CAR-T cell-related toxicity, a non-functional truncated epidermal growth factor receptor (EGFRt) in the construct is designed to enable elimination of BCMA CAR-T cells by administration of an anti-EGFR monoclonal antibody (cetuximab). We will define the peak concentration, duration of persistence, and preserved anti-myeloma cell function of the 4-1BB/CD3ζ BCMA CAR-T cell construct in this trial. The identification of a safe dose in two cohorts of patients stratified by disease burden prior to cell infusion (10-30% $CD138^+$ vs >30%) will be used to inform decision-making about cell dose in future studies. Results from this trial will also begin to formally address whether BCMA CAR-T cell therapy might represent a viable alternative to ASCT.
Interventions and Duration: This Phase I clinical trial for relapsed or treatment refractory MM patients will evaluate the safety and antitumor activity of adoptively transferred autologous T cells transduced to express a BCMA-targeting CAR. Study enrollment will be completed over the course of 3 years.
Study Population: This Phase I trial, will enroll 25 MM patients age 21 or older including any gender, ethnicity or race with a confirmed diagnosis of $BCMA^+$ (flow cytometry demonstrating ≥10% expression on CD138+ plasma cells) disease that 1) is relapsed or refractory following ASCT or 2) among ASCT ineligible patients with disease that persists after ≥4 cycles of therapy and is double refractory (persistence/progression after therapy) to both a proteasome inhibitor and an immunomodulatory drug (IMiD) administered either concurrently or in sequence. At enrollment, patients will be stratified into 2 cohorts based on tumor burden (10-30% and >30% marrow involvement by malignant plasma cells by IHC [$CD138^+$ staining]).

GLOSSARY

ADA	anti-drug antibody
AE	adverse event
AESI	adverse event of special interest
ALL	acute lymphoblastic leukemia
ALT	alanine aminotransferase
ASCT	autologous stem cell transplant
AST	aspartate aminotransferase
AUC	area under the curve
BCMA	B cell maturation antigen
BM	bone marrow
CAR	chimeric antigen receptor
CBC	complete blood count
CCO	Clinical Coordinators Office
CFR	Code of Federal Regulations
CL	clearance
Cmax	maximum concentration
CMC	Chemistry, Manufacturing, and Controls
CNS	central nervous system
CPF	Cell Processing Facility
CR	complete response or complete remission
CRA	Clinical Research Associate
CRF	case report form
CRM	continuous reassessment method
CRP	C-reactive protein
CRS	cytokine release syndrome
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cell Therapy Laboratory
CTLA-4	cytotoxic T lymphocyte-associated protein 4
Cy/Flu	cyclophosphamide + fludarabine
DCR-24w	disease control rate at 24 weeks
DLBCL	diffuse large B-cell lymphoma
DLCO	diffusing capacity of the lung for carbon monoxide
DLT	dose-limiting toxicity

DOR	duration of response
DSMB	Data Safety Monitoring Board
ECG	electrocardiogram
ECHO	echocardiogram
EDC	electronic data capture
EEG	electroencephalogram
EGFR	epidermal growth factor receptor
EGFRt	truncated epidermal growth factor receptor
FDA	Food and Drug Administration
FEV1	forced expiratory volume in 1 second
FHCRC	Fred Hutchinson Cancer Research Center
FLC	free light chain (or serum free light chain)
GCP	Good Clinical Practice
GFR	glomerular filtration rate
GI	gastrointestinal
GLP	Good Laboratory Practice
HCC	hepatocellular carcinoma
HCT	hematopoietic stem cell transplant
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IBC	Institutional Biosafety Committee
ICF	informed consent form
ICH	International Conference on Harmonization
ICU	intensive care unit
IFNγ	interferon gamma
IHC	immunohistochemistry
IL-6	interleukin 6
IMWG	International Myeloma Working Group
IND	Investigational New Drug
IP	investigational product
irAE	immune-related adverse event
IRB	Institutional Review Board
IRO	Institutional Review Office
ISS	International Staging System
IV	intravenous(ly)

IVIG	intravenous immunoglobulin
LDH	lactate dehydrogenase
LTFU	long-term follow-up
LVEF	left ventricular ejection fraction
mAb	monoclonal antibody
MAS	macrophage activation syndrome
mCR	molecular complete remission
MHC	major histocompatibility complex
MRI	magnetic resonance imaging
M-spike	monoclonal protein spike
MTD	maximum tolerated dose
mTPI	modified toxicity probability interval
MUGA	multiple uptake gated acquisition
NCI	National Cancer Institute
NHL	non-Hodgkin lymphoma
NIH	National Institutes of Health
NOAEL	no-observed-adverse-effect level
NOS	not otherwise specified
NSCLC	non-small cell lung cancer
NT	neurotoxicity
NYHA	New York Heart Association
ORR	objective response rate
OS	overall survival
PBMC	peripheral blood mononuclear cell
PC	plasma cell
PCP	pneumocystis pneumonia
PCR	polymerase chain reaction
PD	progressive disease
PD-1	programmed cell death protein 1
PD-L1	programmed cell death ligand 1
PD-L2	PD-L2 programmed cell death ligand 2
P[DLT]	probability of dose-limiting toxicity
PET	positron emission tomography
PFS	progression-free survival
PI	Principal Investigator

PK	pharmacokinetic(s)
PMBCL	primary mediastinal B-cell lymphoma
PO	per os
PR	partial response
PT	prothrombin time
PTT	partial thromboplastin time
Q2W	every 2 weeks
Q4W	every 4 weeks
Q28D	every 28 days
qPCR	quantitative polymerase chain reaction
R/R	relapsed/refractory
RCL	replication-competent lentivirus
R-ISS	Revised International Staging System
SAE	serious adverse event
SCCA	Seattle Cancer Care Alliance
SCCHN	squamous cell carcinoma of the head and neck
scFv	single-chain variable fragment
sCR	stringent complete remission
sCRS	severe cytokine release syndrome
SCT	stem cell transplant
SEM	standard error of the mean
SMC	Safety Monitoring Committee
SNP	single nucleotide polymorphism
SOP	standard operating procedure
SPEP	serum protein electrophoresis
TCR	T-cell receptor
TIL	tumor-infiltrating lymphocyte
TLS	tumor lysis syndrome
Tmax	time to peak concentration
TME	tumor microenvironment
TNFα	tumor necrosis factor alpha
ULN	upper limit of normal
UPEP	urine protein electrophoresis
UPN	Unique Patient Number
VGPR	very good partial response

1. INTRODUCTION, BACKGROUND AND RATIONALE

1.1 Introduction

Patient derived T cells can be engineered through *ex vivo* gene transfer to generate tumor specific T cells for adoptive immunotherapy. Through such processes, various synthetic chimeric antigen receptor T cells (CAR-T) have been rapidly generated. These CAR-T cells typically harbor a single chain antibody, which confers their tumor specificity, linked to T cell signaling domains. Recent studies performed by our group have demonstrated dramatic responses in patients with advanced stage acute lymphocytic leukemia and non-Hodgkin lymphoma following CD19 targeting CAR therapy. Plasma cell restricted B cell maturation antigen (BCMA) appears to be a similarly promising target for CAR-T cell therapy directed against multiple myeloma (MM). Preclinical studies performed *in vitro*, and using an immunodeficient mouse model bearing disseminated disease, have validated the anti-MM efficacy of a comparable BCMA CAR construct and early reports from clinical trials targeting BCMA appear to validate the target. This phase I clinical trial will evaluate the safety and antitumor activity of a defined composition of adoptively transferred autologous $CD4^+$ and $CD8^+$ T cells genetically modified to express a BCMA-specific chimeric antigen receptor

1.2 Background on Multiple Myeloma

Multiple myeloma (MM) is the second most common hematologic malignancy in the United States with an estimated prevalence of 100,000 and approximately 30,000 new cases diagnosed each year. New treatments introduced over the past two decades have dramatically improved initial response to therapy and median overall survival (OS), which has increased from less than 3 years to greater than 7 years among patients without "high risk" cytogenetic or clinical features at diagnosis. The immunomodulatory agents (IMiDs; thalidomide and its analogs lenalidomide and pomalidomide) and proteasome inhibitors (PIs; bortezomib, carfilzomib, and ixazomib), have demonstrated efficacy in both newly diagnosed and relapsed MM and are largely responsible for the recent improvements in outcome.[1,2] Three-drug combinations of IMiD, PI and dexamethasone are now preferred induction regimens in most patients. Intensive chemotherapy followed by autologous stem cell transplant (ASCT) can increase complete remission (CR) rates and prolong survival. Nonetheless, MM remains incurable and sequential lines of treatment for each inevitable relapse results in progressively shorter durations of response due to acquired resistance by MM cells to available therapy.[3] Ultimately, almost all patients develop treatment unresponsive MM [4-8] which causes the death of over 11,000 patients in the US annually.

Autologous stem cell transplant (ASCT), performed in over 6000 MM patients each year, is a standard of care for treatment of MM in the United States, and the most frequent indication for this procedure. Yet the benefit of ASCT does not extend to the majority of MM patients who are deemed ineligible by virtue of age or comorbidities. The median age of diagnosis is 69 years while the benefit of ASCT after age 70 remains to be established in randomized trials [9-11].

Recently, monoclonal antibodies targeting SLAMF7 (elotuzumab) and CD38 (daratumumab) have demonstrated impressive anti-myeloma activity, particularly when combination daratumumab/bortezomib[12] or daratumumab/lenalidomide[13] have been administered in the relapsed or treatment refractory setting. Immune checkpoint inhibitors, which facilitate effective interactions between anti-tumor T-cells and their targets, are also demonstrating early promise in MM when combined with immunomodulatory agents.[14] Nonetheless, the impact of these combinations on long-term survival remains uncertain and no current data suggests that these new approaches ultimately prevent relapse. Further, the

survival benefits from recent advances have disproportionately favored patients with standard risk disease while the 20% to 40% of individuals harboring high-risk features at diagnosis continue to face short durations of response, early relapse and death[15,16].

1.3 Rationale

1.3.1 Rationale for Immunotherapy in MM

Early trials involving myeloablative conditioning therapy, followed by allogeneic hematopoietic stem cell transplant (allo-HCT) for MM, resulted in two significant but discordant findings: 1) the incidence of treatment related toxicity, as a consequence of myeloablative allo-HCT conditioning, prolonged immunosuppression and post-transplant graft versus host disease (GVHD), was unacceptably high (>50% mortality in some studies)[5]; and 2) among individuals not succumbing to transplant associated complications, responses were often durable with long term overall survival (OS) after allo-HCT that was superior to auto-HCT recipients. This durability is likely a consequence of a "graft versus myeloma" effect (GVM). The role of GVM is supported by the observation that early studies of myeloablative allo-HCT resulted in more frequent molecular remissions than auto-HCT[17,18] with a 5 year relapse risk of 0% among allo-HCT recipients achieving a MRD negative status by PCR for IgH-gene rearrangement.[17] Donor lymphocyte infusion has also reversed relapse after allo-HCT in select cases.[19-22] Collectively, GVM appears to be an immunologic approach that not only impacts disease outcome, but unlike other anti-MM therapies, appears agnostic to cytogenetic risk.[23] However, despite longer PFS in MM patients who have undergone allo-HCT after reduced-intensity conditioning, rather than a second ASCT, higher transplantation-related mortality likely contributed to an absence of statistical difference in PFS and OS with this approach.[24] The Blood and Marrow Transplant Clinical Trials Network recently confirmed this finding in patients receiving single autografts followed by nonmyeloablative allografts if a matched donor was available, versus a second autograft in the absence of an appropriate donor.[25] Nonetheless, deletion of malignant plasma cells through immune surveillance provides proof of principle as an approach capable of disease eradication. The adoptive T cell therapy (ACT) approach under study in this trial represents a highly specific form of MM immunotherapy designed to safely exploit the capacity of the immune system to selectively eliminate MM cells and thereby improve patient outcomes.

1.3.2 Rationale for Engineering Therapeutic T Cells with CARs

Early attempts at adoptive T cell therapy (ACT) employed native, tumor-reactive T cells collected from patients, but these can be difficult to obtain and/or difficult to expand to therapeutically useful numbers. Autologous T cells can be redirected by expressing a transgene that encodes a TCR specific for a tumor-associated antigen and have produced transient clinical responses.[26,27] However, TCR targeting strategies generally require major histocompatibility complex (MHC) restricted peptide presentation by tumor cells. Unfortunately, many tumors express low levels of MHC molecules (known as human leukocyte antigens, HLA, in humans) and thereby avoid T cell recognition.[28]

An alternative strategy is to express an artificial non-HLA restricted CAR that recognizes a tumor cell surface molecule.[29,30] A CAR construct is typically comprised of a fusion gene that encodes a monoclonal antibody-derived single chain variable fragment (scFv), with heavy (V_H) and light (V_L) chain variable domains joined by a flexible linker, fused through a transmembrane domain to a cytoplasmic signaling moiety including CD3ζ alone, or CD3ζ combined with activation domains from one or more costimulatory molecules such as CD28, 4-1BB or OX40.[30,31] Such CARs with specificity for tumor cell-surface epitopes are generally considered "universal" in that they bind antigen in an HLA-independent fashion, and one receptor construct can be used to treat a range of patients with tumors that express the CAR-targeted molecule. T cells obtained from the blood of cancer patients can be modified

with CARs to generate anti-tumor, CAR-T effector cells that can recognize tumor cells that have down-regulated HLA molecules, thereby avoiding the need to isolate rare HLA-restricted tumor-reactive T cells. CARs have been constructed for many tumor-associated cell surface molecules.[30-35] *In vitro* studies have demonstrated that both $CD4^+$ and $CD8^+$ T cell effector functions can be triggered via CARs, and studies in animal models and in small numbers of patients have demonstrated the capacity of adoptively transferred CAR-T cells to eradicate established tumors.[36-39]

Impressive clinical results have been achieved with the adoptive transfer of T cells modified to express CARs that target the B-cell lineage CD19 molecule. A subset of patients with refractory B-cell malignancies, including acute lymphoblastic leukemia, chronic lymphocytic leukemia, and non-Hodgkin lymphoma have been induced into a complete remission after receiving an infusion of autologous T cells modified with a CD19-specific CAR.[40-43]

CARs are typically constructed with an scFv ligand-binding domain linked via spacer and transmembrane sequences to a signaling module containing CD3ζ and one or more costimulatory domains. For this trial, we have designed a lentiviral vector encoding a BCMA-specific CAR containing a target-specific scFv, and a 4-1BB/CD3ζ signaling domain.

1.3.3 Rationale for Targeting BCMA in MM

Optimal targets for successful CAR-T cell therapy should: 1) be expressed on the tumor cell surface, 2) not demonstrate off-tumor expression, even at low levels, on any essential organ or cell type (i.e., hematopoietic stem cells), and 3) be uniformly distributed on the tumor to avoid antigen escape or, alternatively, the target must be essential for maintenance of the tumorigenic phenotype.

Various potential MM antigen targets for CAR T-cell therapy (BCMA, CD38, CD138, CD40, CD74, SLAMF7, cancer-testes antigens and others) were extensively assessed. BCMA (CD269; TNFRSF 17) based on a number of factors including its target cell-restricted expression profile. BCMA is a tumor necrosis factor (TNF) receptor family protein that is upregulated at the terminal stages of plasma cell differentiation and promotes survival of long-lived BM PC.[44,45] Apart from low levels of mRNA detected on dendritic cells, BCMA expression appears absent on other normal tissues, but is widely and highly expressed on MM cells where it is thought to play a critical role in protecting against apoptosis. The APRIL (TNFSF13; CD256) proliferation-inducing molecule seems to be the primary BCMA ligand;[46] cells of the tumor microenvironment, osteoclasts in particular, secrete APRIL and the B cell activating factor (BAFF) ligand.[47-49] Anti-BCMA antibodies have been documented in MM patients whose disease had responded to donor lymphocyte infusion following relapse after allogeneic transplant[50] and anti-BCMA antibodies have shown promising *in vitro* activity against MM cell lines and primary patient samples.[51] Further, a BCMA targeting antibody drug conjugate (GSK2857916) delivering microtubule inhibitor monomethyl auristatin phenyalanine to MM cells has demonstrated limited toxicity and anti-tumor activity at higher doses in 24 patients enrolled on a Phase I safety trial (https://clinicaltrials.gov/ct2/show/NCT02064387).

In a preclinical study using an immunocompromised mouse model, BCMA-targeting CAR-T cells exhibited BCMA-specific functions including cytokine production, proliferation, and cytotoxicity leading to the elimination of established plasmacytomas derived from human multiple myeloma cell lines.[49] BCMA signals seemed essential to the maintenance of the tumor phenotype while a wide range of normal human tissues demonstrated no BCMA expression by immunohistochemistry except in lymphoid tissue. Primary human $CD34^+$ hematopoietic cells were BCMA negative. These observations suggest that an anti-BCMA CAR will have quite favorable efficacy and toxicity profiles.

National Cancer Institute (NCI) investigators initiated a Phase I clinical trial of BCMA CAR-T cells for adult patients with previously treated MM (https://clinicaltrials.gov/ct2/show/study/NCT02215967). The NCI CAR incorporates an anti-BCMA scFv, a CD28 domain and a CD3-zeta T-cell activation domain. Patients received one round of conditioning chemotherapy (300 mg/m^2 of cyclophosphamide plus 30 mg/m^2 of fludarabine, daily for 3 days) before receiving one infusion of CAR+ T-cells at one of four dose levels, starting at 0.3 x 10^6 CAR+ T-cells/kg of body weight. The team treated twelve patients with advanced disease and a median of seven prior lines of therapy. Overall, of the six patients treated at the two lowest dose levels, one patient had a transient partial remission (of 2 weeks duration), while the other five patients achieved stable disease. At the third dose level, two patients obtained stable disease, and one patient obtained a very good partial response, with complete elimination of multiple myeloma bone disease based on PET scan results. This patient also had normalization of serum-free light chains and clearance of BM PC. Two patients have been treated on the highest dose level of 9x10^6 CAR+ T cells/kg. One was in stringent complete remission lasting 16 weeks.[52] Although the number of patients treated is small, anti-tumor responses in this trial appeared to correlate with CAR-T cell dose. Two patients at the highest dose level experienced reversible cytokine release syndrome (CRS). Anotherphase 1 dose finding trial of BCMA directed CAR-T cells harboring a 4-1BB/ζ costimulatory domain administered to MM patients with relapsed/refractory disease after ≥3 prior lines of therapy, has been reported by Bluebird Bio Incorporated (Berdeja JG. et al. Abstract #3010, American Society of Clinical Oncology-ASCO-Annual Meeting, Chicago 2017) [https://clinicaltrials.gov/ct2/show/NCT02658929]. Patients with <50% BCMA expression on CD138+ plasma cells were excluded. The overall response rate reported in eighteen evaluable patients was 89%, including sCR/CR (4), VGPR (7), and PR (5). The percentage of patients achieving a VGPR or better at >5 x 10^7 CAR-T cells was 73%, with 27% CRs. In this trial, 15/21 (71%) of patients experienced CRS, including 2 events of Grade 3 CRS. Four patients with CRS were treated with toxcilizumab and 1 patient also received steroids.

In another early stage BCMA targeted CAR-T cell clinical trial from Nanjing Legend Biotech (Fan FX. et al. Abstract 3001, American Society of Clinical Oncology-ASCO-Annual Meeting, Chicago 2017) [https://clinicaltrials.gov/ct2/show/NCT03090659] 19 patients with relapsed/refractory MM received LCAR-B38M (reported to be an anti-BCMA CAR-T cell therapy) at a median cell dose of 4.7 x 10^6 cells/kg. The investigators reported a 100% objective response rate with 95% (18/19 patients) reaching CR or near CR, and no evidence of disease relapse up to 6 months following treatment. Cytokine release syndrome was reported 14 patients (74%) and was generally described as “mild and manageable”, the events include 9 Grade 1 events, 2 Grade 2, 1 Grade 3 and 1 Grade 4; all patients recovered and 5 patients experienced no detectable CRS.

Currently, there is no uniform approach to the detection and quantitation of BCMA on malignant plasma cells and reports describing the fraction of MM patients expressing BCMA have been inconsistent. Kochenderfer and the NCI group report 61% of patients (52/85) met screening criteria by IHC staining and eligibility criteria for this NCI trial included uniform expression of BCMA by either IHC or flow cytometry (patients with clearly identified populations of BCMA negative plasma cells were excluded). Flow cytometry was reported to have superior sensitivity to IHC by the NCI team and all treated patients were reported to have uniform BCMA expression by flow cytometry. Bluebird Bio reports that 60% of pre-screened patients (n=75) had ≥ 50% BCMA$^+$ CD138$^+$ cells by IHC. Separately, our group measured BCMA expression by flow cytometry in

bone marrow aspirates obtained from 50 consecutive MM patients reflecting the spectrum of disease status (prior to, during, and following therapy). We detected BMCA in 100% of cases; however the absolute level of expression was variable based on a semi-quantitative scale. Similarly, Seckinger has reported surface BCMA antigen expression by multidimensional flow cytometry in 100% of untreated (n=31) and relapsed (n=12) myeloma patients.[53] Gene expression profiling (n=712) and RNA sequencing (n=263) has detected BMCA in all MM patient samples (untreated and relapsed).

We have evaluated antitumor effects of primary T cells transduced with a vector encoding an anti-BCMA 4-1BB/ζ CAR in NSG mice engrafted with firefly luciferase transduced RPMI 8226 MM cells (8226^{luc}). All CAR-T cell infusions were at approximately a 1:1 ratio of CD4:CD8 CAR-T cells. Whole body bioluminescence imaging (BLI) demonstrated complete tumor elimination after a minimum CAR-T cell infusion threshold was achieved (Fig. 1).

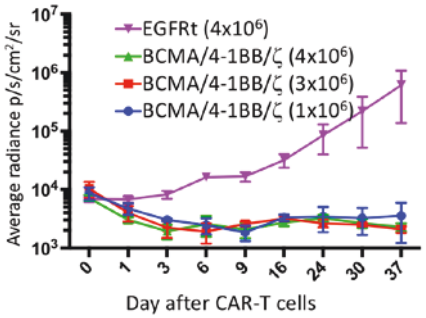

Fig. 1 BCMA CAR-T cells eliminate myeloma in NSG mice. Tumor burden measured by BLI in cohorts of mice engrafted with 8226 MM cells and treated with control (EGFRt) or BCMA 4-1BB/ζ CAR-T cells at various cell doses.

1.3.4 Rationale Rationale for Optimized CAR Lentiviral Vectors and Transduction of T Cell Subsets

The initial human trials of CAR-modified T cells employed first generation constructs that linked the scFv to the CD3ζ or FcR gamma epsilon chain as the only intracellular signaling domain.[54,55] To enhance potency, costimulatory endodomains have been added to CD3ζ to enhance CAR signaling.[38,39,56-58] Methods for selecting and transducing T cells of defined phenotype have also been improved and now do not require long-term culture.[38,39] Our group has shown that the non-signaling extracellular spacer domain can be decisive for *in vivo* antitumor activity of CAR-T cells.[59]

We have previously constructed a CD19-specific CAR comprised of an scFv, derived from a murine IgG1 mAb and fused in tandem to the human IgG4 hinge region, the human 4-1BB costimulatory domain and CD3ζ. The construct also encodes a truncated epidermal growth factor receptor (EGFRt), which can serve as a marker for cell selection and for tracking transduced T cells *in vivo*.[60] Based on the practical and theoretical advantages of lentiviral vectors (LV), a self-inactivating (SIN) LV vector (ZRX-014-LV provided by Juno Therapeutics, Seattle) that encodes the CD19-4-1BB-CD3ζ CAR-EGFRt under transcriptional control of the elongation factor 1 alpha (EF1α) promoter was produced under GMP conditions, and the virus supernatant subjected to quality control analysis for clinical applications. We have demonstrated that primary human T cells were efficiently transduced with the CD19-4-1BB-CD3ζ -EGFRt SIN lentiviral vector, expressed the CAR and EGFRt, and conferred recognition of CD19+ target cells, including primary CLL and lymphoma cell lines.

Our group has also focused on developing methods by which T cell products of defined composition can be engineered with tumor-targeting receptors.[61] Following up on animal model data, we examined individual CD8$^+$ and CD4$^+$ T cell subsets from normal donors and from patients with B-cell malignancies, including naïve, central memory (T_{CM}), and effector memory subsets. All were efficiently transduced with a CD19 CAR and expanded, but showed clear differences in effector function and proliferation *in vitro* and *in vivo* in immunodeficient mice engrafted with human B cell tumors.[62] Importantly, the most potent CD4$^+$ and CD8$^+$ CAR-expressing subsets produced synergistic antitumor effects when combined *in vivo*.[62] Moreover, we recognized that CAR-T cell products generated from defined T cell subsets have the potential to provide uniform potency, compared with products derived from unselected

PBMC-derived T cells that can vary widely in phenotypic composition, and thereby allow CAR-T cell dosing to potentially be adjusted based on clear cell dose/tumor burden/toxicity relationships.

We are currently conducting the first-in-man phase I/II clinical trial to test the feasibility and safety of administering CD19 CAR-T cells of a defined composition of $CD8^+$ and $CD4^+$ T cells in patients with $CD19^+$ B cell malignancies (https://clinicaltrials.gov/ct2/show/NCT01865617). $CD8^+$ T_{CM} (or $CD8^+$ T cells) and CD4+ T cells were separately enriched from each patient, transduced with a CD19 CAR lentivirus and expanded *in vitro*. We administered chemotherapy prior to the T cell infusion, to reduce tumor burden and induce lymphopenia to improve T-cell persistence.

As of April 2017, one hundred and sixty-seven patients have been treated on the CD19 CAR T cell trial, the safety and efficacy data is described briefly for these patients. Enrollment includes 58 patients with relapsed or refractory acute lymphoblastic leukemia (ALL), 82 patients with non-Hodgkin's lymphoma (NHL), and 27 patients with chronic lymphocytic leukemia (CLL) have been treated on this trial with lymphodepleting chemotherapy followed by infusion of CD19 CAR-T cells since initiation of the study. Mixtures of CD4+ plus CD8+ CD19 CAR-T cells had potent anti-tumor activity at cell doses 1-2 $\log_{10}$ lower than the cell doses reported in prior studies in which unselected cell products were used. With regard to safety, the overall incidence of $\geq$ grade 3 CRS in the ALL cohort was 33% and the incidence of $\geq$ grade 3 neurotoxicity was 38%. With risk adapted dosing consisting of administering a lower dose of CAR T cells to patients with high tumor burden, the incidence of $\geq$ grade 3 CRS and $\geq$ grade 3 neurotoxicity were reduced. We had 3 deaths in the ALL cohort felt to be related to CAR T cells, one during the dose escalation phase at the $2x10^7$/kg dose level, and one due to neurotoxicity (cerebral edema) at dose level 2. Patients with NHL had an 11% incidence of $\geq$ grade 3 CRS and a 23% incidence of $\geq$ grade 3 neurotoxicity. There were 2 deaths in the NHL cohort felt to be related to CAR T cells. In the CLL cohort, the incidence of $\geq$ grade 3 CRS was 30% and the incidence of $\geq$ grade 3 neurotoxicity was 26%. There was 1 death felt to be related to the CAR T cell infusion. The toxicity rates may be acceptable given the lack of treatment options for these patients with advanced, refractory B cell malignancies. To reduce toxicity, we have focused on understanding the pathogenesis of toxicity and designing pre-emptive strategies.

With regard to efficacy, we reported a complete remission (CR) rate of 90% in 29 evaluable patients with refractory B-ALL.[63] Lymphodepletion with cyclophosphamide/fludarabine (Cy/Flu), compared with Cy alone (or Cy/etoposide), was associated with better CAR T-cell peak proliferation and persistence and with improved outcomes. The data for the initial 32 NHL patients has been published.[64] Forty-nine of 80 NHL patients have now completed response assessment with an overall response rate (ORR) of 61%, and a CR rate of 37.5%. An additional 11 patients had stable disease. 52% of patients remain alive and the median progression free survival of the patients that achieved a CR has not been reached. The ORR in the CLL cohort is 71% with a CR rate of 30%.

Our study of CD19 CAR T cells demonstrates the feasibility of using GMP-compliant cell selection methods to derive $CD4^+$ and $CD8^+$ T cells for clinical application, and show that administration of uniform cell products is useful in defining safe and effective regimens for CAR-T cells. However, we were unable to select sufficient $CD8^+$ T_{CM} cells for approximately 1/3 of patients enrolled on this trial because many heavily pre-treated patients had low absolute numbers of this cell subset in the blood. These patients instead had $CD8^+$ T cells selected and were treated with a 1:1 mixture of $CD8^+$and $CD4^+$ T cells, with antitumor efficacy observed.

Based in part on the data described above, the proposed trial of BCMA-targeting ACT will also use a defined composition of transduced T cells, unlike the NCI BCMA-targeting CAR-T trial that uses bulk T cells. In the proposed trial, for BCMA4ζ CAR we will enrich $CD8^+$ and $CD4^+$ T cells separately from

the leukapheresis product of each patient, transduce with the CAR lentivirus independently, expand and then pool $CD8^+$ and $CD4^+$ CAR-T cells delivered in approximately a 1:1 ratio to achieve the specified cell dose, before cryopreserving the therapeutic products.

1.3.5 Measuring Response to MM Therapy

Criteria defining response to treatment have been established by the International Myeloma Working Group (IMWG) (see **Appendix F**). These responses, including stringent complete response (sCR), complete response (CR), very good partial response (VGPR), partial response (PR), stable disease (SD), and progressive disease (PD) are based on laboratory, radiographic, and clinical evaluations. Stringent complete remission (sCR) has been demonstrated to serve as an independent prognostic factor for survival in MM patients. Patients in sCR have a projected 5-year survival (OS) of 80% compared to 53% for patients who experience a near CR after high dose therapy. Unfortunately, less than 25% of patients currently achieve a sCR with current treatment,[65] underscoring the need for new approaches.

The advent of increasingly effective MM therapies, in conjunction with the development of more sensitive tools to detect occult disease, have led to recognition of the impact that post-treatment minimal residual disease (MRD) status plays in predicting patient survival.[66,67] MRD^- molecular CR (mCR) has previously been described in a subset of MM patients following allogeneic SCT, and in these settings MRD elimination is associated with a significant improvement in progression free survival (PFS) and OS.[68,69]

Recent clinical data have demonstrated that MRD identified through high-throughput next-generation sequencing (NGS; clonoSEQ) can effectively stratify patients who achieve a CR into two groups with strikingly different prognoses: MRD^- patients demonstrated a time to progression of 131 months compared to just 35 months for those who remained MRD^+ (P=.0009).[67] In this trial, the capacity for our 4-1BB/ζ BCMA CAR-T cell construct to eliminate MRD will be assessed.

The International Myeloma Working Group recently issued consensus recommendations regarding incorporation of ^{18}fluorodeoxyglucose (^{18}F-FDG) PET/CT imaging into the assessment of patients with MM.[70] This imaging modality enables detection of extramedullary sites of proliferating clonal plasma cells. Based on the ability of PET/CT imaging to discern active versus inactive sites of disease, the technique is now, "the preferred functional imaging modality to evaluate and monitor the effect of therapy on myeloma cell metabolism". Molecular and cell based techniques for detecting MRD are complemented by PET imaging, because, as a consequence of inhomogeneous PC infiltration of the bone marrow, the probability of a false negative finding is relatively high. In addition, MRD testing without PET may not identify extramedullary disease. To establish complete eradication of the tumor clones, PET imaging will be integrated into response assessments. All patients will undergo functional imaging during screening (within 30 days of receiving therapy) by PET/CT scan (or if patients have previously demonstrated PET negative (FDG negative) disease by bone marrow MRI). PET without contrast enhanced CT scan can be substituted in patients with iodinated contrast allergy or renal insufficiency upon consultation with the study PI.

1.3.6 Safety Considerations for Adoptive Therapy with Genetically Modified T Cells

There are several potential toxicities of adoptive therapy with CAR-T cells. The first relates to transformation of T cells by retroviral insertional mutagenesis. T cell leukemias have been reported in a subset of patients on two gene therapy trials for X-linked severe combined immunodeficiency syndrome (SCID), in which BM derived CD34+ hematopoietic progenitor cells were transduced with a retroviral vector encoding the common cytokine receptor gamma chain.[71-76] Four of the five cases were associated with activation of the LMO2 oncogene. However, animal studies have shown that mature T

cells are resistant to transformation after retroviral integration.[77] Leukemia has never been observed in clinical trials involving gene transfer into mature T cells, despite more than 10 years of follow-up in some studies. In our CAR-targeting strategies, not only do we modify mature T cells, but we also: 1) use a lentiviral vector (not a retrovirus) to genetically modify cells, which reduces the risk of integrating into a transcriptionally active site, and 2) do not use CARs that constitutively express a functional growth factor receptor, as was the case with transduced cells in the X-linked SCID trial.

Second, adoptive CAR-T cell therapies might cause toxicities in normal tissues that express the target molecule. To date, immunohistochemistry has only shown BCMA expression in normal and malignant lymphoid cells. As such, experience with CD19 immunotargeting cells is relevant. CD19 is expressed on B-cell leukemias and lymphomas and on all human B cells, from initial lineage commitment until terminal differentiation into PC, but not on any other normal tissue. Depletion of normal B cells is thus the only anticipated on-target, off-tumor toxicity with CD19 CAR-T cells, potentially resulting in long-term B-cell deficiency.[38,39,41,57,78] Of note, prolonged suppression of normal B cells is common in patients receiving rituximab therapy and does not appear to result in significant complications, providing Ig levels are maintained by IVIG therapy.[79] In fact, in univariate analyses of results from 50 patients treated on six phase I clinical trials with CAR T cells targeting CD19 in B-lineage malignancies, B-cell aplasia was statistically significantly associated with PFS, along with conditioning chemotherapy and durable persistence of CAR T cells.[80]

Finally, cytokine release syndrome (CRS) can occur after CAR-T cell engagement of target cells and/or tumor lysis syndrome can occur upon rapid and extensive tumor cell death. Both toxicities have been observed in clinical trials of CD19 CAR-T cell therapy, although tumor lysis syndrome is relatively rare. CRS occurs most frequently in acute lymphocytic leukemia patients with high tumor burdens. In the systematic review of six phase I clinical trials of CD19 CAR-T cells in B-lineage malignancies, the most common adverse events were fever, hypotension, rigor, fatigue, bacteremia, chill, dyspnea, and headache, in most cases reactions were temporary and resolved.[80] Among 161 adults with B-ALL, NHL or CLL treated with anti-CD19 CAR-T cells at our center, ≥3 neurotoxicity (NT) was observed at a median of 4.5 days following CAR-T cell administration in 24% of patients. CRS and NT were reversible with the exception of 6 pts who died, 4 during the dose-finding phase of the study. In multivariable analyses, higher CAR-T cell dose and malignant B cells in BM were associated with CRS; and CAR-T cell dose, malignant BM B cells, more intensive lymphodepletion, and prior neurologic comorbidities were associated with neurotoxicity.

Based on unpublished observations suggesting that neoplastic PC express low levels of CD19 more frequently than has previously been reported and that CD19 CAR-T cells can be cytotoxic for cells with low level CD19 expression, the University of Pennsylvania group initiated a pilot trial of CD19-targeting ACT that, along with high-dose melphalan and ASCT, depletes the majority, CD19-negative PC.[81] They had previously treated one MM patient with a CD19 CAR-T cell dose of 5×10^8 cells on day 2 after ASCT, on a compassionate-use protocol. The patient had a very good partial response complicated by severe CRS and neurotoxic effects that were attributed to a robust *in vivo* CTL expansion. As a result of these toxic effects and their previous demonstration of schedule-dependent effects on *in vivo* expansion of adoptively transferred T cells after ASCT,[82] the clinical trial specified a lower CTL dose, with infusions scheduled on day 12, 13 or 14, rather than day 2, after transplantation. To date, they have published results for the first patient treated on this protocol. This patient, with advanced refractory MM, realized a durable CR despite the absence of CD19 expression in the vast majority of neoplastic cells.[81] They also note in that report that six of the total 10 treated patients remain progression-free, and that the only CTL-attributable toxic effects have been one instance of grade 1 CRS and one instance of grade 3 enterocolitis due to autologous graft-versus-host disease.

In the recent NCI study, higher doses of BCMA CAR-T cells were linked to better responses, and were also associated with more adverse events. Toxicity among patients on the first three dose levels was mild, including cytopenias attributable to chemotherapy and fever. One patient treated at the highest cell dose achieved a stringent complete remission and experienced CRS that included fever, tachycardia, dyspnea, acute kidney injury, coagulopathy, hypotension requiring vasopressor support, and muscle damage manifesting as an elevated creatine kinase level and weakness. This patient also experienced prolonged thrombocytopenia. The other patient treated at the highest cell dose also experienced signs of CRS, with toxicities including fever, tachycardia, hypotension, delirium, hypoxia and coagulopathy. Four weeks after CAR T-cell infusion, BM PC were undetectable in this patient. And, all symptoms of toxicity resolved within 2 weeks, the group reported. Similarly, in other BCMA CAR-T cell studies (Bluebird and Nanjing Legend trials detailed above) the incidence of CRS was 71% and 74% respectively.

Of note, patients with advanced MM who have failed conventional lymphodepleting chemotherapy and/or ASCT are likely to already have a B-cell deficiency. B-cell lymphopenia may thus be an acceptable side effect of T cell therapy, if it is accompanied by a significant anti-tumor effect. Similarly, CRS risks are real, but may be associated with better clinical responses than can be achieved with other available salvage regimens. We do not expect any additional or more severe toxicities with BCMA-targeting than with CD19-targeting CAR T cells.

1.3.7 Description and Preclinical Data for a Fully Human BCMA-Specific CAR

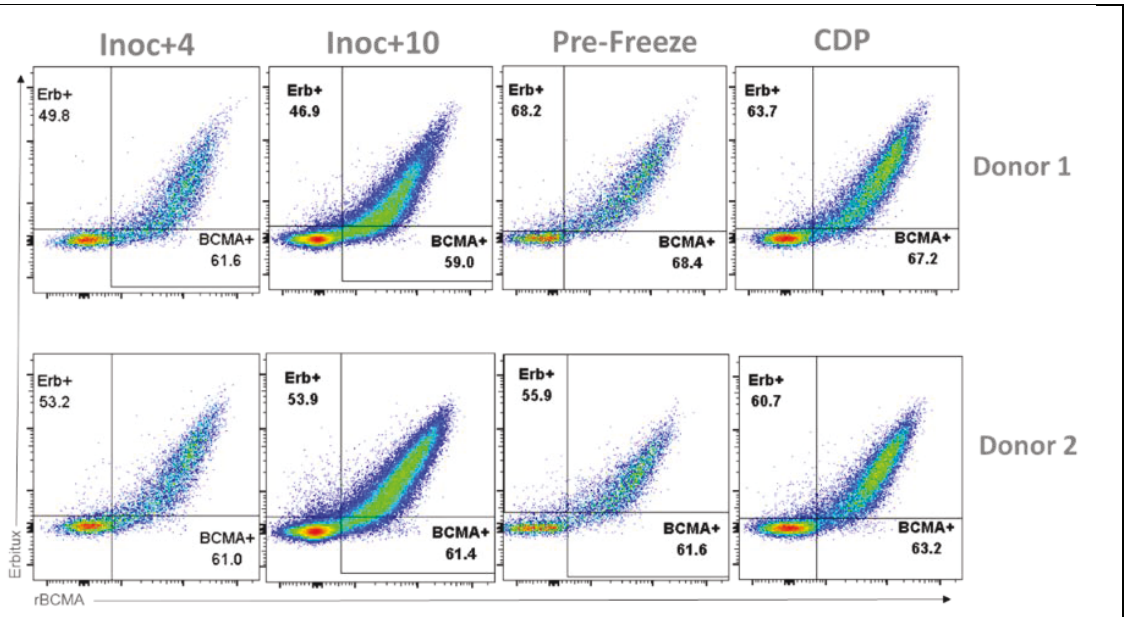

Fig. 2 Evaluation of CAR and EGFRt Expression on CD3+ Cells During CAR T Cell Production. Primary human T cells from two donors were stimulated with CD3/CD28 activation beads at a bead/cell ratio of 1 for 24 hours, transduced with CAR-encoding lentiviral vector, and expanded for 10 days before cryopreservation. The cells were stained with fluorochrome labeled anti-CD4, anti-CD8, and anti-EGFR antibodies and recom-binant BCMA and DAPI (for live/dead). Staining was performed 3 days and 9 days post transduction (Inoc+4 and Inco+10) before (Pre-freeze) and after (CDP)-cryopreservation). Data is representative of that obtained using cells from 10 different donors.

This section describes preclinical data obtained with cells expressing a comparable anti-BCMA CAR as compared to the investigational product. For these studies, CD4+ and CD8+ T cells were transduced with a genetically-engineered replication incompetent, self-inactivating lentiviral vector encoding a BCMA-specific chimeric antigen receptor (CAR). Similar to the investigational product, the CAR expressed in cells evaluated in the preclinical studies included a single chain variable fragment (scFv)

binding domain containing fully-human variable domains selected from a human scFv phage display library (Eureka), a modified IgG4-CH2-CH3 hinge region, a CD28 transmembrane domain, a 4-1BB (CD137) co-stimulatory domain, and CD3ζ signaling domain (BCMA/4□). Several of the domains of the CAR construct were modified and/or selected to provide unique characteristics relative to other CAR constructs previously reported [83]. These modifications included design of the "hinge" length to provide a particular degree of proximity between target BCMA epitope and CAR binding domains, e.g., within the synapse, use of a CD28 trans-membrane domain, which may, for example, limit potential auto-activation, and use of a CD137 endodomain, which may contribute to improved outcomes compared to other constructs such as potentially enhanced cytotoxicity and/or provide lower risk of toxicity, given the favorable cytokine production profile.

A third generation self-inactivating (SIN) lentiviral vector, which encodes the CAR domains described above is used to transduce T cells. Additionally, this lentiviral construct also encodes a truncated human epidermal growth factor receptor (EGFRt) that is expressed separate from the CAR on the cell surface and serves as a non-functional marker [60,84], providing the potential to directly measure transduced T cells in the product.[85] For evaluation of both lentiviral integration and CAR expression, staining for EGFRt is combined with staining with a fluorochrome conjugated recombinant BCMA reagent. Binding of recombinant BCMA within the live T cell populations is used to estimate CAR frequency in final product. Staining of CAR and EGFRt on primary human T cells transduced with CAR-encoding lentivirus at different time points throughout the production process is shown in Figure 2.

Proliferation, cytokine production, and cytolytic function in response to BCMA expressing target cells were evaluated to confirm the specificity of the product. CAR+ CD4+ and CD8+ T cells proliferated specifically in response to K562 cell lines expressing BCMA (orange), but not cells lacking BCMA antigen expression (blue). In contrast, non-transduced T cells (Mock) cultured with BCMA-expressing target cells and CAR+ CD4+ and CD8+ T cells cultured with target cells negative for BCMA did not proliferate (Figure 3).

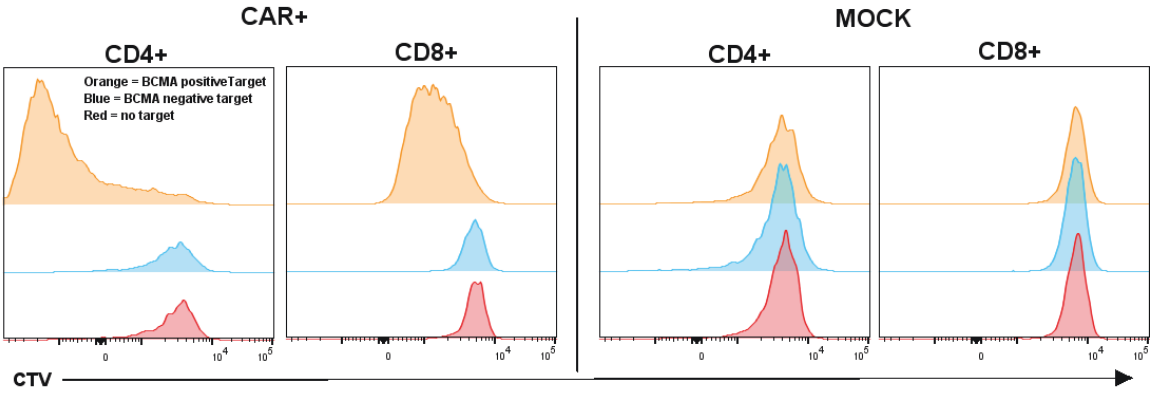

Fig. 3. Proliferation of CAR+ CD4+ and CD8+ T Cells *In Vitro*. CAR+ (left) Mock (right) T cells were labeled with Cell trace violet (CTV) and cultured in the presence of target cells, BCMA+K562 (positive target- orange) and wild type+K562 (negative target - blue) and results compared to cultures of T cells alone (Red). After four days, proliferation was measured as decrease in fluorescent signal (from right to left) and is displayed in the histograms. Cells were also stained for CD4, CD8 and Live dead IR; gated on live CD4 or CD8+ T cells. (n>5).

CAR-expressing T cells were observed to release cytokines specifically upon encountering target cells expressing BCMA. The amount of cytokine production observed increased proportionally to the amount of available antigen, as shown in studies using tumor cell targets that express variable levels of antigen density (Daudi<RPMI-8226<K562-BCMA). In contrast, non-transduced T cells stimulated

with the different target cells did not produce substantial levels of cytokines (Figure 4).

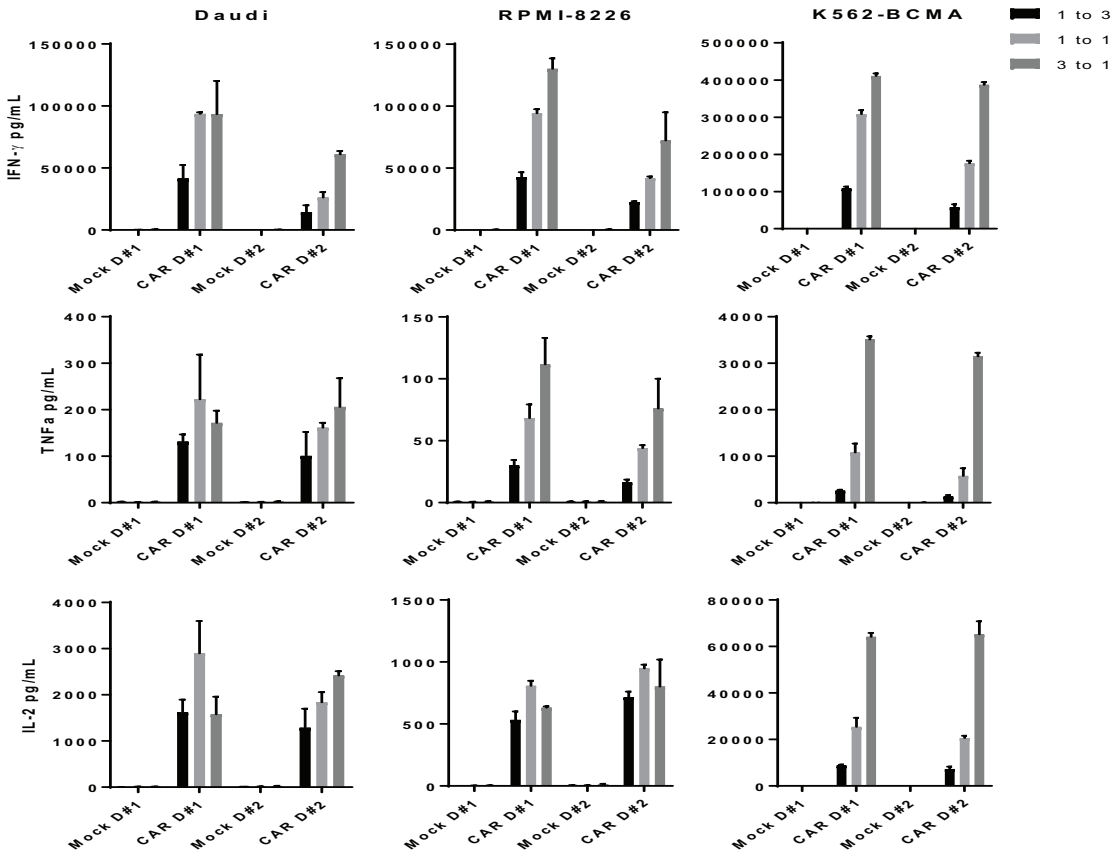

Fig. 4 Cytokine Production by CAR-expressing T Cells *In Vitro* in Response to Targets with Varying Antigen Density. T cells expressing the anti-BCMA CAR assessed in these preclinical studies (donors 1 and 2) produced increasing levels of cytokines in response to target cells expressing increasing levels of BCMA (Daudi<RPMI-8226<K562-BCMA), whereas non-transduced Mock T cells do not. Cells were cultured with target cells for 24 hours at which point supernatants were collected and analyzed for the production of cytokines by MSD analysis. T cells cultured with target cells lacking BCMA produced no cytokine (data not shown).

We measured the lytic capacity of CAR-expressing T cells when they were cultured with target cells expressing different levels of BCMA antigen. CAR-expressing T cells displayed similar cytotoxic activity when cultured with target cells expressing BCMA at variable levels, while mock transduced T cells did not lyse any BCMA+ target cell lines (Figure 5). Target cells negative for BCMA expression were not lysed by CAR-expressing T cells (data not shown).

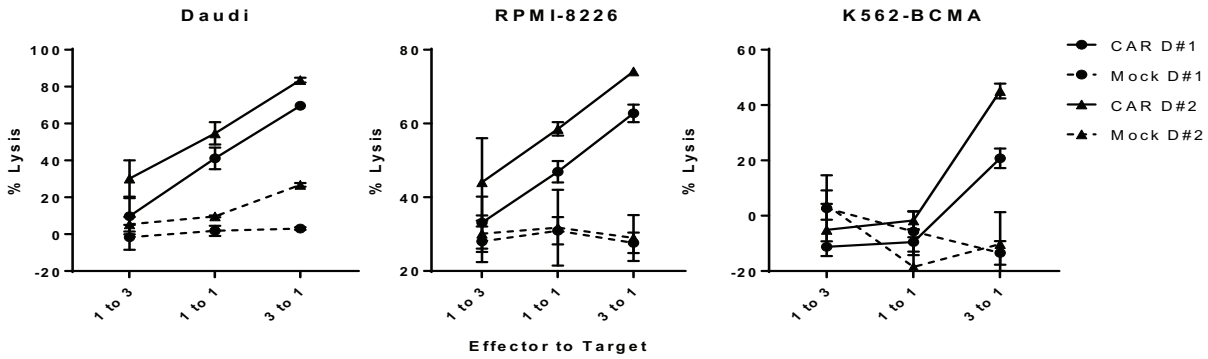

Fig. 5 Cytolytic Activity of CAR T Cells *In Vitro* to Targets with Variable Antigen Levels. The cytolytic activity of CAR T cells was evaluated by a flow based lysis assay. The BCMA-positive target cell lines were stained with CFSE and the target negative cells lines were stained with cell trace violet. The BCMA+ and BCMA- cell lines were mixed 1 to 1 and then incubated with CAR+ T cells at varying effector to target ratios (calculated based on the BCMA+ targets). After 24 hours, the remaining BCMA+ vs BCMA- target cells were measured by flow cytometry and the cytotoxicity was calculated.

1.3.8 Summary of Preclinical In Vivo Studies of exemplary anti-BCMA CAR

Antitumor activity of the anti-BCMA CAR assessed in *in vitro* as described above was assessed using NOD/Scid IL-2Rγnull (NSG) mice engrafted with the disseminated luciferase (ffluc) labeled OPM-2 multiple myeloma cell line. Fourteen days after tumor engraftment, anti-BCMA CAR+ cells, at a dose of 1×10^6 or 3×10^6 CAR T cells per mouse were adoptively transferred intravenously. Survival and tumor burden were assessed over 90 days.

Tumor growth in immune-deficient mice was measured by *in vivo* bioluminescence imaging of ffluc-positive OPM-2 myeloma cells. In mice that did not receive human T cells or that were treated with human T cells transduced with an irrelevant CAR, myeloma cells rapidly expanded within the first week and continued without plateau until the mice became moribund. In contrast, treatment of immune-deficient mice with 1×10^6 or 3×10^6 CAR+ cells significantly suppressed expansion of OPM-2 myeloma cells up to 22 days post CAR T cell transfer ($p= 0.0160$ mock vs. CAR+ 1×10^6 or 3×10^6, two-way ANOVA). Up to Day 49, myeloma expansion continued to be suppressed in all mice treated with 3×10^6 CAR cells and in 5 out of 8 mice treated with 1×10^6 CAR+ cells ($p = 0.1253$ CAR 1×10^6 vs. 3×10^6, two-way ANOVA). OPM-2 multiple myeloma was fatal in all mice that did not receive T cells by Day 28 and in all mice receiving T cells transduced with an irrelevant CAR construct on Day 42.

In mice with OPM-2 myeloma that were administered CAR+ cells, 87.5% of mice survived to Day 79 with 1×10^6 CAR expressing cells and 67.5% of mice survived to Day 79 with 3×10^6 CAR cells. The percent survival was not significantly different for mice with OPM-2 myeloma injected with 1×10^6 or 3×10^6 CAR expressing cells produced from Donor ($p = 0.2278$ log-rank (Mantel-Cox) test).

The *in vivo* anti-myeloma activity of CAR+ cells was also tested in immune-deficient mice engrafted with multiple myeloma engrafted as a tumor mass (RPMI-8226). Primary efficacy was assessed by survival and tumor burden, and the secondary endpoint was the evaluation of the pharmacokinetics of cells expressing the CAR. NOD.Cg-*Prkdc*^{scid}*IL-2rg*^{tm1Wjl}/SzJ mice were injected subcutaneously into the right flank with RPMI-8226 multiple myeloma cells on Day 0. On Days 27 or 35, mice were randomized into groups based on a minimum mean tumor volume. On Days 29 or 36, anti-BCMA CAR T cells from two independent donors were injected intravenously at 1×10^6 or 3×10^6 cells per mouse. In

all studies, control mice did not receive T cells or received 3×10^6 nontransduced human T cells from the corresponding donor. In all studies, mice were monitored for survival and tumor growth. In the RPMI-8226 studies, myeloma and blood cells were evaluated by flow cytometry for CAR T and non-CAR T cells, for BCMA expression, and for the presence of RPMI-8226 myeloma cells.

Mice receiving CAR+ cells had a significant survival advantage over mice receiving mock T cells. In treated mice, 100% of mice survived following administration of 1×10^6 CAR cells, and 87.5% of mice survived following administration of 3×10^6 CAR+ cells up to Day 108 post CAR T-cell injection. For mice treated with CAR cells up to Day 98 post CAR T-cell injection, 75% of mice survived following administration of 1×10^6 cells, and 12.5% of mice survived with 3×10^6 CAR+ cells. The percent survival was significantly better for mice with RPMI-8226 myeloma injected with 1×10^6 cells than with 3×10^6 CAR+ cells likely because the high-dose group developed a high incidence of graft-versus-host disease. Mice treated with both doses of CAR+ cells from two donors had complete regression of tumor growth by 20 days post CAR T-cell transfer which continued up to Day 60. Total circulating CAR+ T cells peaked at Day 14 post CAR T-cell transfer. Total numbers of T cells generally were skewed towards greater numbers of CD8+ than CD4+ CAR T cells. The intensity of BCMA-targeting CAR expression (as measured by BCMA staining) on CD4+ and CD8+ CAR T cells from both donors and doses was readily detectable and remained stable. Thus, in two xenograft models of multiple myeloma, the pre-clinical CAR candidate assessed, produced at small-scale from two healthy donors, significantly prolonged the survival and control of tumor growth in mice.

2. STUDY DESIGN

The trial schema is shown **in Figure 6**.

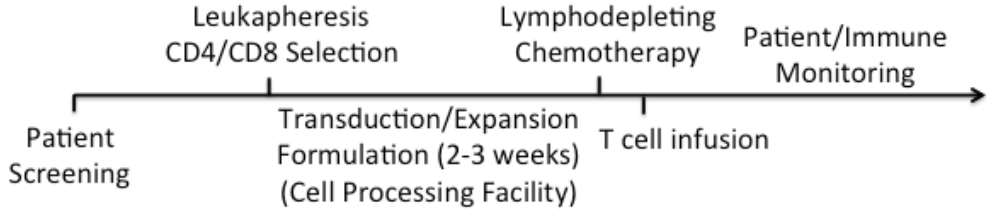

Figure 6: Overall Treatment Schema

Eligible patients will undergo collection of autologous lymphocytes by steady state apheresis. CD4+ and CD8+ cells will be selected using immunomagnetic selection, transduced with the BCMA-targeting

CAR construct, expanded in culture, and administered to patients in approximately a 1:1 ratio 36-96 hours after Cy/Flu lymphodepleting chemotherapy. The CAR construct will encode a BCMA-specific scFv, a CD3ζ signaling moiety and a costimulatory molecule, 4-1BB (BCMA4ζ). Reports from various other clinical trials have not always provided well-characterized correlations between CAR T cell dose, toxicity and response, which may in part be a consequence of variable phenotypic composition (CD4, CD8) of the CAR product in individual patients when unselected T cell populations are used. In this trial, we will formulate CAR-T cell products of a defined CD4:CD8 ratio based on prior experience at our center with CD19 specific CAR-T cells showing that an equal target ratio of CD4 to CD8 T cells was associated with a dose response and dose toxicity relationship.

Based on preclinical and clinical findings, we hypothesize that cyclophosphamide in combination with fludarabine (Cy/Flu) will produce lymphodepletion sufficient for CAR-T cell proliferation and persistence, and that we can identify a dose of T cells transduced with the BCMA/4ζ CAR that is safe and well-tolerated when administered 36 to 96 hours after lymphodepletion. A CAR-T cell dose escalation/de-escalation design will be used in order to identify a dose with acceptable toxicity and clinical efficacy. We hypothesize that CD8+ and CD4+ BCMA CAR T cells will persist, migrate to bone marrow and mediate antitumor activity. CAR-T cell measures will include peak concentration, duration of persistence, functional assays on the CAR product and persisting CAR T cells in the patient, and various measures of anti-myeloma response.

The impact of BCMA CAR therapy on plasma cell numbers and function will be monitored including serial quantitative immunoglobulin (Ig) measurements. Myeloma patients are often deficient in normal Ig levels even prior to CAR T cell therapy, but if needed may be supported with IVIG infusion. The CAR construct also includes a truncated epidermal growth factor receptor if prolonged Ig deficiency associated with recurrent infections or other serious treatment-related toxicities occur, BCMA CAR-T cells could potentially be eliminated by administration of an anti-EGFR monoclonal antibody (cetuximab).

3. STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of this phase I clinical trial is to evaluate the safety of adoptive therapy with *ex vivo* expanded autologous $CD8^+$ plus $CD4^+$ T cells transduced to express a human BCMA-targeting CAR for patients with relapsed or treatment refractory multiple myeloma.

3.2 Secondary Objectives

The secondary objectives of this trial are:

1. To determine the duration of *in vivo* persistence and the phenotype of long lived CAR-T cells,
2. To determine the degree to which adoptively transferred T cells traffic to MM cells in the BM and function *in vivo,* and
3. To estimate the antitumor activity of adoptively transferred BCMA CAR-T cells

3.3 Projected Target Accrual

The number of patients required using the CRM model to estimate the MTD depends on observed toxicity. We estimate that defining the dose level with acceptable toxicity will take up to 25 patients. Based on institutional experience, we expect enrollment as follows:

Table 1: Targeted / Planned Enrollment

Ethnic Category	Patient Numbers		
	Females	**Males**	**Total**
Hispanic or Latino	1	1	2
Not Hispanic or Latino	9	14	23
Ethnic Category Total of All Subjects*	10	15	25
Racial Category			
American Indian / Alaska Native	0	1	1

Asian	1	2	3
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	1	2	3
White	7	9	16
More than One Race	1	1	2
Racial Categories: Total of All Subjects*	10	15	25

4. SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria

MM patients who:

1. Are over 21 years of age and have the capacity to give informed consent
2. ECOG Performance Status score ≤ 2
3. Have measurable disease by International Myeloma Working Group (IMWG) criteria based on one or more of the following findings:
   a. Serum M-protein ≥ 1 g/dL
   b. Urine M-protein ≥ 200 mg/24 hour
   c. Involved serum free light chain (sFLC) level ≥ 10 mg/dL with abnormal κ/λ ratio
   d. Measurable biopsy-proven plasmacytomas (≥ 1 lesion that has a single diameter ≥ 2 cm)
   e. Bone marrow plasma cells ≥ 30%
4. Have a diagnosis of $BCMA^+$ MM (≥5% $BCMA^+$ by flow cytometry on CD138 co-expressing plasma cells obtained within 45 days of study enrollment). The MM diagnosis must be confirmed by internal pathology review of a fresh biopsy specimen at the FHCRC/SCCA
5. Have relapsed or treatment refractory disease with ≥10% CD138+ malignant plasma cells (IHC) on BM core biopsy*, either:

   Following ASCT

   Or, if a patient has not yet undergone ASCT, the individual must:

   Be transplant ineligible, due to age, comorbidity, patient choice, insurance reasons, concerns of rapidly progressive disease, and/or discretion of their attending physician and principal investigator

   And,

   Demonstrate disease that persists after >4 cycles of induction therapy and that is double refractory (persistence/progression) after therapy with both a proteasome inhibitor and immunomodulatory drug (IMiD) administered either in tandem, or in sequence; >4 cycles of therapy are not required for patients with a diagnosis of plasma cell leukemia.

   *Patients receiving retreatment do not need to meet the >10% CD138+ malignant plasma cells (IHC) on BM core biopsy.
6. Male and female patients of reproductive potential must be willing to use an effect contraceptive method before, during, and for at least 4 months after the CAR T cell infusion (see Appendix I).

4.2 Exclusion Criteria

1. History of another primary malignancy that requires intervention beyond surveillance or that has not been in remission for at least 1 year (the following are exempt from the 1year limit: non-melanoma skin cancer, curatively treated localized prostate cancer, and cervical carcinoma in situ on biopsy or a squamous intraepithelial lesion on PAP smear)
2. Active hepatitis B, hepatitis C at the time of screening
3. Patients who are (HIV) seropositive
4. Subjects with uncontrolled active infection
5. >1 hospital admission (lasting 5 days or more) for documented infection in prior 6 months
6. Presence of acute or chronic graft-versus-host disease (GVHD) requiring active treatment unless limited to skin involvement and managed with topical steroid therapy alone
7. History of any one of the following cardiovascular conditions within the past 6 months: Class III or IV heart failure as defined by the New York Heart Association (NYHA), cardiac angioplasty or stenting, myocardial infarction, unstable angina, or other clinically significant cardiac disease as determined by the PI or designee
8. History of clinically relevant or active CNS pathology such as epilepsy, seizure, paresis, aphasia, stroke, severe brain injuries, dementia, Parkinson's disease, cerebellar disease, organic brain syndrome, psychosis, active central nervous system MM involvement and/or carcinomatous meningitis; subjects with previously treated central nervous systems involvement may participate, provided they are free of disease in the CNS (documented by flow cytometry performed on the CSF within 14 days of enrollment) and have no evidence of new sites of CNS activity.
9. Pregnant or breastfeeding females
10. Allogeneic HSCT or donor lymphocyte infusion within 90 days of leukapheresis
11. Use of any of the following:
    - Therapeutic doses of corticosteroids (defined as >20 mg/day prednisone or equivalent) within 7 days prior to leukapheresis. Physiologic replacement, topical, and inhaled steroids are permitted
    - Cytotoxic chemotherapeutic agents within 1 week of leukapheresis. Oral chemotherapeutic agents are allowed if at least 3 half-lives have elapsed prior to leukapheresis.
    - Lymphotoxic chemotherapeutic agents within 2 weeks of leukapheresis
    - Daratumumab (or other anti-CD38 therapy) within 30 days of leukapheresis
    - Experimental agents within 4 weeks of leukapheresis unless progression is documented on therapy and at least 3 half-lives have elapsed prior to leukapheresis
12. ANC < 1000/mm^3, or per PI discretion if cytopenia thought to be related to underlying myeloma
13. Hgb < 8 mg/dl, or per PI discretion if cytopenia thought to be related to underlying myeloma
14. Platelet count < 50,000/mm^3, or per PI discretion if cytopenia thought to be related to underlying myeloma

15. Active autoimmune disease requiring immunosuppressive therapy
16. Major organ dysfunction defined as:
    a. Creatinine clearance < 20 ml/min.
    b. Significant hepatic dysfunction (SGOT >5x upper limit of normal; bilirubin >3.0 mg/dL)
    c. FEV1 of <50% predicted or DLCO (corrected) <40% (patients with clinically significant pulmonary dysfunction, as determined by medical history and physical exam should undergo pulmonary function testing)
17. Anticipated survival of <3 months
18. Contraindication to cyclophosphamide or fludarabine chemotherapy
19. Patients with known AL subtype amyloidosis
20. Uncontrolled medical, psychological, familial, sociological, or geographical conditions that do not permit compliance with the protocol, as judged by the PI; or unwillingness or inability to follow the procedures required in the protocol

4.3 Study Enrollment Procedures

4.3.1 Patient Evaluation and Counseling

Patients will be seen at the Seattle Cancer Care Alliance (SCCA) for consideration of treatment options for their disease. The protocol will be discussed thoroughly with the patient and other family members if appropriate, and all known and potential risks to the patient will be described. The procedure and alternative forms of therapy will be presented as objectively as possible, and the risks and hazards of the procedure explained to the patient. Signed consent will be obtained from the patient using forms approved by the FHCRC IRB. The consent form describes the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A summary of the clinic visit detailing what was covered will be dictated for the medical record.

4.3.2 Protocol Registration

Potentially eligible subjects will be identified and registered into the system by the Clinical Coordinators Office (CCO) / Intake Office and assigned a UPN (Unique Patient Number). The CCO will register enrolled subjects on to the protocol through the Data Management Office. A screening/leukapheresis consent must be signed prior to the performance of study related assessments or procedures. The T cell therapy treatment consent will be signed after successful collection of T cells and prior to chemotherapy and treatment with BCMA CAR-T cells.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

A schematic of the treatment plan is shown in **Figure 6** in Section 2, above. All methods used to derive BCMA CAR-T cells from a patient's $CD8^+$ and $CD4^+$ T cells, and release tests of the cell products prior to infusion are detailed in the Chemistry, Manufacturing and Controls (CMC) section of the Investigational New Drug (IND) application to the FDA. Modifications to the CMC section during the course of the study will be submitted for FDA review.

5.1.1 Leukapheresis or Blood-Draw to Obtain T Cells for BCMA CAR-T Cell Manufacturing

1. After eligibility is confirmed, each patient will undergo leukapheresis to obtain T cells for transduction. Leukapheresis will be performed in the Apheresis Unit at the SCCA using standard operating procedures for obtaining peripheral blood mononuclear cells. Should a technical issue arise during the procedure or in the processing of the product, or insufficient BCMA CAR-T cells be manufactured for the prescribed BCMA CAR-T cell dose, a second procedure may be performed.

   Patients ineligible for a vein-to-vein apheresis may elect to have a percutaneous central venous access catheter inserted to support this collection. Patients ineligible for leukapheresis and who have a hematocrit of >38 and a total non-malignant (normal) lymphocyte count of >2000 may undergo phlebotomy of 400 ml of blood to obtain PBMCs necessary to establish the T cell cultures. This approach will only be taken in patients that would be enrolled at dose levels 1 and 2 and if the absolute CD8 and CD4 T lymphocyte counts are deemed to be sufficient by the Clinical PI to obtain enough T cells for successful manufacturing.

2. The leukapheresis or phlebotomy product will be delivered to the Cell Processing Facility (CPF) at the FHCRC. CD8+ and CD4+ cell selections may be performed in the FHCRC CPF. If lymphocyte subset counts are considered adequate, the product will undergo enrichment for CD8+ T cells and for CD4+ T cells using clinical grade reagents and SOPs developed at the FHCRC. Subsequent processing, after selection of CD8+ and CD4+ T cells and cryopreservation (if required), is performed in the FHCRC CPF. If processing of CD8+ and CD4+ T cells is not considered suitable due to lymphopenia, low CD8+ T and CD4+ cell counts or other reasons, we may use other strategies to manufacture BCMA CAR-T cells without selection of subsets that are approved for use in this protocol by the FDA. PBMC not required for cell selections may be archived for research studies by FHCRC or Juno Therapeutics.

3. Patients will be scheduled for lymphodepleting chemotherapy to be completed 36 to 96 hours prior to the first CAR-T cell infusion.

5.1.2 Interim Therapy after Leukapheresis

1. Patients may receive interim therapy after leukapheresis to control disease during production of BCMA CAR T cells. Decisions regarding interim therapy in this interval should be discussed with the PI
2. See section 5.1.3 below for therapy duration restrictions
3. Patients may undergo disease restaging if they received interim therapy. A bone marrow procedure may be repeated if necessary for disease restaging. If the patient has less than 10% CD138+ malignant plasma cells (IHC) and/or less than 5% BCMA expression, or a decrease in measurable disease by International Myeloma Working Group (IMWG) criteria based on one or more of the findings in section 4.1 prior to lymphodepleting chemotherapy, the patient will remain eligible to receive BCMA CAR T cells.

5.1.3 Criteria for Initiation of Lymphodepleting Chemotherapy

1. Chemotherapy given after leukapheresis to maintain disease control must be stopped ≥ 7 days prior to initiation of lymphodepleting chemotherapy
2. Therapeutic doses of corticosteroids (defined as >20mg/day prednisone or equivalent)

must be stopped ≥ 7 days prior to lymphodepleting chemotherapy. Physiologic replacement, topical, and inhaled steroids are permitted

3. Daratumumab (or other anti-CD38 therapy) must be stopped ≥ 25 days prior to lymphodepleting chemotherapy
4. Investigational agent(s) must be stopped ≥ 25 days prior to lymphodepleting chemotherapy
5. ECOG Performance Status score ≤ 2 within 72 of lymphodepleting chemotherapy
6. MMSE-2 completed within 72 hours of the start of lymphodepleting chemotherapy
   a. If there is a decrease of > 4 points from pre-leukapheresis MMSE-2 score the treatment may be delayed
   b. If treatment is delayed, MMSE-2 may be reevaluated to proceed with treatment per criteria as defined above (5.1.3.6a)
7. Negative serum pregnancy test performed within 2 weeks of starting lymphodepleting chemotherapy for females of reproductive potential (see Appendix I)
8. Adequate renal function, defined as:
   a. eGFR > 20 ml/min (within 1 week prior to lymphodepletion)
9. Absence of uncontrolled active infection (bacterial, fungal, viral, mycobacterial) not responding to treatment with antibiotics, antiviral agents, or antifungal agents

5.1.4 Lymphodepleting Chemotherapy

1. Prior to the infusion of BCMA CAR-T cells, patients will receive cyclophosphamide and fludarabine (Cy/Flu) chemotherapy. The objectives of administering chemotherapy are to provide lymphodepletion to facilitate BCMA CAR-T cell survival and it may additionally reduce the tumor burden.
2. Lymphodepleting chemotherapy should be administered 36-96 hours before the CAR T cell infusion
3. Refer to **Appendix A** for Cy/Flu regimen. In patients who are otherwise eligible but are not able to tolerate Cy/Flu, alternative lymphodepleting regimens may be considered at the discretion of the PI.

5.1.5 BCMA CAR T Cell Infusion

1. BCMA CAR-T cells should be administered between 36 and 96 hours after the completion of lymphodepleting chemotherapy
2. On the day of scheduled T cell infusion the patients should undergo a clinical evaluation and a clinical determination for appropriateness to proceed with CAR T cell administration
3. Patients who receive lymphodepleting chemotherapy, but are deemed not clinically appropriate for CAR-T cell administration or who were unable to undergo CAR T cell infusion due to CAR T cell manufacturing or release issues may be eligible to receive the cell infusion outside of the 36-96 hour window, for up to 7 days after the end of the 36-96 hour window, if clinical concerns and problems with manufacturing or release criteria are resolved. Discussion with the study PI is required.

5.1.6 Infusions of BCMA CAR-T cells

Cell dose escalation/de-escalation

Dose level	*BCMA CAR T Cell Dose*
0	*25 x 10^6 EGFRt$^+$ cells*
*1 **(Starting dose level)***	*50 x 10^6 EGFRt$^+$ cells*
2	*150 x 10^6 EGFRt$^+$ cells*
3	*300 x 10^6 EGFRt$^+$ cells*
4	*450 x 10^6 EGFRt$^+$ cells*
5	*800 x 10^6 EGFRt$^+$ cells*

Table 5.1 Dose levels to be used during dose escalation
[EGFRt is co-expressed in the CAR vector and serves as a transduction marker to determine the number of CAR-T cells in the cell product to define cell dose.]

The primary objective of this study is to estimate the MTD of BCMA CAR-T cells will be infused 36-96 hours following chemotherapy at one of six target dose levels (Table 5.1). A modification of the continual reassessment method (CRM) will be used to estimate the MTD separately for each cohort (low: 10-30% and high: >30% marrow involvement by malignant plasma cells by IHC [using CD138+ staining]).

Treatment of patients in the dose-escalation/de-escalation groups will be staggered with a minimum of a 14-day interval between patients within each cohort. DLTs for dose-finding purposes will be evaluated for 28 days. Refer to the Safety Assessment for DLT criteria (**Section 10.4.1**).

The rationale for starting at 50x10^6 CAR-T cells in each cohort is based on others' experience in MM with BCMA CAR-T cells demonstrating that this dose level is well tolerated, with no reports of neurotoxicity or severe CRS.[86] The stratification plan is shown **Fig. 7**

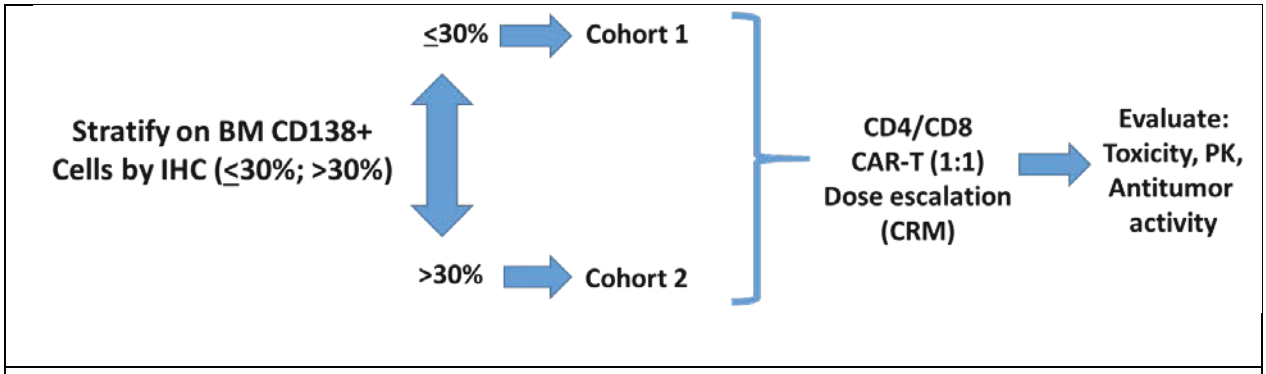

Figure 7. Schematic for stratification by disease burden

Products that cannot be formulated to meet cell dose specification

The intent for each infusion is to provide a cell product that reaches at least 85% of the targeted cell dose and contains approximately 50% (+/- 15%) of BCMA CAR-modified CD4+ T cells and 50% (+/- 15%) of BCMA CAR-modified CD8+ T cells (i.e. CAR-modified T cells in approximately 1:1 CD4+/CD8+ target ratio) If a T cell product cannot be formulated to meet this cell dose specification because of low transduction efficiency, suboptimal growth of one of the subsets, or failure of either the CD4+ or CD8+ T cell product to meet release criteria, the cell product should be infused at or as close as possible to the specified phenotype allocation and total T cell dose providing:

i. The total EGFRt+ CAR-T cell dose exceeds 5x10^4 cells/kg,

ii. The cell product passes other release criteria including endotoxin, gram stain, sterility, mycoplasma, RCL and viability.

iii. Toxicity and safety data will be collected and reported for subjects receiving such products.

Any such patient will not be considered evaluable for safety analysis in the assigned dose cohort and a replacement subject will be added to the cohort.

Cell administration

After passing release criteria, BCMA CAR-T cells will be formulated to provide a single cell product at the specified cell dose.

Each T cell infusion should be administered intravenously over approximately 20 – 30 minutes at the specified T cell dose. At least the first patient treated at each dose level will receive the T cell infusion at the University of Washington Medical Center.

All patients will be monitored during each T cell infusion. Patients should receive the T cells under physician guidance and nursing supervision, with vital signs including oxygen saturation being monitored and recorded before, approximately every 15 minutes during and at the conclusion of the infusion and then approximately hourly for 2 hours after the infusion.

5.1.7 Concomitant Medications

Patients should be discouraged from use of illicit drugs, herbal remedies, self-prescribing drugs, tobacco products, or alcohol consumption at any time during the clinical study.

6. RETREATMENT OF PATIENTS ON THE STUDY

Patients enrolled in the study may be eligible for retreatment with BCMA CAR-T cells, with or without additional cytoreductive chemotherapy, at the same dose or up to the highest dose level cleared for individuals in the cohort to which the patient would now be assigned (≤30% or >30% bone marrow involvement by CD138 IHC) and as long as adequate BCMA CAR-T cells can be produced and the following criteria are met:

a. There is evidence of persistent or progressive disease as defined by IMWG criteria (Appendix F) after the first T cell infusion.

b. There were no toxicities attributed to the first infusion that were dose-limiting or required dose de-escalation (Section 10.4.1).

c. The patient is greater than 21 days from the first T cell infusion.

d. The patient meets eligibility criteria (Section 4.1)

    1. With the exception of inclusion criteria 3 and 5, which are not relevant for patients in this group because they either have disease persistence or have met IMWG progression criteria (Appendix F)

Subjects undergoing retreatment will follow the same assessment schedule. Toxicity data after retreatment with BCMA CAR-T will be recorded, but not used for evaluation of first infusion DLT or dose escalation.

The decision to retreat any patient who meets the above criteria will be at the discretion of the Principal Investigator or their designee (Sub-Investigator) based on a comprehensive assessment of the patient's status at the time retreatment is under consideration.

7. CLINICAL AND LABORATORY EVALUATIONS

7.1 Schedule/Timing of Evaluations (also see **Appendix D** – Study Calendar)

Please note that results of tests and procedures conducted as per standard of care purposes prior to signing of Consent R may be used for research purposes if conducted within the protocol-defined window.

7.2 Specific Evaluations

7.2.1 Screening Evaluations (to be completed within 45 days of enrollment)

- Screening consent & HIPAA signing
- Medical history, including:
  - Hematologic, cytogenetic, flow cytometric, and histologic findings at diagnosis and at the time of enrollment on the study
  - Prior therapies and response to therapy
  - Medical history will be updated from screening to Day 28 post CAR T cell infusion
- Bone marrow aspirate and core biopsy
  - Confirmation of multiple myeloma diagnosis by internal pathology review of initial or subsequent biopsy or other pathologic material at the FHCRC/SCCA and a core bone marrow obtained after the patient's most recent line of therapy and within 45 days of enrollment demonstrating $\geq$10% CD138+ plasma cells by IHC enumeration.
  - Confirmation of BCMA expression on tumor cells ($BCMA^+$ MM comprising $\geq$5% of $CD138^+$ cells) by flow cytometry from bone marrow aspirate, core biopsy washings or single cell suspension from tissue biopsy obtained following the patient's most recent line of therapy and within 45 days of enrollment.
- Research samples (see **Appendix E** for time points)
  - Bone Marrow Aspirate and core biopsy

7.2.2 Evaluations following Screening (to be completed within 45 days prior to leukapheresis)

- Consent A
- Physical exam
- ECOG Performance Status (see Appendix B for scale)
- Mini-Mental State Examination (MMSE 2nd edition, Appendix J)
- CXR
- ECG
- MUGA scan or echocardiogram
- Lumbar puncture with CSF evaluation
  - Required for any patient with a history of CNS disease or signs and symptoms of CNS or epidural disease.

- Performed within 14 days of enrollment.

- Serum Pregnancy test for females of reproductive potential
- Chemistries/Hematology
  - **CBD** (to include: white blood cell count, red blood cell count, hemoglobin, hematocrit, platelet count, absolute neutrophils, lymphocytes, monocytes, eosinophils, basophils, immature granulocytes)
  - **Renal/Hepatic Function Panel** (to include: sodium, potassium, chloride, total carbon dioxide, anion gap, glucose, urea nitrogen, creatinine, *eGFR, total protein, albumin, total bilirubin, direct bilirubin, calcium, phosphate, AST (GOT), Alkaline phosphatase, ALT (GPT))*
  - Magnesium and lactate dehydrogenase (LDH)
- ABO blood typing and antibody screen
- G6PD screening
- PSBC recipient/donor battery panel (HBsAg; anti-HIV-1, 2; anti-HBc; anti-HTLV-1, 2; anti-HCV)
- Immunoglobulins (IgG, IgA, IgM)
- SPEP with immunofixation
- Serum free light chain (FLC) measurement including kappa to lambda ratio
- Creatinine clearance
- Bence Jones Quantitation
- PET/CT scan or bone marrow MRI (if necessary, to meet IMWG defined enrollment criteria only – at this time point)

7.2.3 Evaluation Prior to Leukapheresis

- Eligibility verified
- Height/Weight
- Vital Signs (heart rate, blood pressure, temperature, oxygen saturation)
- Chemistries/Hematology
  - CBD
  - Renal/Hepatic Function Panel
  - Magnesium and lactate dehydrogenase (LDH)
- C-Reactive Protein (CRP)
- Research samples (see Appendix E)
  - Blood
    - Including T-Cell Subset (within 7 days of leukapheresis)

7.2.4 Evaluations Prior to Lymphodepletion

- Criteria for initiation of Lymphodepleting Chemotherapy section 5.1.3
- Physical exam
- ECOG
- Vital Signs
- MMSE-2 (within 72 hours of lymphodepleting chemotherapy)
- Serum Pregnancy test for females of reproductive potential
- Chemistries/Hematology
    - CBD
    - Renal/Hepatic Function Panel
    - Magnesium and lactate dehydrogenase (LDH)
    - Uric Acid
- Serum Ferritin and CRP
- Coagulation panel (PT, PTT, fibrinogen, and D-dimer)
- Immunoglobulins (IgG, IgA, IgM)
    - Repeat only if more than 60 days since last result or if patient received intervening cytotoxic therapy
- SPEP with immunofixation
- Serum free light chain (FLC) measurement including kappa to lambda ratio
- Bence-Jones Protein Quantitation
- Bone marrow aspirate and biopsy
    - Repeat if patient received interim therapy prior to lymphodepletion and has greater than 50% reduction from screening values of serum FLC or monoclonal spike (M spike/protein) or if patients receive a multi drug myelosuppressive treatment regimen (eg. PACE or hyper CVAD) or ≥ 30 days have passed since leukapheresis or at PI discretion.
        - Hyposecretory / Nonsecretory patients who receive interim therapy require a repeat BMA/BMB
    - Evaluation should include pathology analysis including IHC [using CD138+ staining], flow cytometry
- PET/CT scan or bone marrow MRI
    - Repeat imaging prior to starting lymphodepleting chemotherapy if patient received interim therapy or more than 30 days since last PET/CT or bone marrow MRI
- Osseous skeletal survey
- Tissue biopsy (if applicable; may be performed at any time point prior to LD chemotherapy)

- Research samples (see Appendix E)
  - Blood
  - Bone marrow aspirate/biopsy
  - Tissue biopsy

7.2.5 Evaluations Prior to BCMA CAR T Cell Infusion (Day 0)

- Physical exam
- ECOG Performance Status
- Vital Signs
- Chemistries/Hematology
  - CBD
  - Renal/Hepatic Function Panel
  - Magnesium and lactate dehydrogenase (LDH)
  - Uric Acid
- Coagulation panel (Coagulation PT, PTT, fibrinogen, and D-dimer)
- Serum Ferritin and CRP
- Research samples (see Appendix E)
  - Blood

7.2.6 Evaluations Following each BCMA CAR T Cell Infusion (see Appendix D Study Calendar)

- Physical exam (Days +1, 3, 7, 10, 14, 21)
- ECOG Performance Status (Days +7, 14, 21)
- Vital Signs (Days +1, 3, 7, 10, 14, 21)
- Chemistries/Hematology (Days +1, 3, 7, 10, 14, 21)
  - CBD
  - Renal/Hepatic Function Panel
  - Magnesium and lactate dehydrogenase (LDH)
- Uric acid (Days +3, 10, 14, 21)
- Coagulation panel (Days +1, 3, 7, 10, 14, 21)
- Serum Ferritin and CRP (Days +1, 3, 7, 10, 14, 21)
- SPEP with immunofixation (Day +14)
- Serum Free Light Chains (Day +14)
- Bone marrow aspirate and core biopsy (Day +14, +/- 3 days)
- Research samples (see Appendix E)

- Bone marrow aspirate and core biopsy (Day +14, +/- 3 days)
- Blood (Days +1, 3, 7, 10, 14, 21)

- If patients become febrile or develop symptoms of cytokine release or tumor lysis between the indicated time points, we may measure serum ferritin, CRP, coagulation panel, and tumor lysis markers at additional times

7.2.7 Response Assessment (Day 28 +/- 3 days)

- Physical exam
- ECOG Performance Status
- Vital Signs
- Chemistries/Hematology
  - CBC, differential and platelet count
  - Renal/Hepatic Function Panel
  - Magnesium and lactate dehydrogenase (LDH)
- Coagulation panel (Coagulation PT, PTT, fibrinogen, and D-dimer)
- Serum Ferritin and CRP
- Immunoglobulins (IgG, IgA, IgM)
- Serum free light chains (FLC) measurement including kappa to lambda ratio
- SPEP with immunofixation
- Bence-Jones Protein Quantitation
- PET/CT scan or bone marrow MRI
- Bone marrow aspirate and core biopsy
  - Evaluation should include pathology analysis including IHC [using CD138+ staining], flow cytometry
  - Karyotyping, FISH studies [if clinical evidence of relapse only]
- Tissue biopsy (if applicable)
- Research samples (see Appendix E)
  - Bone marrow aspirate and core biopsy
  - Blood
  - Tissue biopsy (if applicable)

7.2.8 Post Treatment Follow-Up *(see Study Calendar for Time-points Window)*

- Physical exam (Day 60, 90, 120, 180, 365)
- Laboratory testing (Day 60, 90, 120, 180, and 365) including:
  - CBD (CBC w/ differential)

- Renal/Hepatic Function Panel
- Magnesium and lactate dehydrogenase (LDH)

- Immunoglobulins - IgG, IgA, IgM (Day 60, 90, 180, and 365)
- Serum free light chains (Day 60, 90, 180, and 365)
- SPEP with immunofixation (Day 60, 90, 180, and 365)
- Bone marrow aspirate and core biopsy (Day 60, 90, 180, and 365)
  - Evaluation should include pathology analysis including IHC [using CD138+ staining], flow cytometry
  - Karyotyping, FISH studies [if clinical evidence of relapse only]
- Osseous skeletal survey (Days 180 and 365)
- Bence-Jones protein quantitation (Day 60, 90, 180, and 365)
- PET/CT or bone marrow MRI (Day 90)
- Research samples (see Appendix E)
  - Bone marrow aspirate and core biopsy (Day 60, 90, 180, and 365)
  - Blood (Day 60, 90, 120, 180, and 365)

7.2.9 **Suspected Relapse** +/- 3 months (ideally prior to systemic therapy)

- Physical exam
- Laboratory testing including:
  - CBD (CBC w/ differential)
  - Renal/Hepatic Function Panel
  - Magnesium and lactate dehydrogenase (LDH)
- Serum free light chains
- SPEP with immunofixation
- Bence-Jones Protein Quantitation
- Bone marrow aspirate and core biopsy
  - Evaluation should include pathology analysis including IHC [using CD138+ staining], flow cytometry
  - Karyotyping, FISH studies [if clinical evidence of relapse only]
- PET/CT or bone marrow MRI (as clinically warranted)
- Research samples (see Appendix E)
  - Bone marrow aspirate and core biopsy
  - Blood
  - Tissue biopsy (if applicable)

7.2.10 Long Term Follow-up (see also Appendix G)

Enrolled patients who receive BCMA CAR-T cells will be asked to participate in long-term follow-up (LTFU) according to the guidelines set forth by the FDA's Biologic Response Modifiers Advisory Committee that apply to gene transfer studies. Current recommendations from the FDA suggest a minimum of 15 years of follow-up. See **Appendix G** for recommendations.

7.2.11 Research Samples (see Appendix E for time-points, volumes, and lab delivery)

If biopsies, sampling of tissues or specimens are performed for clinical indications then additional tissue/specimen may be obtained during the same procedure for research. Please discuss the planned procedure with the PI.

PBMC Immunophenotyping

- Blood samples will be obtained during screening and repeated if necessary, to confirm subject feasibility and/or facilitate cell selection of CD4, and CD8 T cells.

Evaluation of Persistence and Phenotype of BCMA CAR-T Cells:

- Blood samples will be obtained prior to the T cell infusion to provide controls for analysis of the presence of transferred T cells by q-PCR for vector sequences and/or for expression of the EGFRt and BCMA CAR transgenes on $CD8^+$ and $CD4^+$ T cells by flow cytometry, if sufficient PBMC can be obtained.

Serum Cytokines

- Samples should be obtained for measurement of serum inflammatory cytokine levels using Luminex bead array.
- If patients become febrile, develop symptoms of cytokine release, or assessment of cytokines is clinically appropriate at times other than those indicated, we may measure cytokine levels at additional times.

B-Cell Reconstitution

- BCMA is not expressed on mature normal B cells or PC in the peripheral blood, but is expressed at a stage of B cell development in the BM. The absolute numbers of normal B cells in the peripheral blood (and abnormal BCMA+ B cells in patients with MRD or relapse) should be determined at various time points after the last T cell infusion. If a deficiency of B cells develops by day 90 or there are persisting BCMA CAR-T cells, we will continue to monitor B cell numbers every 6 months. Blood samples should be sent to SCCA Hematopathology Laboratory for analysis.

Ig Levels

- Patients receiving CAR T cells who develop significant B/plasma cell depletion may develop an IgG deficiency. Recommendations may be made for monitoring Ig (IgG, IgA, IgM) levels more frequently and administering IVIG, as clinically indicated.
- Monitoring of Ig levels may be discontinued if endogenous normal PCs are detected in blood or if the patient does not have evidence of persisting BCMA CAR-T cells in the blood.

Evaluation of persistence and phenotype of BCMA CAR-T Cells

- Blood samples should be obtained at various time points after the T cell infusion for analysis of the persistence of transferred T cells. Additional samples may be collected at other times than those indicated if required for evaluation of persistence of CAR-T cells. Persistence monitoring may be discontinued beyond day 28 in patients who proceed to allogeneic hematopoietic stem cell transplantation and/or do not have detectable transgene-expressing T cells on two consecutive occasions.
- A subset of blood samples obtained after the infusion should also be analyzed by multi-parameter flow cytometry for the phenotype of persisting CD8+ and CD4+ BCMA CAR-T cells. Markers that may be analyzed include but are not limited to: CD62L, CCR7, CD28, CD27, CD127, PD1, Lag3, Tim3 and PDL1.
- If patients become febrile, develop possible signs of toxicity, or assessment of CAR-T cell persistence is clinically appropriate at times other than those indicated, we may measure the persistence of transferred T cells at additional times.

Evaluation of Transgene Immunogenicity

- If loss of persistence of transferred T cells by flow cytometry for the EGFRt marker/and or BCMA-Fc marker (<0.01% of $CD3^+$ cells) is noted, patients will be evaluated for antibody or T cell-mediated transgene immune responses:
- Blood will be collected and serum extracted for evaluation of antibody-mediated immune responses.
- Blood will be collected and PBMC isolated for evaluation of cellular immune responses.

Archival Samples for Future Studies of T Cell Function at FHCRC or Juno Therapeutics

- Blood may be obtained from patients for serum and plasma archival purposes.

Circulating Tumor DNA Analysis

- Blood will be collected for circulating tumor DNA analysis

Evaluation of migration of adoptively transferred BCMA CAR-T cells

- If aspirations and/or biopsies of BM are performed for evaluation of tumor response or other clinical indications then additional aspirates (5-10 ml) will be obtained for research.

8. POTENTIAL RISKS, TOXICITY MONITORING, AND CARE

Acute infusion toxicities may occur during or shortly after the T cell infusion. In addition, cytokine release syndrome, tumor lysis syndrome, and neurologic toxicity may occur after the infusion of BCMA CAR-T cells. Management of these complications are addressed in the sections below. A table of proposed grading criteria for CRS and neurotoxicity is provided in **Appendix H**.

8.1 Acute Toxicity Associated with T Cell Infusion

The results of our prior studies of adoptive immunotherapy for CMV, HIV, leukemia and lymphoma, and melanoma suggest that serious acute toxicities resulting simply from infusing the numbers of T cells proposed in this study are unlikely to occur. However, fevers and other transient constitutional symptoms are often observed with T cell infusions, and can occur very shortly after the T cell infusion in patients with a high antigen (tumor) burden.

1. Examples of potential symptoms/signs due to T cell infusions and their initial management are:
   - Fever, chills, and temperature elevations >38.3 degrees Celsius may be managed with acetaminophen 650 mg orally q 4-6 hrs. All subjects who develop fever or chills should have a blood culture drawn.
   - Headache may be managed with acetaminophen.
   - Nausea, vomiting may be managed with diphenhydramine 25-50 mg IV or other antiemetics (excluding corticosteroids).
   - Hypotension should be managed initially by fluid administration.
   - Hypoxemia should be managed initially with supplemental oxygen.
2. If the following signs appear during T cell infusion, the infusion should be paused and the patient assessed:
   - Systolic BP < 80 mmHg OR > 30 mmHg fall from baseline
   - Heart rate > 140/min OR increase from baseline > 40/min (confirmed by palpation or EKG)
   - Respiratory rate > 35/min OR increase from baseline of > 10/min
   - Arterial O2 saturation < 88% on air OR fall from baseline > 5%
3. If after assessment by the PI or designee the patient's condition is stable then the infusion may be resumed.
4. If an infusion is terminated due to acute toxicity, the residual T cells should be returned to FHCRC Therapeutic Products Program Quality Control Department for analysis. Investigation of possible causes of observed signs should proceed and, if necessary, additional medical treatment will be instituted.
5. Patients requiring discontinuation of T cell infusion may be eligible for re-treatment if the cause is deemed not related to the T cell infusion.

8.2 Cytokine Release Syndrome

1. Administration of CAR T cells such as BCMA CAR T cells may be associated with CRS. CRS may be characterized by high fever, fatigue, nausea, headache, dyspnea, tachycardia, rigors, hypotension, hypoxia, myalgia/arthralgia, anorexia, coagulation abnormalities, organ dysfunction, and neurologic abnormalities.
2. If a patient becomes febrile or develops symptoms of CRS, cytokine levels, serum ferritin, CRP, coagulation studies, and/or markers of tumor lysis syndrome (eg., chemistry, uric acid, LDH may be measured, and persistence and/or phenotype of the transgene-expressing cells may be evaluated, as clinically indicated.
3. Any patient who develops clinical evidence of symptoms related to CRS will have a work-up to exclude infection or other causes, as clinically appropriate. Initial treatment should consist of supportive measures as dictated by the clinical and laboratory findings, and may

include fluid replacement, medications to support blood pressure, antipyretics, oxygen supplementation, anti-seizure medications, and broad-spectrum antibiotics if infection cannot be excluded as a potential etiology for the signs and symptoms.

4. A modification of the CTCAE CRS grading scale has been established to better reflect CAR T cells associated CRS (Lee 2014), as detailed in Appendix C.
    a. Patients with Grade ≥ 3 CRS (severe CRS; sCRS) and/or Grade 2 CRS with progressive symptoms and signs should be treated with tocilizumab 4-8 mg/kg IV and corticosteroids (dexamethasone 10 mg IV every 12 hours).
        i. Higher doses of steroids may be given after discussion with the PI or designee and repeated doses of tocilizumab may be given if necessary.
5. Guidelines for management of CRS are provided in Appendix H.
6. Other cytokine-directed therapies may be considered after discussion with the PI.

8.3 Neurologic Toxicity

1. Neurotoxicity, manifest as delirium, seizures, focal neurologic deficits, and/or coma, has been reported after CAR T cell therapy. Neurotoxicity is usually reversible, but can be irreversible or fatal. Levetiracetam (500 mg bid PO starting dose) or other anti-seizure medication should be considered prophylactically prior to treatment.
2. For patients who develop mild neurologic manifestations (CTCAE Grade 1), symptomatic care and levetiracetam are recommended. Discussion with the PI or designee is recommended.
3. For patients with worsening neurologic changes, the addition of corticosteroids should be considered (eg, dexamethasone 10 mg IV every 6 to 24 hours). Tocilizumab (4-8 mg/kg IV) or other cytokine-directed therapies may be used based on clinical judgment; at this time, it is unclear if these approaches are of benefit to patients with neurotoxicity. Cerebrospinal fluid (CSF) assessments and CNS imaging should be considered if clinically indicated.
4. Guidelines for management of neurotoxicity are provided in Appendix H.

8.4 Tumor Lysis Syndrome

1. All patients will be considered at risk for tumor lysis and should receive allopurinol prophylaxis before chemotherapy begins, unless contraindicated. Allopurinol should be continued for as long as the medical team determines appropriate after the T cell infusion. They may receive additional hydration and urine alkalinization for the first 2 weeks after the T cell infusion.
2. If tumor lysis syndrome develops, as defined by the Cairo Bishop criteria[87], the Attending Physician will direct patient management with guidance from the study staff[88]. Conservative therapy, including allopurinol, urinary alkalinization, and IV fluid hydration may be instituted immediately for suspected tumor lysis syndrome. Hyperkalemia may be treated with potassium binding resins, diuresis, or insulin/dextrose therapy. Hyperphosphatemia may be treated with phosphate binding resins. In severe cases, rasburicase (in non-G6PD-deficient individuals) or renal dialysis may be necessary.

8.5 Macrophage Activation Syndrome

Macrophage activation syndrome (MAS) is a serious disorder potentially associated with uncontrolled activation and proliferation of CAR T cells and subsequent activation of macrophages. MAS is typically characterized by high-grade, non-remitting fever, cytopenias, and hepatosplenomegaly, and may be difficult to distinguish from CRS. Laboratory abnormalities found in MAS include elevated inflammatory cytokine levels, serum ferritin, soluble IL-2 receptor (sCD25), triglycerides, and decreased circulating NK cells. Other findings include variable levels of transaminases, signs of acute liver failure, coagulopathy, and disseminated intravascular coagulopathy. While there are no definitive diagnostic criteria for MAS, it is typically diagnosed using published criteria for hemaphagocytic lymphohistiocytosis (Schulert 2015).

8.6 Other Toxicities

1. If a new onset CTCAE v4.0 grade ≥3 toxicity is observed following any T cell infusion, the patients will receive investigation and medical treatment appropriate for the physiological abnormalities.
2. Grade ≥3 toxicity that is attributed to the T cell infusion/s and is unresponsive to supportive measures or persists for > 7 days may be treated with corticosteroids (e.g. dexamethasone 10 mg IV q 4-12 hours) after discussion with the Principal Investigator or designee.
3. Uncontrolled proliferation of CAR-T cells has not been observed in clinical trials to date. However, in the unlikely event uncontrolled proliferation of BCMA CAR-T cells occurred in a study subject, initial therapy may involve treatment with corticosteroids (e.g. methylprednisolone 1 g IV). Anti-lymphocyte globulin or cytotoxic drugs would also be considered in serious cases. If we observe an increase in CAR positive cells to greater than 10% of T cells at more than 3 months after last infusion we will analyze for clonal expansion by deep sequencing of the TCR beta gene (ImmunoSeq Adaptive Biotechnology).
4. The vector encodes EGFRt and our preclinical data in animal models demonstrates that administering two doses of cetuximab can eliminate CAR-T cells that express EGFRt and reverse on-target toxicity not attributed to CRS for which there is no suitable animal model. Thus, in patients without CRS who have an ongoing organ toxicity that is attributed to BCMA CAR-T cells, we could consider administering up to two doses one week apart of intravenous cetuximab in standard dosing
    a. Cetuximab 400 mg/m^2 (dose 1) and 250 mg/m^2 (dose 2) with premedication per standard practice.
    b. Blood samples should be collected prior to the cetuximab infusions, and on day +1, 4, 7, 14, and 21 after cetuximab infusions to determine if cetuximab resulted in a decline or elimination of BCMA CAR-T cells.
        i. 40 ml on each designated day
5. BCMA is expressed by normal plasma cells and required for their long term survival[89]. Thus, a deficiency of plasma cells but not B cells, which are BCMA$^-$, is anticipated if CAR-T cells persist. We will measure serum Ig every 6 months, and patients will receive intravenous Ig (IVIG) if the IgG level is <400 mg/dl. If patients achieve a complete molecular remission for >1-year duration, are deficient in normal plasma cells, and have persisting BCMA CAR-T cells, we would offer the patient treatment with 2 doses of cetuximab one week apart to determine if CAR-T cells could be ablated as we have shown in murine model

9. CRITERIA FOR INTERVENTION DISCONTINUATION

9.1 Removal of Patients from Study

A patient may be removed from the study for any of the following reasons:

- Patient did not receive BCMA CAR-T cells
- Patient withdrawal of consent
- Study termination by the PI, the FHCRC Institutional Review Board (IRB), or the FDA
- Loss to follow-up
- Death
- Continued participation is not in the patient's best interests

Patients who have received BCMA CAR-T cells and are subsequently removed from study treatment and research evaluations will be asked if they consent to participate in long-term follow-up.

Patients who are withdrawn from the study because of failure to generate a BCMA CAR-T cell dose that meets the required quality control and criteria for product release may re-enroll in the study at a later time provided the patient meets all eligibility criteria.

10. STATISTICAL CONSIDERATION

10.1 Type of Study

This is a phase 1 study to assess the safety and estimate the antitumor activity of adoptive T cell therapy with autologous $CD4^+$ T cells and $CD8^+$ T cells transduced to express a BCMA-specific CAR for patients with $BCMA^+$ multiple myeloma.

10.2 Outcomes

10.2.1 General Toxicity Assessment

- History and physical exam before and at intervals after T cell infusions
- CBC and chemistry laboratory values before and at intervals after the T cell infusion
- Toxicity grading according to NCI CTCAE Version 4.0
- Serum cytokine levels
- B cell reconstitution
- Serum Ig levels
- Replication competent lentivirus
- Adverse event reporting

10.2.2 Efficacy Assessment

- Evaluation of the duration of persistence of adoptively transferred BCMA CAR-T cells
- Evaluation of the migration of adoptively transferred BCMA CAR-T cells
- Evaluation of antitumor activity of adoptively transferred BCMA CAR-T cells by evaluating the objective response rate of complete remission and partial remission, and determining PFS and OS.

10.3 Sample Size and Accrual

Patients are planned to be treated in cohorts of two patients at one or more of six dose levels of BCMA CAR-T cells starting at dose level 1 (**Section 5.1.5**) to determine cell doses associated with an estimated dose limiting toxicity rate of less than 25%. Dose adjustments will be made separately for the high- and low-disease-burden cohorts.

10.4 Endpoint Monitoring/Analyses

10.4.1 Safety Assessment

1. **DLT**

The primary endpoint for this study is dose-limiting toxicity (DLT). DLT is defined as the occurrence of any of the following that is attributed as at least possibly related to the T cell infusion. Grading will be done in accordance with the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 unless otherwise specified.

a) Grades 3-5 allergic reaction related to the BCMA CAR T cell infusion.

b) Grades 3-5 autoimmune reactions, excluding B cell depletion, which is expected to occur as a consequence of elimination of $BCMA^{+}$ cells.

c) Any Grade 3 or 4 non-hematologic event definitely or probably related to the BCMA CAR T cells, that has not resolved to < grade 3 by day 28 post BCMA CAR T cell infusion.

d) Grade ≥ 3 neurotoxicity of greater than 7 days duration.

e) Grade ≥ 3 neurotoxicity lasting > 7 days in duration that does not revert to Grade 1 or baseline by day 28 post BCMA CAR T cell infusion.

f) Grade ≥ 3 seizures that do not resolve to < grade 3 within 3 days.

g) Grade ≥ 4 CRS (using criteria modified from Lee 2014, Appendix C).

h) Grade 3 CRS that does not resolve to < grade 3 within 7 days (using criteria modified from Lee 2014, Appendix C).

i) Any other toxicity not meeting the above criteria that is deemed by the PI to represent a DLT.

- The DLT evaluation period is 28 days from infusion. All DLT events will be considered evaluable. However, if a patient does not experience a DLT (within the DLT window) and received a cell infusion that did not meet specifications, the absence of a DLT in that patient will not be considered relevant nor included in the estimation of the MTD. The target DLT rate is 25%.

A modification of the continual reassessment method (CRM) will be used to estimate the MTD. The modifications include treating patients in cohorts of two and not allowing any dose levels to be skipped. The number of patients with a DLT at each dose level will be used to determine dose escalation or de-escalation, as defined by the CRM algorithm. Treatment of patients in the dose-escalation/de-escalation groups will be staggered with a minimum of a 14-day interval following infusion between patients within each cohort. DLTs for dose-finding purposes will be evaluated for 28 days.

The CRM algorithm will be followed until up to 25 patients are treated, and the MTD will be estimated after the 25th patient is evaluable for DLT. As noted above, the high- and low-disease-burden groups will be treated separately in terms of dose modifications.

The original version of this protocol used a gamma prior distribution with shape and scale parameters of 20 and 0.05, respectively, and a skeleton prior of .05, .10, .25, .40, and .60 for 5 doses. In this revision, we have added a dose level (see section 5.1.5) and we have additionally changed the prior distribution and the prior skeleton as detailed below, the new prior being much less informative than the original gamma prior.

The functional form of the dose-toxicity curve that will be used in the modified CRM is often referred to as the power model, namely the probability, p, that a particular patient experiences a DLT at dose d is given by $p=d^{\exp(\alpha)}$, where α is a parameter that is estimated existing data and updated as data are accumulated. The initial estimates of DLT at the 6 dose levels (also known as the "skeleton") will be taken to be 0.16, 0.25, 0.35, 0.46, .56, and 0.65, with the starting dose level the second of these. The prior distribution for α will be taken to be a normal distribution with mean zero and variance 0.63, this variance providing the least informative prior for the current situation. The study will stop if the lower limit of a 90% confidence interval for the estimated DLT rate at the lowest dose level exceeds the target DLT rate of 25%.

The table below summarizes the operating characteristics under several assumed-true scenarios.

True DLT probability:	0.05	0.1	0.2	0.4	0.55	0.7
MTD selection percentage:	0.2	10.2	59.5	28.3	1.7	0.0
Average number of DLTs:	0.1	0.6	2.2	2.4	0.6	0.1
Average number of patients:	1.04	6.24	11.39	6.08	1.11	0.12
Percentage stopped for safety:	0.1					

True DLT probability:	0.05	0.1	0.15	0.2	0.25	0.3
MTD selection percentage:	0.2	4.2	22.5	32.4	26.1	14.5
Average number of DLTs:	0.1	0.4	1.0	1.3	1.1	0.8
Average number of patients:	0.92	4.72	6.93	6.52	4.26	2.64
Percentage stopped for safety:	0.1					

True DLT probability:	0.05	0.2	0.4	0.5	0.65	0.8
MTD selection percentage:	5.2	60.7	31.8	2.0	0.0	0.0
Average number of DLTs:	0.2	2.6	3.0	0.6	0.1	0.0
Average number of patients:	3.55	13.46	7.48	1.28	0.16	0.01
Percentage stopped for safety:	0.3					

True DLT probability:	0.01	0.07	0.11	0.15	0.2	0.25
MTD selection percentage:	0.0	1.3	7.9	27.5	31.8	31.5
Average number of DLTs:	0.0	0.2	0.5	1.0	1.0	1.1
Average number of patients:	0.41	3.59	5.27	6.67	5.25	4.81
Percentage stopped for safety:	0					

True DLT probability:	0.3 0.5	0.55	0.65	0.75	0.85	
MTD selection percentage:	64.4	6.5	0.4	0.0	0.0	0.0
Average number of DLTs:	4.7	2.5	0.5	0.1	0.0	0.0
Average number of patients:	15.98	5.07	0.89	0.13	0.00	0.00
Percentage stopped for safety:	28.7					

2. Stopping and Suspension Rules and Criteria

If there ever exists sufficient evidence to suggest that the true probability of treatment-related death by day 100 exceeds 20% (regardless of T cell dose), enrollment of patients will be suspended pending review by the PI, DSMB, and statistician. Sufficient evidence will be defined as any observed outcome whose lower 80% confidence limit exceeds 20%. Operationally, any of the following ratios of treatment-related deaths to patients treated would trigger such a rule: 2/2-4, 3/5-7, 4/8-11, 5/12-15, or 6/16-20. If the true probability of treatment-related death is 0.10, then the probability of stopping after 10 or 20 patients is approximately .07 and .08, respectively. If the true probability of treatment related death is 0.40, then the probabilities of stopping after 10 or 20 patients are approximately 0.73 and 0.92, respectively (probabilities estimated from 5,000 simulations).

Additionally, any death in the first two dose levels, independent of cohort, will trigger a study pause to evaluate the incident to assure the safety of the study participants.

10.4.2 Assessments of Efficacy of Transferred T Cells

Data should be collected for persistence, migration and efficacy of transferred T cells and descriptive statistics will be used to summarize the changes from baseline, where possible. Responses will be evaluated using modified IWMG response criteria (see Appendix F).

11. DATA COLLECTION, SITE MONITORING, ADVERSE EVENT REPORTING

11.1 Records, Data Management

The medical record containing information regarding treatment of the patient will be maintained as a confidential document, within the guidelines of the Fred Hutchinson Cancer Research Center, the University of Washington Medical Center and the Seattle Cancer Care Alliance. The investigators will ensure that data collected conform to all established guidelines. Each patient is assigned a unique patient number to assure patient confidentiality. Patients will not be referred to by name or by any other personal identifier in any publication or external presentation. The licensed medical records departments, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents. The primary research records are kept in access controlled office spaces or password protected computer based applications. Information gathered from this

study regarding patient outcomes and adverse events may be made available to the U.S. Food and Drug Administration, NIH and Juno Therapeutics. All precautions to maintain confidentiality of medical records will be taken.

11.2 Adverse Event Reporting

- Adverse Event

  An Adverse Event (AE) is any undesirable experience associated with the use of a medical product in a patient. The NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40) will be used for grading and analysis of adverse events. All grade 3 or greater adverse events will be collected during and for 48 hours post leukapheresis and again from the start of lymphodepleting chemotherapy through day 28 after each T cell infusion. The collection of adverse events will stop at the time of commencement of new anti-tumor therapy.

- Serious Adverse Event

  According to FDA guidelines, 21 CFR 312.32, IND Safety Reports, and Definitions and Standards for Expedited Reporting, a Serious Adverse Event (SAE) is defined as an adverse event that results in one or more of the following outcomes:

  1. Result in Death
  2. Is life-threatening
  3. Requires inpatient hospitalization or prolongation of existing hospitalization
  4. Results in a persistent or significant disability/incapacity
  5. Is a congenital anomaly or birth defect
  6. Is an important medical event, as determined by the investigator that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above.

11.2.1 Assessment of Causality

To ensure that investigative treatment-related conditions are distinguished from disease related conditions, attribution of causality will be established in grading adverse events. For each event, the Principal Investigator or designee, in conjunction with the physician or research nurse who examined and evaluated the research participant, will assign the attribution. Data managers who are removed from the clinical assessment of the research participant should not perform this.

Attribution of adverse events attributed to the infused genetically modified T cells should be determined using the following criteria:

Definite (must have all 3)	• Has a reasonable temporal relationship to the intervention • Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions • Follows a known pattern of response to intervention
Probable (must have 2)	• Has a reasonable temporal relationship to the intervention

	• Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions • Follows a known pattern of response to intervention
Possible (must have 2)	• Has a reasonable temporal relationship to the intervention • Could not have readily been produced by the subject's clinical state • Could not readily have been due to environmental or other interventions • Follows a known pattern of response to intervention
Unlikely (must have 2)	• Does not have a temporal relationship to the intervention • Could readily have been produced by the subject's clinical state • Could have been due to environmental or other interventions • Does not follow a known pattern of response to intervention • Does not reappear or worsen with reintroduction of intervention

Deaths occurring 30 days or later from a T cell infusion and death due to progression of known malignancy will be scored as a grade 5 toxicity, but with a low level of attribution, unlikely or not related, unless a serious toxicity that was attributed to the T cell infusion occurred prior to day 30, did not resolve and was considered a significant factor in the patient's demise.

11.2.2 Reporting to Sponsor

The principal investigator or designee must report events to the sponsor of the study as outlined below.

Classification of an event as serious or non-serious determines the reporting procedures to be followed by the site for reporting the event to the IND Sponsor.

PI to IND Sponsor Reporting Requirements for Adverse Events

Classification		Reporting Time	Reporting Action
Serious Adverse Event (SAE)	Fatal or life-threatening	Within 24 hours of research team awareness	Email notification to Sponsor & PI
	All SAEs	Within 2 business days of research team* awareness	Email notification to Sponsor & PI
Non-serious Adverse Event		Per CRF completion guidelines	Record information on appropriate CRFs

*Research team is defined as the individuals listed on the delegation of authority log. Physicians listed on the study's delegation of authority log as attending physicians with delegated authority to administer informed consent will not be considered part of the research team unless additional responsibilities related to the conduct of the study have been delegated to them by the Principal Investigator.

The IND Sponsor will report safety information to the FDA in accordance with regulations under 21 CFR 312.32.

The FHCRC PI and Research Nurse and study personnel should meet regularly (in person or via teleconference) to review all reported events.

11.2.3 Reporting to IRB

1. Definitions associated with reportable events and reporting requirements can be found on the FHCRCs Institutional Review Office (IRO) extranet website (Table 1).

2. The FHCRC IRB will be notified of reportable events by the FHCRC Principal Investigator (PI) or study nurse according to current reporting obligations as found on the FHCRC Institutional Review Office extranet website.

11.2.4 Data and Safety Monitoring Plan

Institutional support of trial monitoring will be in accordance with the Fred Hutch/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, Fred Hutch Clinical Research Support coordinates data and compliance monitoring conducted by consultants, contract research organizations, or Fred Hutch employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Fred Hutch Scientific Review Committee (SRC) and the Fred Hutch/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating patients. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state, and federal guidelines.

Monitoring and Personnel Responsible for Monitoring

1. The Principal Investigator (P.I.) and IND Sponsor are responsible for every aspect of the design, conduct and final analysis of the protocol. Regulations defining the responsibilities for assessment and reporting of adverse events (AE), serious AE and unexpected AE are defined by the Code of Federal Regulations: 21 CFR 312.32 and Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 published by the Cancer Therapy Evaluation Program (CTEP), a division of the NCI/NIH.
2. This clinical study will rely upon the monitoring of the trial by the P.I. in conjunction with a Study Physician(s), Physician Assistant(s) (PA) or Nurse Practitioner(s), Research Nurse(s), Research Coordinator(s), statistician, and an independent Study Monitor assigned by the FHCRC Clinical Research Support Office.
3. Continuous monitoring of the data and safety of this study should be performed by the Protocol Management Team (PMT), which consists of the Principal Investigators, Research Nurse, and Statistician. Monitoring by the PMT should be performed at least monthly or more often if necessitated by the development of adverse events, and should include review of the data on all enrolled patients with a summary of grade 3+ adverse events and follow up information for each patient. This list of patients should be included with the PMT report form. The PMT will be responsible for implementation of the stopping rules for safety if necessary.
4. A Case Report Form (CRF) should be completed for every patient that was registered for participation in the study.
    a. Forms should be completed as information becomes available on a visit-by-visit or course-by-course basis.
    b. The Principal Investigator or a Sub-Investigator will sign and date CRF of all patients at the close of the study.

5. All laboratory specimens, evaluation forms, reports, video recordings, and other records that leave the site will be identified only by the Study Identification Number (SID) to maintain subject confidentiality. All records will be kept in a secure access protected file cabinet. All computer entry and networking programs will be done using SIDs only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB or the FDA.

Data and Safety Monitoring Board (DSMB)

The study will be monitored by the Immunotherapy Integrated Research Center (IIRC) DSMB. The DSMB will be responsible for safeguarding the interests of trial participants and assessing the safety and efficacy of the interventions during the trial. This responsibility will be exercised by providing recommendations about stopping or continuing the trial. To contribute to enhancing the integrity of the trial, the DSMB may also formulate recommendations relating to the selection, recruitment and retention of participants and their management; adherence to protocol-specified regimens; and the procedures for data management and quality control.

The DSMB will be advisory to the study Sponsor and the PI, who will be responsible for prompt review of the DSMB recommendations to guide decisions regarding continuation or termination of the trial and whether amendments to the protocol or changes in study conduct are required.

The DSMB is an independent, multidisciplinary group consisting of clinical experts and a statistician who collectively have experience in leukemia, lymphoma, hematology, biostatistics, and the conduct and monitoring of clinical trials. The DSMB will meet approximately every 6 months to review data. The current members are listed in the IIRC DSMB charter.

12. HUMAN SUBJECTS

12.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol will be subject to approval by the FHCRC Institutional Review Board, the FHCRC Scientific Review Committee, the UW and SCCA Institutional Biosafety Committees (IBC), and registered with the NIH Office of Science Policy.

Subjects who are eligible for the proposed protocol will be identified by referring primary care providers or community oncologists and referred for participation on this study where they will be provided with study information according to standard institutional practices. At FHCRC/SCCA/UW, this includes discussion by the attending oncologist in the outpatient department about the general rationale for the research study and the procedures involved, as well as alternative treatment options (standard chemotherapy, observation alone, treatment on other studies, etc.). Patients interested in participating in this study will be provided additional detailed information by one of the investigators on the trial. The known potential risks involved in these studies as well as potential benefits will be presented by the investigator and in a detailed consent form approved by the Consortium IRB and other appropriate oversight committees. All patients must sign an IRB-approved consent form before protocol-specific procedures and treatments begin. As the principal investigator of the protocol, Dr. Green will review eligibility for each patient enrolled.

12.2 Subject Confidentiality

The investigators will ensure that data collected conform to established guidelines for coding collection, key entry and verification. Institutional and program standards for patient privacy and security have kept pace with federal standards (e.g., HIPAA implementations) and are considered highly ef-

fective in providing appropriate protections. Each patient is assigned a unique patient number to assure patient confidentiality. Patients will not be referred to by name or by any other personal identifier in any publication or communications with outside agencies such as the FDA and NIH. The Clinical Statistics Departments at FHCRC maintain a patient database to allow storage and retrieval of patient data collected from a wide variety of sources. The licensed medical records departments, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents. The primary research records are kept in access controlled office spaces or password protected computer based applications. Information gathered from this study regarding patient outcomes and adverse events may be made available to the U.S. Food and Drug Administration and the NIH. All precautions to maintain confidentiality of medical records will be taken.

All investigators on this grant have participated in NIH-mandated courses on "The ethical conduct of research with humans" at the University of Washington or the Fred Hutchinson Cancer Research Center. These tutorials included reviews of the ethical principles pertaining to research with humans, a review of the history of abuses of human subjects, requirements for regulatory oversight, informed consent, risk-benefit evaluations, equitable selection of subjects, and examples of case studies in research ethics.

12.3 Study Modification/Discontinuation

The study may be modified or terminated at any time by the IND Sponsor, Protocol PI, the FHCRC IRB, or the FDA or other government agencies as part of their duties to ensure that research subjects are protected.

13. PUBLICATION OF RESEARCH FINDINGS

We will share data generated from this research trial in publications (via PubMed Central and/or publication in open-access journals) and at national and international meetings.

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15. APPENDICES

APPENDIX A: LYMPHODEPLETION CHEMOTHERAPY REGIMENS

The following is a guide to chemotherapy regimens to be used immediately prior to infusion of BCMA CAR T cells. The selection of the appropriate chemotherapy regimen to provide lymphodepletion and tumor cytoreduction must be discussed with the study PI. The priority chemotherapy regimen for all patients on the trial is Regimen A. However, other regimens may be used depending on PI discretion. Chemotherapy regimens that employ prolonged administration of corticosteroids that would continue after the T cell infusion cannot be used as lymphodepletion regimens.

Regimen A (IV)
Cyclophosphamide 300 mg/m^2 on days 1-3; fludarabine 25 mg/m^2 on days 1-3

Alternative Regimens (IV):

(B) Cyclophosphamide 1000 mg/m^2 IV on day 1

(C) Cyclophosphamide 60 mg/kg on day 1; fludarabine 25 mg/m^2 on days 2-4

APPENDIX B: ECOG PERFORMANCE STATUS SCALE

GRADE	SCALE
0	Fully active, able to carry out all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

APPENDIX C: GRADING CRITERIA FOR CRS

Grade	Description of Symptoms
1: Mild	Not life-threatening, require only symptomatic treatment such as antipyretics and anti-emetics (e.g., fever, nausea, fatigue, headache, myalgia, malaise)
2: Moderate	Require and respond to moderate intervention: • Oxygen requirement < 40%, or • Hypotension responsive to fluids or low dose of a single vasopressor, or • Grade 2 organ toxicity (by CTCAE v4.03)
3: Severe	Require and respond to aggressive intervention: • Oxygen requirement ≥ 40%, or • Hypotension requiring high dose of a single vasopressor (e.g., norepinephrine ≥ 20 µg/min, dopamine ≥ 10 µg/kg/min, phenylephrine ≥ 200 µg/min, or epinephrine ≥ 10 µg/min), or • Hypotension requiring multiple vasopressors (e.g., vasopressin + one of the above agents, or combination vasopressors equivalent to ≥ 20 µg/min norepinephrine), or • Grade 3 organ toxicity or Grade 4 transaminitis (by CTCAE v4.03)
4: Life-threatening	Life-threatening: • Requirement for ventilator support, or • Grade 4 organ toxicity (excluding transaminitis)
5: Fatal	Death

Adapted from Lee et al., 2014 (Lee 2014)

APPENDIX D: STUDY CALENDAR

Schedule of Assessments	Screening Evaluations	Evaluations following screening	Eval prior to Leukapheresis	Eval prior to Lympho-	Eval prior to BCMA CAR T	Evaluations following each T cell Infusion						Response Assessment	Post Treatment Follow-Up						Suspected Relapse
Study Day	**-45**				**0**	**1**	**3**	**7**	**10**	**14**	**21**	**28**	**60**	**90**	**120**	**180**	**365**	**LTFU**	
Visit Window	<45 days prior to apheresis	<45 days prior to apheresis	<7 days prior to apheresis	<14 days prior to LD chemo	Evals prior to CAR		+/- 1 day	+/- 1 day	+/- 1 day	+/- 2 days	+/- 3 days	+/- 3 days	+/- 15 days	+/- 15 days	+/- 15 days	+/- 30 days	+/- 30 days	+/- 3 Month	+/- 3 Month
Obtain consent	x	x																	
I/E criteria			x	x															
Medical history[1]	x																		
Physical exam [2]	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
Height/Weight			x																
ECOG	x	x		x	x			x		x	x	x							
MMSE		x		x [17]															
Vital signs (HR, BP, Oxygen saturation, temperature)			x	x	x	x	x	x	x	x	x	x							
12-lead ECG		x																	
MUGA or echocardiogram		x																	
CXR		x																	
Lumbar puncture with CSF evaluation		x [3]																	
Confirmed BCMA+ MM >5% (CD138+ flow cytometry)[4]	X																		

Schedule of Assessments	Screening Evaluations	Evaluations following screening	Eval prior to Leukapheresis	Eval prior to Lympho-	Eval prior to BCMA CAR T	Evaluations following each T cell Infusion						Response Assessment	Post Treatment Follow-Up						Suspected Relapse
Study Day	**-45**				**0**	**1**	**3**	**7**	**10**	**14**	**21**	**28**	**60**	**90**	**120**	**180**	**365**	**LTF U**	
Visit Window	<45 days prior to apheresis	<45 days prior to apheresis	<7 days prior to apheresis	<14 days prior to LD chemo	Evals prior to CAR		+/- 1 day	+/- 1 day	+/- 1 day	+/- 2 days	+/- 3 days	+/- 3 days	+/- 15 days	+/- 15 days	+/- 15 days	+/- 30 days	+/- 30 days	+/- 3 Month	+/- 3 Month
PSBC recipient/donor battery panel		x																	
Serum pregnancy test [5]		x		x															
ABO blood typing and antibody screen		x																	
G6PD screening		x																	
Chemistry/Hematology labs[6]		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
Uric Acid				x	x		x		x	x	x								
Coagulation Panel [7]				x	x	x	x	x	x	x	x	x							
C-reactive Protein			x	x	x	x	x	x	x	x	x	x							
Serum Ferritin				x	x	x	x	x	x	x	x	x							
Quantitative IgG, IgA, IgM		x		x [8]								x	x	x		x	x		
SPEP with immunofixation		x		x						x		x	x	x		x	x		x
Serum Free Light Chains		x		x						x		x	x	x		x	x		x
Bence Jones Quantitation		x		x								x	x	x		x	x		x
PET/CT or bone marrow MRI [9]		x [10]		x [10]								x		x					X [18]
Tumor Biopsy [11, 16]				x [11]								x							X [18]

Schedule of Assessments	Screening Evaluations	Evaluations following screening	Eval prior to Leukapheresis	Eval prior to Lympho-	Eval prior to BCMA CAR T	Evaluations following each T cell Infusion						Response Assessment	Post Treatment Follow-Up						Suspected Relapse
Study Day	**-45**				**0**	**1**	**3**	**7**	**10**	**14**	**21**	**28**	**60**	**90**	**120**	**180**	**365**	**LTFU**	
Visit Window	<45 days prior to apheresis	<45 days prior to apheresis	<7 days prior to apheresis	<14 days prior to LD chemo	Evals prior to CAR		+/- 1 day	+/- 1 day	+/- 1 day	+/- 2 days	+/- 3 days	+/- 3 days	+/- 15 days	+/- 15 days	+/- 15 days	+/- 30 days	+/- 30 days	+/- 3 Month	+/- 3 Month
Osseous Survey				x												x	x		
Bone marrow aspirate and bone marrow core biopsy [12]	x			x [13]						x [14]		x	x	x		x	x		x
Leukapheresis			x																
Lymphodepleting chemotherapy [15]				x															
BCMA CAR-T cell administration					x														
Research Samples	Refer to Appendix E																		

Footnote:

[1] History to include hematologic, cytogenetic, flow cytometric and histologic findings at diagnosis and enrollment, as well as prior therapies and response to treatment.

[2] Physical exam to include neurologic assessment.

[3] Perform if prior history of CNS involvement or based on clinical assessment/unexplained neurologic symptoms and within 14 days of enrollment.

[4] Bone marrow aspirates/biopsies should be sent for pathology analysis as clinically indicated and have testing done according to the protocol (see section 7, the aspirate/biopsy research specimen should be sent to the Green Lab at FHCRC).

[5] If clinically indicated; refer to Appendix I.

[6] Chemistry/Hematology labs include: CBD (white blood cell count, red blood cell count, hemoglobin, hematocrit, platelet count, absolute neutrophils, lymphocytes, monocytes, eosinophils, basophils, immature granulocytes). Renal/Hepatic Function Panel (sodium, potassium, chloride, total carbon dioxide, anion gap, glucose, urea nitrogen, creatinine, eGFR, total protein, albumin, total bilirubin, direct bilirubin, calcium, phosphate, AST (GOT), Alkaline phosphatase, ALT (GPT), magnesium, lactate dehydrogenase (LDH)).

[7] Coagulation panel includes: PT, PTT, fibrinogen, and D-dimer

[8] Repeat only if more than 60 days since last result or patient received interim cytotoxic therapy.

Schedule of Assessments	Screening Evaluations	Evaluations following screening	Eval prior to Leukapheresis	Eval prior to Lympho-	Eval prior to BCMA CAR T	Evaluations following each T cell Infusion						Response Assessment	Post Treatment Follow-Up						Suspected Relapse
Study Day	**-45**				**0**	**1**	**3**	**7**	**10**	**14**	**21**	**28**	**60**	**90**	**120**	**180**	**365**	**LTF U**	
Visit Window	<45 days prior to apheresis	<45 days prior to apheresis	<7 days prior to apheresis	<14 days prior to LD chemo	Evals prior to CAR		+/- 1 day	+/- 1 day	+/- 1 day	+/- 2 days	+/- 3 days	+/- 3 days	+/- 15 days	+/- 15 days	+/- 15 days	+/- 30 days	+/- 30 days	+/- 3 Month	+/- 3 Month

[9] PET/CT preferred in patients with FDG avid disease
[101] May be done prior to leukapheresis if necessary to meet IMWG defined enrollment criteria, otherwise may be done after leukapheresis. Repeat imaging prior to starting lymphodepleting chemotherapy if patient received interim therapy or more than 30 days since last PET/CT or bone marrow MRI
[11] May be performed at any time point prior to LD chemotherapy and at day 28 to characterize extramedullary sites of disease; additional research samples may be collected with clinical biopsies when performed
[12] Bone marrow aspirates and core biopsies should be sent for pathology analysis as clinically indicated and according to the protocol (see Section 7). The aspirate/biopsy research specimen (in RPMI media) should be sent to the Green Lab at FHCRC. BCMA antigen expression may be examined on all specimen collections.
[13] Repeat only if patient received interim therapy prior to lymphodepletion and has greater than 50% reduction from screening values of serum FLC or monoclonal spike (M spike/protein) or if patient received interim therapy for ≥14 days or ≥ 30 days have passed since leukapheresis or per PI discretion.
[14] If patient is clinically unstable, this bone marrow sample may be collected +/-3 days from day 14; please contact PI regarding this procedure if there are concerns.
[15] Cyclophosphamide 300 mg/m2 and fludarabine 25 mg/m2 concurrently x 3 days; see Appendix A for alternative regimens
[16] Specimen should be sent to SCCA Hematopathology for BCMA expression analysis
[17] Perform within 72 hours of lymphodepleting chemotherapy
[18] As clinically warranted

***Follow up data collection: cancer, autoimmune, neurologic, hematologic disorders will be monitored on an annual basis for 15 years following therapy.*

APPENDIX E – RESEARCH SAMPLE CHECKLIST

Time	Sample	Test	Tube	Volume (approx)	Lab
Screening	BM Aspirate and BM Core Biopsy	BCMA expression analysis	Sodium heparin (green)	5 mL	Heme-path (SCCA)
		MM characterization; TME assessment; MRD evaluation[5]	Sodium heparin (green)	5 mL	Green
Within 7 days prior to leu-kapheresis	Blood	T-cell Subset	EDTA (lavender)	10 mL	Heme-path (SCCA)
Preleuka-pheresis	Blood	Circulating tumor DNA analysis	Streck Tube	9 mL	Coffey
		Serum inflammatory cytokines [1]	Serum separator	10 mL	RCB
		Soluble BCMA	Serum separator	2 mL	
		RCL Testing	Sodium heparin (green)	10 mL	
		Plasma Archive	Sodium citrate (blue)	5 mL	
Pre-Lym-phodeple-tion	Blood	Baseline PBMC isolation	Sodium heparin (green)	60 mL	RCB
	BM Aspirate and BM Core Biopsy	BCMA expression analysis	Sodium heparin (green)	5 mL	Heme-path (SCCA)
		MM characterization; TME assessment; MRD evaluation[5]	Sodium heparin (green)	5 mL	Green
	Tissue Biopsy	Research	Media (RPMI)	≥1cm length	
Day 0 Pre-T cell infusion	Blood	Serum inflammatory cytokines [1]	Serum separator	10 mL	RCB
		Plasma Archive	Sodium citrate (blue)	5 mL	
Day 1	Blood	T-cell persistence/phenotyping[2]	Sodium heparin (green)	30 mL	RCB
		Serum inflammatory cytokines [1]	Serum separator	10 mL	
Day 3	Blood	T-cell persistence/phenotyping[2]	Sodium heparin (green)	30 mL	RCB
		Serum inflammatory cytokines [1]	Serum separator	10 mL	
Day 7	Blood	T-cell persistence/phenotyping[2]	Sodium heparin (green)	30 mL	RCB
		Serum inflammatory cytokines [1]	Serum separator	10 mL	
		Plasma Archive	Sodium citrate (blue)	5 mL	
Day 10	Blood	T-cell persistence/phenotyping[2]	Sodium heparin (green)	30 mL	RCB
		Serum inflammatory cytokines [1]	Serum separator	10 mL	
		Soluble BCMA	Serum separator	2 mL	
		PBMC Transgene immunogenicity As-say[4]	Sodium heparin (green)	20 mL	
Day 14	Blood	B-cell immunophenotyping	EDTA (purple)	5 mL	Heme-path (SCCA)
		T-cell persistence/phenotyping[2]	Sodium heparin (green)	30 mL	RCB
		Soluble BCMA	Serum separator	2 mL	
		Plasma Archive	Sodium citrate (blue)	5 mL	
	BM Aspirate and BM core biopsy	BCMA expression analysis	Sodium heparin (green)	5 mL	Heme-path (SCCA)
		BCMA CAR-T migration and phenotype; TME assessment; MRD evaluation[5]	Sodium heparin (green)	5 mL	Green
Day 21	Blood	Serum inflammatory cytokines [1]	Serum separator	10 mL	RCB
		T-cell persistence/phenotyping[2]	Sodium heparin (green)	30 mL	

		PBMC Archive	Sodium heparin (green)	20 mL	
		Serum Archive	Serum separator	20 mL	

Time	Sample	Test	Tube	Volume (approx)	Lab
Day 28	Blood	Circulating Tumor DNA analysis	Streck tube	10 mL	Coffey Lab
		B-cell immunophenotyping	EDTA (purple)	5 mL	Heme-path (SCCA)
		Serum inflammatory cytokines [1]	Serum separator	10 mL	RCB
		T-cell persistence/phenotyping[2]	Sodium heparin (green)	30 mL	
		Serum Transgene immunogenicity Ab[3]	Serum separator	10 mL	
		PBMC Transgene immunogenicity As-say[4]	Sodium heparin (green)	20 mL	
		Soluble BCMA	Serum separator	2 mL	
		Plasma Archive	Sodium citrate (blue)	5 mL	
	BM Aspirate and BM core biopsy	BCMA expression analysis	Sodium heparin (green)	5 mL	Heme-path (SCCA)
		BCMA CAR-T migration and phenotype; TME assessment; MRD evaluation[5]	Sodium heparin (green)	5 mL	Green
	Tissue biopsy	Research	Media (RPMI)	≥1cm length	
Day 60	Blood	B-cell immunophenotyping	EDTA (purple)	5 mL	Heme-path(SCCA)
		T-cell persistence/phenotyping[2]	Sodium heparin (green)	30 mL	RCB
		Serum Transgene immunogenicity Ab[3]	Serum separator	10 mL	
		PBMC Transgene immunogenicity As-say[4]	Sodium heparin (green)	20 mL	
		Soluble BCMA	Serum separator	2 mL	
		Plasma Archive	Sodium citrate (blue)	5 mL	
	BM Aspirate and BM core biopsy	BCMA expression analysis	Sodium heparin (green)	5 mL	Heme-path (SCCA)
		BCMA CAR-T migration and phenotype; TME assessment; MRD evaluation[5]	Sodium heparin (green)	5 mL	Green
	Tissue biopsy	Research	Media (RPMI)	≥1cm length	
Day 90	Blood	Circulating Tumor DNA analysis	Streck tube	10 mL	Coffey Lab
		B-cell immunophenotyping	EDTA (purple)	5 mL	Heme-path(SCCA)
		T-cell persistence/phenotyping[2]	Sodium heparin (green)	30 mL	RCB
		PBMC Archive	Sodium heparin (green)	40 mL	
		Serum Archive	Serum separator	20 mL	
		RCL Testing	Sodium heparin (green)	10 mL	
	BM Aspirate and BM core biopsy	BCMA CAR-T migration and phenotype; TME assessment; MRD evaluation[5]	Sodium heparin (green)	5 mL	Green
		BCMA expression analysis	Sodium heparin (green)	5 mL	Heme-path (SCCA)

Day 120	Blood	T-cell persistence/phenotyping[2]	Sodium heparin (green)	30 mL	RCB
		Serum Transgene immunogenicity Ab[3]	Serum separator	10 mL	
		PBMC Transgene immunogenicity Assay[4]	Sodium heparin (green)	20 mL	
		Soluble BCMA	Serum separator	2 mL	
Day 180	Blood	T-cell persistence/phenotyping[2]	Sodium heparin (green)	30 mL	RCB
		Serum Transgene immunogenicity Ab[3]	Serum separator	10 mL	
		PBMC Transgene immunogenicity Assay[5]	Sodium heparin (green)	20 mL	
		PBMC Archive	Sodium heparin (green)	40 mL	
		Serum Archive	Serum separator	20 mL	
		Soluble BCMA	Serum separator	2 mL	
		RCL Testing	Sodium heparin (green)	10 mL	
	BM Aspirate and BM core biopsy	BCMA CAR-T migration and phenotype; TME assessment; MRD evaluation[5]	Sodium heparin (green)	5 mL	Green
		BCMA expression analysis	Sodium heparin (green)	5 mL	Heme-path (SCCA)
Day 365	Blood	T-cell persistence/phenotyping[2]	Sodium heparin (green)	30 mL	RCB
		Serum Transgene immunogenicity Ab[3]	Serum separator	10 mL	
		PBMC Transgene immunogenicity Assay[4]	Sodium heparin (green)	20 mL	
		PBMC Archive	Sodium heparin (green)	40 mL	
		Serum Archive	Serum separator	20 mL	
		Soluble BCMA	Serum separator	2 mL	
		RCL Testing	Sodium heparin (green)	10 mL	
	BM Aspirate and BM core biopsy	BCMA CAR-T migration and phenotype; TME assessment; MRD evaluation[5]	Sodium heparin (green)	5 mL	Green
		BCMA expression analysis	Sodium heparin (green)	5 mL	Heme-path (SCCA)
Suspected relapse [6]	Blood	Serum Transgene immunogenicity Ab[3]	Serum separator	10 mL	RCB
		PBMC Transgene immunogenicity Assay[4]	Sodium heparin (green)	20 mL	
		T-cell persistence/phenotyping[2]	Sodium heparin (green)	38 mL	
		Soluble BCMA	Serum separator	2 mL	
	BM Aspirate and BM core biopsy	BCMA expression analysis	Sodium heparin (green)	5 mL	Heme-path (SCCA)
		BCMA CAR-T migration and phenotype; TME assessment; MRD evaluation[5]	Sodium heparin (green)	5 mL	Green
	Tissue biopsy	Research	Media (RPMI)	≥1cm length	
LTFU *optional per PI discretion Section 7.2.10 guidelines [6]	Blood	T-cell persistence/phenotyping[2]	Sodium Heparin (green)	38 mL	RCB
		Soluble BCMA	Serum separator	2 mL	
	BM Aspirate and BM core biopsy	BCMA CAR-T migration and phenotype; TME assessment; MRD evaluation[5]	Sodium heparin (green)	5 mL	Green
		BCMA expression analysis	Sodium heparin (green)	5 mL	Heme-path (SCCA)

[1] Cytokines to be monitored: IFN-γ, TNF-α, GM-CSF, and IL-6 by Luminex bead array. If symptomatic cytokine release syndrome, more frequent assessments may be necessary

[2] PK of CD4+ and CD8+ CART cells in blood collected after infusion [flow cytometry to detect EGFRt+ CD4+ and CD8+ T cells, and qPCR for vector sequences]; PD1, CTLA4, LAG3, TIM3, CD27, CD28, TIGIT, CD62L, KLRG1 will be evaluated on EGFRt+ CART cells; RNAseq on the 10X Genomics gel bead emulsion barcoding platform at peak and single late time-point or progressive disease

[3] To be performed by Juno Therapeutics

[4] Single cell RNAseq on the 10X Genomics gel bead emulsion barcoding platform pretreatment and on days 10, 28 and 60.

[5] MDSC and CD4+ Tregs; PD-L1 on CD138+ MM; RNAseq on the 10X Genomics pretreatment/screening, at peak and one late time-point; MRD Clonal IgH (Clonoseq)

[6] Laboratory testing may change per PI discretion, based on patient clinical status

APPENDIX F: DEFINITIONS OF RESPONSE AND PROGRESSION (MODIFIED IMWG)

Response Subcategory	Response Criteria
Stringent Complete Response (sCR)	CR, as defined below, plus the following: Normal FLC ratio[b] and absence of clonal cells[c] in bone marrow by immunohistochemistry or immunofluorescence.
Complete Response (CR)[b]	Negative immunofixation of serum and urine and disappearance of any soft tissue plasmacytomas, and < 5% plasma cells in bone marrow.
Very Good Partial Response (VGPR)[b]	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥ 90% reduction in serum M-protein level plus urine M-protein level < 100 mg per 24 hour.
Partial Response (PR)	≥ 50% reduction of serum M-protein and reduction in 24-hour urinary M-protein by ≥ 90% or to < 200 mg per 24 hour. If serum and urine M-protein are unmeasurable, a ≥ 50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria. In addition to the above criteria, if present at baseline, ≥ 50% reduction in the size of soft tissue plasmacytomas is also required
Minor (Minimal) Response (MR)	25-49% reduction of serum M-protein and reduction in 24-hour urine M-protein by 50-89%, which still exceeds 200 mg per 24 hours. In addition, if present at baseline, 25-49% reduction in the size of soft tissue plasmacytomas is also required. No increase in the size or number of lytic bone lesions (development of compression fracture does not exclude response).
Stable Disease (SD)	Not meeting criteria for CR, VGPR, PR, MR, or progression. Revised
Progressive Disease (PD)	Any of the following: Increase of 25% from lowest response value in any one or more of the following: 1. Serum M-component (absolute increase must be ≥0.5 g/dL (5g/L))[d] and/or 2. Urine M-component (absolute increase must be ≥ 200 mg (0.2g) per 24 h) and/or 3. Only in patients without measurable serum and urine M-protein levels: the difference between involved and uninvolved FLC levels (absolute increase must be > 10 mg/dL (100mg/L)) 4. Bone marrow plasma cell percentage (absolute % must be ≥ 10%) Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas or Development of hypercalcemia (corrected serum calcium > 11.5 mg/100 mL) that can be attributed solely to the plasma cell proliferative disorder.

[a] All response categories require 2 consecutive assessments made at any time before the institution of any new therapy; all categories also require no known evidence of progressive or new bone lesions if radiographic studies were performed. Radiographic studies are not required to satisfy these response requirements. Bone marrow assessments need not be confirmed.

[b] Note clarification to IMWG criteria for coding CR and VGPR in patients in whom the only measurable disease is by serum FLC levels: CR in such patients is defined as a normal FLC ratio of 0.26-1.65 in addition to CR criteria listed above. VGPR in such patients is defined as a > 90% decrease in the difference between involved and uninvolved FLC levels.

[c] Presence or 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 of > 4:1 or < 1:2.

[d] For progressive disease, serum M-component increase of ≥ 1 g/dL (10g/L) is sufficient to define progression if starting M-component is ≥ 5 g/dL (50 g/L).

APPENDIX G - LONG TERM FOLLOW-UP

Study participants should be asked to participate in long term follow-up, as directed by the FDA guidance for industry – gene therapy clinical trials: observing subjects for delayed adverse events. See also: http://www.fda.gov/biologicsbloodvaccines/guidancecompliancere gulatoryin-formation/guida nces/cellularandgenetherapy/ucm072957.htm#5

Long term follow-up should commence one year after the BCMA CAR T cell infusion. The planned recommendations for follow-up are as follows:

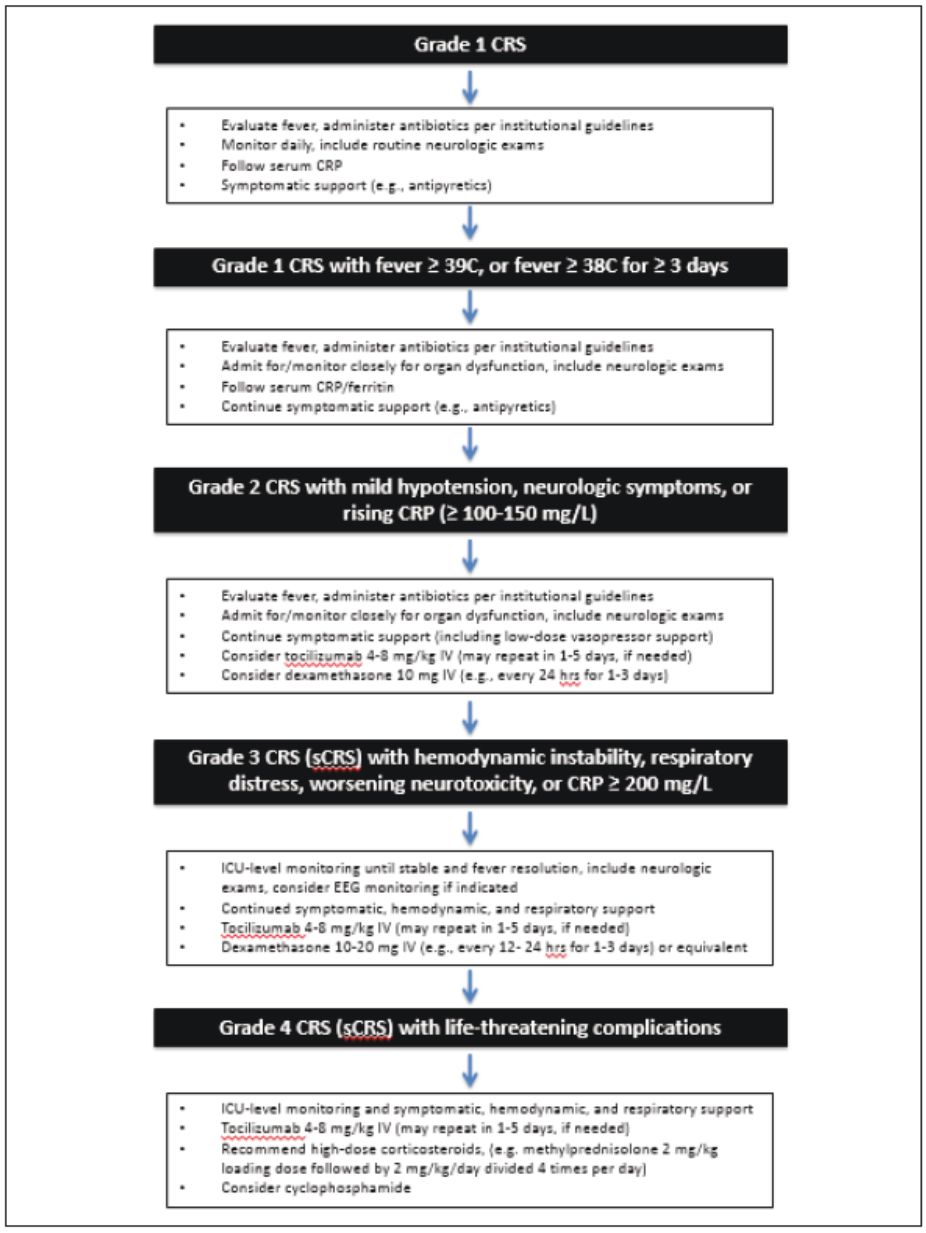
Years 1 - 15:
1. Recommendation that patients undergo at least annual history and physical examination with their primary physician:
Adverse event screening guidance for the primary physician in the form of a gene therapy LTFU-directed screening survey may be available.
A request for the study team to be notified of all new malignancies and unexpected illnesses.
The primary physician may be provided with a blood draw courier kit to enable samples to be returned to the FHCRC Green lab for archival purposes, and for analysis for transgene and vector persistence, and RCL, as dictated by studies of transferred t cell persistence.
2. Annual phone call survey or questionnaire to the participant to screen for adverse events.
3. Offer the opportunity to return to FHCRC for an annual LTFU clinic visit.
4. Compliance with 21 CFR 312.32 in adverse event reporting.
5. Research studies

Evaluation for transgene vector sequence by PCR of PBMC every 6 months for years 1 to 5 and every year for years 6-15 until the transgene becomes undetectable.

If > 1% of cells express the transgene or if clonality is suggested, vector integration sites or TCR-B sequence utilization may be analyzed in PBMC, CAR-T cells or other tissue. If clonality is suggested, repeat testing may be performed 3 months later. Persistent monoclonality, clonal expansion or vector integration near a known oncogenic locus should precipitate careful attention to the possibility of malignancy. However, the need for additional intervention should be guided by the clinical circumstances and not solely by the presence of these factors.

Annual testing of PBMC for RCL by VSVG qPCR. If there is no evidence of transgene persistence, RCL assays may be suspended after one year and samples may be archived.

APPENDIX H - RECOMMENDED MANAGEMENT GUIDELINES FOR CRS AND NEUROTOXICITY



APPENDIX I – Reproductive Potential and Approved Contraception Methods

Reproductive Potential:
Any female patient who does not meet at least one of the following criteria will be considered to be of childbearing potential:

- Post-menopausal for at least 12 consecutive months (i.e. no menses)
- Undergone a sterilization procedure
    1. hysterectomy, salpingectomy, or bilateral oophorectomy; tubal ligation is not considered a sterilization procedure

Approved Contraception methods:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation: oral; intravaginal; transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation: oral; injectable; implantable
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomized partner
- sexual abstinence
- barrier contraception (i.e. condom, diaphragm, cervical cap)

Protocol 9762

APPENDIX J – Mini-Mental State Examination

MMSE-2

Standard Version

Blue Form

Date of examination ___/___/___ Examiner _____

Name _____ Age _____ Sex _____

Years of school completed _____ Purpose of exam _____

Assessment of level of consciousness

Alert/ Responsive | Drowsy | Stuporous | Comatose/ Unresponsive

Instructions: Words in boldface type should be read aloud clearly and slowly to the examinee. Item substitutions appear in parentheses. Administration should be conducted privately and in the examinee's primary language. Unless otherwise specified, circle 0 if the response is incorrect or 1 if the response is correct. Begin by introducing the test:

Now I'd like to ask you some questions about your memory.

	RESPONSE	SCORE (circle one)
REGISTRATION		
Listen carefully. I am going to say three words. You say them back after I stop. Ready? Here they are... MILK [*pause*], **SENSIBLE** [*pause*], **BEFORE** [*pause*]. **Now repeat those words back to me.** [*Repeat up to 3 times, but score only the first trial.*]		
MILK		0 1
SENSIBLE		0 1
BEFORE		0 1
Now keep those words in mind. I am going to ask you to say them again in a few minutes.		
ORIENTATION TO TIME		
What day is today? What is the...		
year?		0 1
season?		0 1
month of the year?		0 1
day of the week?		0 1
date?		0 1
ORIENTATION TO PLACE*		
Where are we now? What is the...		
state (or province)?		0 1
county (or city/town)?		0 1
city/town (or part of city/neighborhood)?		0 1
building (name or type)?		0 1
floor of the building (room number or address)?		0 1

*Alternative place words that are appropriate for the setting and increasingly precise may be substituted and noted.

RECALL		
What were those three words I asked you to remember? [*Do not offer any hints.*]		
MILK		0 1
SENSIBLE		0 1
BEFORE		0 1

If administering the MMSE-2:SV, copy the MMSE-2:BV total raw score to the space provided at the top of page 2 and continue with administration.

MMSE-2:BV total raw score ☐ (16 max. points)

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MMSE-2:BV total raw score ☐ (16 max. points)

ATTENTION AND CALCULATION [*Serial 7s*]

Now I'd like you to subtract 7 from 100. Then keep subtracting 7 from each answer until I tell you to stop.

What is 100 take away 7?	[93]	_____	0	1
If needed, say: **Keep going.**	[86]	_____	0	1
If needed, say: **Keep going.**	[79]	_____	0	1
If needed, say: **Keep going.**	[72]	_____	0	1
If needed, say: **Keep going.**	[65]	_____	0	1

Score 1 point for each correct answer. An answer is considered correct if it is 7 less than the previous answer, even if the previous answer was incorrect.

NAMING

What is this? [*Point to eye.*]	_____	0	1
What is this? [*Point to ear.*]	_____	0	1

REPETITION

Now I am going to ask you to repeat what I say. Ready? IT IS A LOVELY, SUNNY DAY BUT TOO WARM. Now you say that. [*Wait for examinee response and record response verbatim. Repeat up to one time.*]

IT IS A LOVELY, SUNNY DAY BUT TOO WARM.	_____	0	1

Detach the last page of this form. Tear the detached page in half along the horizontal perforation line. Use the upper half of the detached page, which has three shapes on it, as a stimulus form for the Comprehension task. Use the bottom half of the page as a stimulus form for the Reading ("CLOSE YOUR EYES") task. Use the upper back half of the detached page as a stimulus and response form for the Drawing (intersecting pentagons) task and the bottom half of the page (blank) as a response form for the Writing task.

COMPREHENSION

Listen carefully because I am going to ask you to do something. [*Show examinee the geometric figures stimulus page.*] **Look at these pictures and point to the circle, then point to the square, and then point to the triangle.**

Correct response	Observed response		
○		0	1
□		0	1
△		0	1

READING

[*Show examinee the word stimulus page.*] **Please do what this says to do.**

CLOSE YOUR EYES	_____	0	1

WRITING

[*Place the blank piece of paper in front of the examinee and provide a pen or pencil.*] **Please write a sentence.** [*If examinee does not respond, say:* **Write about where you live.**] 0 1

Score 1 point if the sentence is comprehensible and contains a subject and a verb. Ignore errors in grammar or spelling.

DRAWING

[*Display the intersecting pentagons on the stimulus form and provide a pen or pencil.*] **Please copy this design.** Score 1 point if the drawing consists of two 5-sided figures that intersect to form a 4-sided figure. 0 1

MMSE-2:SV total raw score ☐ (30 max. points)

CLOSE YOUR EYES