

5 FIG. 9 is a series of images showing CD19 protein expression as determined by IHC in bone marrow core biopsies acquired prior to administration (“Pre”), and on Day 28 and Day 90 (“3 month”) post-infusion of CART-BCMA from Patient 13, Patient 14, Patient 15, Patient 16, and Patient 17.

 FIG. 10 is a series of images showing CD20 protein expression as determined by IHC in bone marrow core biopsies acquired prior to administration (“Pre”), and on Day 28 and Day 90 (“3 month”) post-
10 infusion of CART-BCMA from Patient 13, Patient 14, Patient 15, Patient 16, and Patient 17.

 FIGs. 11A and 11B are a series of spectrally unmixed pseudo fluorescent microscopy images showing that BCMA positive cells and CD19 positive cells are separate populations in bone marrow core biopsies acquired from Patient 15 prior to administration (“pre”) and on Day 90 (“3M”) post-infusion of CAR-BCMA.

15 FIGs. 12A and 12B are a series of spectrally unmixed pseudo fluorescent microscopy images showing that CD19+ CD34^{dim} cell population was present in the pretreatment bone marrow core biopsies acquired from Patient 15 and Patient 17, respectively.

 FIG. 13 is a series of spectrally unmixed pseudo fluorescent microscopy images showing that the CD19 population was variably CD138+ and CD138- in the pretreatment bone marrow core biopsies acquired
20 from Patient 15.

 FIG. 14 is a graph comparing the level of tumor burden in a KMS11 tumor model following implant and administration of PBS, untransduced T cells (“UTD”), or T cells transduced with either a tool CAR (“J6MO”), BCMA-4, BCMA-9, BCMA-10 (“MCM998”), BCMA-13, or BCMA-15. BCMA-10 demonstrated the most potent anti-tumor activity.

25 FIG. 15 is a diagram showing the design of a clinical trial (NCT Number: NCT02546167; UPCC 14415) to assess the safety and feasibility of infusion of autologous T cells expressing CART-BCMA in adult patients with multiple myeloma.

 FIG. 16A is a table showing MM patient disease characteristics. FIG. 16B is a table showing the presence of baseline lymphopenia due to disease and prior therapies in MM patients.

30 FIGs. 17A, 17B, and 17C are graphs showing patient response for Cohort 1, Cohort 2, and Cohort 3, respectively.

 FIGs. 18A-1, 18A-2, 18A-3, 18A-4, 18A-5, 18A-6, 18A-7, 18A-8, 18A-9 and 18B-1, 18B-2, 18B-3, 18B-4, 18B-5 are a series of graphs showing expansion of CART-BCMA evaluated by flow cytometry in Cohort 1 patients and Cohort 2/3 patients, respectively.

35 FIGs. 19A-1, 19A-2, 19A-3, 19A-4, 19A-5, 19A-6, 19A-7, 19A-8, 19A-9 and 19B-1, 19B-2, 19B-3, 19B-4, 19B-5 are a series of graphs showing expansion of CART-BCMA evaluated by PCR in Cohort 1 patients and Cohort 2/3 patients, respectively. The plots show the number of detected CART genes per µg of DNA isolated from patient’s blood (y-axis) at the respective day post CART infusion (x-axis).

 FIGs. 20A and 20B are graphs showing that BCMA expansion may correlate with clinical outcomes.

5 FIG. 21A, 21B, 21C, and 21D are graphs showing the fraction of CAR-positive (CAR+) CD4/CD8 cells at various time points post-infusion in Responders compared to Non-Responders.

 FIGs. 22-1 and 22-2 are a series of graphs showing the changes in level of cytokine expression at various time points post infusion of CART-BCMA. The y-axis in each graph shows fold change from Day 0. The x-axis in each graph shows days post-infusion of CART-BCMA.

10 FIGs. 23A and 23B are graphs showing the change in IL-6 expression at various time points post infusion of CART-BCMA. The y-axis in each graph shows fold change from Day 0. The x-axis in each graph shows days post-infusion of CART-BCMA.

 FIGs. 24A and 24B are graphs showing the change in IFN- γ expression at various time points post infusion of CART-BCMA. The y-axis in each graph shows fold change from Day 0. The x-axis in each graph shows days post-infusion of CART-BCMA.

15 FIGs. 25A and 25B are graphs showing the serum level of BCMA in 14 normal donors (FIG. 25A) and 12 myeloma patients (FIG. 25B).

 FIGs. 26A, 26B, 26C, and 26D are graphs showing serum BCMA level at various time points post infusion of CART-BCMA. The y-axis in FIGs. 26A and 26B shows peripheral blood (PB) serum BCMA levels. The y-axis in FIGs. 26C and 26D shows PB serum BCMA level fold change from baseline. The x-axis in each graph shows days post-infusion of CART-BCMA.

20 FIGs. 27A, 27B, and 27C are graphs showing data collected from three multiple myeloma patients who received CART-BCMA treatment. The y-axis on the left shows the percentage of CD4+ or CD8+ CART cells. The y-axis on the right shows the level of serum BCMA (ng/mL) or the number of CART copies (BBz) per μ g DNA, as evaluated by qPCR.

25 FIGs. 28A and 28B are graphs showing CD4+ T cell subsets of normal donors (FIG. 28A) and multiple myeloma (MM) patients (FIG. 28B). FIGs. 28C and 28D are graphs showing CD8+ T cell subsets of normal donors (FIG. 28C) and MM patients (FIG. 28D). FIGs. 28E and 28F are graphs showing CD4+ and CD8+ T cell subsets, respectively, in apheresis samples acquired from MM patients (dots with slashes represent non-responders and white dots represent responders).

30 FIGs. 29-1 and 29-2 are a series of graphs showing T cell differentiation in apheresis samples acquired from MM patients. The x-axis shows CD45RO expression and the y-axis shows CCR7 expression. Signal in the top left quadrant indicates naïve cell phenotype; signal in top right quadrant indicates central memory (T_{CM}) phenotype; signal in bottom right quadrant indicates effector memory (T_{EM}) phenotype; and signal in bottom left quadrant indicates T_{EMRA} . CR stands for complete response. PD stands for progressive disease. VGPR stands for very good partial response.

35 FIGs. 30A and 30B are a pair of graphs showing CD4+ and CD8+ T cell subsets in apheresis samples acquired from MM patients (dots with slashes represent non-responders and white dots represent responders).

FIG. 31 is a graph showing treatment schema.

FIGs. 32A, 32B, and 32C are a set of graphs showing clinical outcomes. FIG. 32A is a Swimmer's plot showing best response and progression-free survival (PFS) for each subject. Arrow indicates ongoing response. FIG. 32B is a pair of PET/CT scan images for subject 03 showing resolution of extramedullary disease and malignant pleural effusion post-treatment. FIG. 32C is a Kaplan-Meier plot showing overall survival for Cohort 1. MR=minimal response; MRD=minimal residual disease; PR=partial response; PD=progressive disease; sCR=stringent complete response; SD=stable disease.

FIGs. 33A-1, 33A-2, 33A-3, 33B, and 33C are a set of graphs showing CART-BCMA expansion and persistence. FIG. 33A is a set of graphs depicting CART-BCMA cell levels over time in peripheral blood for each subject, as measured by flow cytometry (%CAR+ within CD3+ T cells, ▲, left axis) and quantitative PCR for CAR sequence (■, right axis). See FIG. 38 for representative flow cytometry plots. FIG. 33B is a graph showing that peak CART-BCMA levels by qPCR correlate with response: median 102507 vs. 4187 copies/μg DNA for ≥PR vs. <PR, respectively (p=0.016, Mann-Whitney). FIG. 33C is a graph showing that AUC-28 (area under the curve for CART-BCMA levels by qPCR during first 28 days after infusion) correlates with response: median 885181 vs. 26183 (copies)x(days)/μg DNA for ≥PR vs. <PR, respectively (p=0.016, Mann-Whitney).

FIGs. 34-1, 34-2, 34-3 are a set of graphs showing soluble BCMA (sBCMA), BAFF, APRIL levels and B cell frequency after CART-BCMA infusions. Peripheral blood serum levels of sBCMA, BAFF, and APRIL (ng/ml, left axis) were measured by ELISA pre- and post-CART-BCMA infusions for each subject as indicated above. Subjects with deepest clinical responses (01 (sCR), 03 (VGPR), 15 (VGPR)) had greatest declines in sBCMA and reciprocal increases in BAFF and APRIL. Peripheral blood B cell frequency (%CD19+ of CD45+CD14- gate, right axis) was assessed by flow cytometry at indicated time points.

FIGs. 35-1, 35-2 and 35-3 are a set of histograms showing BCMA expression by flow cytometry on gated MM cells in marrow aspirates for each subject, before and after CART-BCMA infusions. Hatched histograms show BCMA; filled histograms show FMO (fluorescence minus one) control. Post-infusion time point is Day 28, unless specified. Percentage of cells expressing BCMA as well as mean BCMA fluorescence intensity (MFI) for each subject are listed in Table 37. Note decreased BCMA expression for subject 03 at relapse (D164). See FIG. 42 for representative gating.

FIGs. 36A, 36B, 36C, and 36D are a set of graphs showing predictors of in vivo CART-BCMA expansion. The ratio of CD4+ to CD8+ T cells (CD4/CD8 ratio) within the apheresis product immediately after collection (FIG. 36A) and within the seed culture at start of manufacturing (i.e. following elutriation step to reduce monocyte contamination) (FIG. 36B) was determined by flow cytometry. In vitro fold expansion (FIG. 36C) was calculated from total cell counts at start and end of

5 manufacturing. The proportion of CD8⁺ T cells within the apheresis product with a CD45RO-CD27⁺ phenotype was assessed by flow cytometry (FIG. 36D). The CD4/CD8 ratio and frequency of CD45RO-CD27⁺CD8⁺ T cells pre-manufacturing, and degree of in vitro expansion were associated with peak in vivo CART-BCMA expansion post-infusion (Spearman correlation r and p -value shown).

FIG. 37 is a CONSORT diagram showing subject enrollment.

10 FIG. 38 is a set of graphs showing representative gating and staining for CART-BCMA cells. Staining is shown for peripheral blood from subject 01, day +7 after first CART-BCMA infusion. Cells are gated by forward and side scatter, then singlets, then CD45⁺CD14⁻ leukocytes, then T cells (CD3⁺CD19⁻). CART-BCMA⁺ cells were identified using biotinylated recombinant human BCMA-Fc and streptavidin-PE. Negative control was an FMO (fluorescence minus one) tube (lacking biotinylated BCMA-Fc) with
15 streptavidin-PE. The % of CD3⁺ T cells expressing CART-BCMA was calculated by subtracting CAR⁺ cells in FMO tube from CAR⁺ cells in tube with biotinylated BCMA-Fc (i.e. in this example $34.7 - 0.9 = 33.8$). Activation status of CART-BCMA⁺ cells was identified by staining for HLA-DR (bottom right panel). The % of CAR⁺ cells that were activated at each time point was calculated by dividing %HLA-DR⁺ by (%HLA-DR⁺ plus %HLA-DR⁻) (i.e. in this example $32.9/(32.9 + 1.5) = 95.6\%$).

20 FIGs. 39-1, 39-2 and 39-3 are a set of graphs showing absolute number of CART-BCMA⁺ T cells for each subject. Absolute # of CD3⁺CAR⁺ cells per μ l of blood was estimated from the absolute lymphocyte count (ALC, reported from the clinical complete blood count (CBC) differential) and the CART-BCMA flow cytometry results (FIG. 38), using the following formula: $(ALC) (\%CD45+CD14-)(\%CD3+CD19-)(\%CAR+)/10000$. For example, for subject 01 at day+7, ALC was 0.08×10^3 cells/ μ l.
25 Estimated absolute # of circulating CAR⁺ T cells at this time point is $(0.08)(48.3)(72.1)(33.8)/10000 = 0.019 \times 10^3$ cells/ μ l.

FIGs. 40-1, 40-2 and 40-3 are a set of graphs showing serum cytokine changes after CART-BCMA treatment. Levels of 30 peripheral blood cytokines were assessed at multiple time points by Luminex assay. Changes in selected cytokines over first 28 days are depicted. Subjects with deepest responses (01, 03, 15)
30 had greatest fold-increase in cytokines, typically at or just before peak CART-BCMA expansion.

FIGs. 41A and 41B are a pair of graphs showing baseline soluble BCMA (sBCMA) levels, peak expansion, and response. Peripheral blood serum levels of sBCMA were measured by ELISA pre-treatment. FIG. 41A is a graph showing that baseline sBCMA levels did not correlate significantly with peak expansion of CART-BCMA by qPCR (Spearman correlation $r=0.43$, $p=0.25$). FIG. 41B is a graph showing that
35 baseline levels of sBCMA were not significantly associated with response ($p=0.56$, Mann-Whitney test).

FIG. 42 is a set of graphs showing representative gating for myeloma cells and BCMA staining. Bone marrow aspirate cells were gated by forward and side scatter, then by singlets, then on CD3-