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Clinical Research and Public Health   Oncology

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RESULTS. CART-BCMA cells were manufactured and expanded in all subjects. Toxicities included cytokine release syndrome and neurotoxicity, which were grade 3–4 in 8 (32%) and 3 (12%) subjects, respectively, and reversible. One subject died at day 24 from candidemia and progressive myeloma, following treatment for severe cytokine release syndrome and encephalopathy. Responses (based on treated subjects) were seen in 4 of 9 (44%) in cohort 1, 1 of 5 (20%) in cohort 2, and 7 of 11 (64%) in cohort 3, including 5 partial, 5 very good partial, and 2 complete responses, 3 of which were ongoing at 11, 14, and 32 months. Decreased BCMA expression […]

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CONCLUSION. CART-BCMA infusions with or without lymphodepleting chemotherapy are clinically active in heavily pretreated patients with MM.

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Introduction

Despite advances in multiple myeloma (MM) therapy, the disease remains incurable in most patients, and novel approaches are needed. B cell maturation antigen (BCMA) is a cell surface receptor expressed primarily by plasma cells. Its ligands are BAFF and APRIL, and it functions to maintain long-lived plasma cell homeostasis (1). BCMA is expressed consistently on MM cell lines and primary patient samples, though intensity of expression is variable (2–5). BCMA is shed from the surface of plasma cells by gamma-secretase–mediated cleavage (6), leading to a soluble form (sBCMA) that is detectable in circulation. Higher concentrations of sBCMA in patients with myeloma are associated with poorer clinical outcomes (7). Ligation of BCMA promotes MM cell proliferation, survival, and drug resistance (8, 9), and antibody-based blockade of BCMA signaling has anti-MM activity in preclinical models (3, 9). Thus, BCMA is a rational target for antimyeloma therapy.

Chimeric antigen receptors (CARs) provide novel specificity to T cells, coupling antibody-based extracellular domains to T cell signaling domains and costimulatory molecules. We previously reported that CD19-specific CAR T cells given after salvage autologous stem cell transplantation could prolong remission duration in some patients with relapsed/refractory MM (10, 11). Carpenter et al. demonstrated preclinical anti-MM activity using BCMA-specific CAR T cells (4), and adoptive transfer of autologous T cells expressing this murine BCMA CAR, following lymphodepletion with fludarabine and cyclophosphamide conditioning, induced objective responses in 4 of 12 heavily pretreated patients with MM. Responses were associated with significant but expected toxicities including cytokine release syndrome (CRS) and neurotoxicity (12), similar to those described with CD19-targeted CAR T cells in patients with B cell malignancies (13, 14).

We developed what we believe is a novel BCMA-targeted CAR with a fully human scFv (single chain variable fragment), fused to the hinge and transmembrane domain of CD8 and the human 4-1BB and CD3ζ intracellular signaling domains and packaged in a lentiviral vector, with demonstrated preclinical activity (15). We now report results from our phase I clinical trial evaluating autologous T cells expressing this fully human BCMA-specific CAR (CART-BCMA) in relapsed/refractory myeloma patients, both with and without lymphodepleting chemotherapy.

Results

This clinical trial was a phase I, single-center, open-label study to evaluate the feasibility, safety, and clinical and biologic activity of manufacturing and administering CART-BCMA cells to patients with relapsed/refractory myeloma. We enrolled 3 sequential dose cohorts as follows: cohort 1, 1×10^8 to 5×10^8 CART-BCMA cells alone; cohort 2, cyclophosphamide (Cy) 1.5 g/m² plus 1×10^7 to 5×10^7 CART-BCMA cells; cohort 3, Cy 1.5 g/m² plus 1×10^8 to 5×10^8 CART-BCMA cells. Enrolled subjects underwent steady-state leukapheresis to collect T cells for CART-BCMA manufacturing, typically a 4-week process. Antimyeloma therapy could resume during manufacturing until 2 weeks prior to first CART-BCMA infusion. CART-BCMA cells were administered in an outpatient research unit over 3 days as split-dose intravenous infusions (10% of dose given on day 0, 30% on day 1, and 60% on day 2). In cohorts 2 and 3, Cy was administered for lymphodepletion 3 days prior to first CART-BCMA infusion (Figure 1).

From November 2015 to December 2017, 34 subjects consented and 29 were eligible and commenced manufacturing, with 25 receiving CART-BCMA infusions. Four were not treated due to rapid disease progression and clinical deterioration during manufacturing and bridging therapy (Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/JCI126397DS1). Baseline characteristics and prior lines of therapy are summarized in Table 1, with individual details shown in Supplemental Table 2. Subjects had a median of 7 prior lines of therapy, with 96% dual refractory to a proteasome inhibitor (PI) and immunomodulatory drug (IMID), 72% refractory to daratumumab, and 44% penta-refractory to bortezomib, lenalidomide, carfilzomib, pomalidomide, and daratumumab. At least 1 high-risk cytogenetic abnormality was found in 96% of the subjects, and 68% of subjects had either deletion 17p or a *TP53* mutation. Baseline tumor burden was high (median 65% myeloma cells on bone marrow biopsy), and 28% had extramedullary disease.

For all subjects, the minimum target goal of CART-BCMA cells was successfully manufactured and formulated, though 1 subject required 2 leukaphereses and manufacturing attempts. Final products comprised a median of 97% CD3+ T cells, with median CD4/CD8 ratio of 1.7. Twenty-one subjects received all 3 planned CART-BCMA infusions, with 4 receiving 40% of planned dose (third infusion held due to early CRS). Further details of manufacturing, product characteristics, and dosing for each subject are shown in Supplemental Table 3.

Grade 3 or higher adverse events, regardless of attribution, were seen in 24 of 25 subjects (96%) and are summarized in Table 2, with individual adverse events for each subject listed in Supplemental Table 4. Twenty-four of 25 subjects (96%) were admitted to the hospital a median of 4 days (range 1–28 days) after first CART-BCMA infusion, with longer time to admission in cohort 2 (1×10^7 to 5×10^7 CART-BCMA cells, median 8 days) than cohorts 1 and 3 (1×10^8 to 5×10^8 CART-BCMA cells, median 3 days for both). CRS was observed in 22 of 25 subjects (88%),
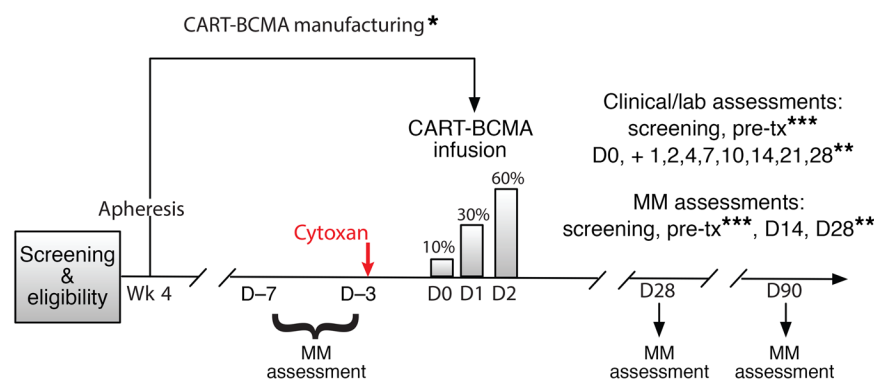
Figure 1. Treatment schema. Cytoxan indicates cyclophosphamide. *Patients may receive therapy during manufacturing to maintain disease control. **After first 28 days, follow-up is every 4 weeks up to 6 months, then every 3 months up to 2 years. *** Pre-tx, pretreatment, 3 to 7 days before CART cell infusion.

Table 1. Subject characteristics

Characteristic (n = 25)	Median (range) or %
Age, years	58 (44–75)
Sex	68% male; 32% female
Median time from diagnosis, years	4.6 (1.8–14.5)
High-risk cytogenetics[A]	96%
Del 17p or TP53 mutation	68%
Prior lines of therapy, no.	7 (3–13)
Len / Bort / Pom / Carf [B]/ Dara, % exposed	100%/100%/92%/96%/76%
Len / Bort / Pom / Carf / Dara, % refractory	76%/ 88%/ 88%/ 80%/ 72%
Dual- / Quad- / Penta-refractory	96% / 56% / 44%
Prior autologous SCT	92%
Extramedullary disease	28%
Bone marrow plasma cells	65% (0–95)
Absolute CD3⁺ T cell count preleukapheresis,[C] cells/μl	538 (151–1529)
Day 0 LDH, U/l	175 (75–385)
Day 0 serum creatinine, mg/dl	0.93 (0.55–2.87)
Day 0 hemoglobin, g/dl	9.1 (6.4–12.4)
Day 0 platelets, × 10³/μl	128 (13–316)

[A]Includes complex karyotype, gain 1q, deletion 17p, t(14;16), and/or t(4;14). [B]Includes 1 patient who received oprozomib. [C]n = 23 (subjects 01 and 02 did not have prepheresis T cell counts done). Normal range, 900–3245 cells/μl. Bort, bortezomib; Carf, carfilzomib; Dara, daratumumab; Del, deletion; Dual-refractory, refractory to both a proteasome inhibitor (PI) and immunomodulatory agent (IMID); LDH, lactate dehydrogenase; Len, lenalidomide; Penta-refractory, refractory to 2 PIs, 2 IMIDs, and Dara; Pom, pomalidomide; Quad-refractory, refractory to 2 PIs and 2 IMIDs; SCT, stem cell transplant.

and was grade 3–4 on the Penn grading scale (16) (Supplemental Table 1) in 8 (32%) subjects, all of whom were treated at the 1 × 10⁸ to 5 × 10⁸ dose. Median time to CRS onset was 4 days (range 1–11 days), with a median duration of 6 days (range 1–18 days), and median duration of hospitalization of 7 days (range 0–40 days). CRS was associated with elevations in ferritin and C-reactive protein, as previously described (14). Seven subjects (28%) received IL-6 blockade with either tocilizumab (n = 6) or siltuximab (n = 1).

Neurotoxicity was seen in 8 of 25 subjects (32%), and was mild (grade 1–2) in 5 (transient confusion and/or aphasia) subjects. Three (12%) had grade 3–4 encephalopathy including 1 subject (subject 03) in cohort 1 with a dose-limiting toxicity (DLT) of PRES (posterior reversible encephalopathy syndrome) with severe obtundation, recurrent seizures, and mild cerebral edema on MRI that fully resolved after treatment with high-dose methylprednisolone (1 g/day times 3) and cyclophosphamide 1.5 g/m². The others had no objective changes on MRI. All 3 subjects with severe neurotoxicity had high tumor burden (2 with extramedullary disease), had received a dose of 5 × 10⁸ CART-BCMA cells, and had grade 3 or 4 CRS. A summary of CRS and neurotoxicity based on cohort is provided in Supplemental Table 5. The other DLT was grade 3 cardiomyopathy and grade 4 spontaneous hemothorax in subject 27 (cohort 3) in the setting of CRS, coagulopathy, thrombocytopenia, and extensive myelomatous rib lesions. Of note, this subject had received siltuximab (anti–IL-6 mAb) and low-dose steroids for grade 3 CRS due to concurrent grade

3 encephalopathy and treating physician's concern that tocilizumab, which blocks the IL-6 receptor and can cause a spike in serum IL-6 concentration (17), might exacerbate the subject's neurotoxicity. All of these toxicities fully resolved. No unexpected off-target toxicities were observed.

There was one grade 5 event during the study. Subject 08 (cohort 1) was a 71-year-old man with IgA lambda MM with complex karyotype. By the time of CART-BCMA infusion, he was progressing rapidly through bridging therapy with worsening kidney function, pancytopenia, and extensive extramedullary disease. He tolerated all 3 CART-BCMA infusions and was admitted on day 4 with grade 1 CRS, grade 1 delirium, and possible pneumonia. He was stable initially with antibiotics and supportive care, but on day 14, he developed grade 4 CRS with hypoxemia, distributive shock and renal failure requiring dialysis, as well as grade 4 encephalopathy requiring intubation. Tocilizumab and steroids were given with improvement, and by day 19, he was extubated with discontinuation of dialysis and steroids. He then worsened on days 21–22 with recurrent pressor-dependent hypotension, hypoxemia, worsening renal function, and confusion. He was reintubated, and his condition worsened despite additional tocilizumab and steroids. Blood cultures identified candidemia. Restaging labs revealed a serum M-spike of 5.1 g/dl (increased from 2.5 g/dl pretreatment), and serum-free lambda light chains 2544 mg/l (increased from 882 mg/l pretreatment), along with 13% circulating plasma cells. His family opted to withdraw aggressive support and pursue comfort care only, and the subject died at day 24. No other deaths occurred during the study.

Objective responses (partial response [PR] or better) were confirmed in 4 of 9 subjects (44%) in cohort 1, 1 of 5 subjects (20%) in cohort 2, and 7 of 11 subjects (64%) in cohort 3 (Figure 2A), including 5 PR, 5 very good partial response (VGPR), 1 complete response (CR), and 1 stringent complete response (sCR). The overall response rate was 12 of 25 (48%), with 11 of 20 subjects (55%) responding at the more effective dose of 1 × 10⁸ to 5 × 10⁸ CART-BCMA cells. Five additional subjects had minimal response (MR). The overall response rate by intention-to-treat, based on the 29 subjects who were eligible and underwent apheresis, was 12 of 29 (41%). Four of 7 subjects with extramedullary disease responded (Figure 2B). Four subjects (subjects 01, 03, 15, 19) had no detectable myeloma by flow cytometry (estimated sensitivity 10⁻⁵) from postinfusion marrow aspirates at month 1 and/or 3. Median time to first response was 14 days. Based on Kaplan-Meier estimates, median duration of response was 124.5 days (range 29–939+) (Supplemental Figure 2). At time of data cut-off, 3 subjects (subjects 01, 19, 33) remained progression free at 953, 427, and 322 days (roughly 32, 14, and 11 months), respectively. All other subjects have progressed, and median progression-free survival (PFS) is 65, 57, and 125 days for cohorts 1, 2, and 3, respectively (Supplemental Figure 2). At time of data cut-off, 13 subjects had expired, with median overall survival of 502 days for all subjects (Supplemental Figure 2), and 359 days, 502 days, and not reached for cohorts 1, 2, and 3, respectively (Figure 2C).

All infused subjects had detectable CART-BCMA cells in peripheral blood by qPCR (Figure 3, A–C), and 24 of 25 had detectable CAR⁺ T cells by flow cytometry (Supplemental Figures 4–6; Supplemental Figure 3 for representative staining). Expan-

Table 2. Grade 3 or higher adverse events, regardless of attribution

Event	Grade 3 n	Grade 4 n	Grade 5 n	All n (%)
Leukopenia	3	8	0	11 (44)
Neutropenia	2	9	0	11 (44)
Lymphopenia	3	6	0	9 (36)
Cytokine release syndrome	7	1	0	8 (32)
Thrombocytopenia	3	4	0	7 (28)
Hypophosphatemia	7	0	0	7 (28)
Febrile neutropenia	6	0	0	6 (24)
Hyponatremia	6	0	0	6 (24)
Anemia	4	1	0	5 (20)
Hypocalcemia	3	2	0	5 (20)
AST increased	2	1	0	3 (12)
Hypokalemia	2	1	0	3 (12)
Neurotoxicity[A]	1	2	0	3 (12)
Hypertension	3	0	0	3 (12)
Lung infection	2	0	0	2 (8)
Hypoalbuminemia	2	0	0	2 (8)
Supraventricular tachycardia	1	0	0	1 (4)
Papilledema	1	0	0	1 (4)
Abdominal pain	1	0	0	1 (4)
Death NOS[B]	0	0	1	1 (4)
Fatigue	1	0	0	1 (4)
Infections – other (fungemia)	0	1	0	1 (4)
Sepsis	0	1	0	1 (4)
Urinary tract infection	1	0	0	1 (4)
Alk phos increased	1	0	0	1 (4)
Ejection fraction decreased	1	0	0	1 (4)
Fibrinogen decreased	1	0	0	1 (4)
Hyperglycemia	0	1	0	1 (4)
Hypoglycemia	1	0	0	1 (4)
Pleural effusion	1	0	0	1 (4)
Pleural hemorrhage	0	1	0	1 (4)

Highest grade toxicity experienced by subject is reported in table. [A]Neurotoxicity includes 1 subject with grade 3 seizures, grade 4 delirium, and grade 4 reversible posterior leukoencephalopathy syndrome (RPLS) (also known as posterior reversible encephalopathy syndrome [PRES]); 1 subject with grade 3 delirium and grade 4 encephalopathy; and 1 subject with grade 3 encephalopathy. [B]Death NOS (not otherwise specified) in subject 08 with candidemia and rapidly progressive myeloma, whose family chose to pursue comfort measures only. *n*, number of subjects who had the event; Alk phos, alkaline phosphatase; AST, aspartate aminotransferase.

sion generally peaked at day 10–14, and appeared most uniform in cohort 3 with Cy conditioning and the higher dose of CART-BCMA cells, while it was more heterogeneous in cohorts 1 and 2, though this difference did not meet statistical significance (Figure 3D). Despite a predominance of CD4$^+$ T cells in the infused product, CART-BCMA cells circulating in blood were predominantly CD8$^+$, and were highly activated, with a median of 94% (range 21%–94%) of CAR$^+$CD3$^+$ cells expressing HLA-DR during peak expansion (Supplemental Table 6). CART-BCMA levels in marrow aspirates generally mirrored those in peripheral blood, and also

were elevated in pleural fluid and cerebrospinal fluid for subject 03, and pleural fluid from subject 27, demonstrating widespread trafficking (Supplemental Table 7). Following peak expansion, CART-BCMA cell levels by qPCR declined in a log-linear fashion in the majority of patients (Figure 3, A–C), and were still detectable 3 months after infusion in 20 of 20 (100%) subjects tested, and at 6 months in 14 of 17 (82%) tested. Subject 01 (in stringent CR) continued to have detectable cells by qPCR when last tested 2.5 years after infusion.

We quantified 30 cytokines in peripheral blood serum before and after CART-BCMA infusion. Nineteen of these were increased more than 5-fold over baseline in more than 1 subject, with the most frequent increases observed for IL-6, IL-10, monokine induced by interferon-gamma (MIG, CXCL9), IP-10, IL-8, GM-CSF, and IL-1 receptor antagonist (IL-1RA) (Supplemental Figure 7). More severe CRS (grade 3–4, or grade 2 receiving tocilizumab) was associated with increases in multiple cytokines (Supplemental Table 8), most significantly with IFN-γ, IL-2 receptor alpha (IL2-Rα), macrophage inflammatory protein 1 alpha (MIP-1α), and IL-15 (Figure 4, A–D). Neurotoxicity was most strongly associated with increases in IFN-γ, IL-1RA, and MIP-1α (Figure 4, E–G, see Supplemental Table 9 for full analysis). Peak fold-increase in IL-6 was strongly associated with both severe CRS and neurotoxicity (Supplemental Table 8, Supplemental Table 9); however, tocilizumab and siltuximab administration can artificially elevate serum IL-6 levels (17). When only IL-6 values obtained prior to administration of tocilizumab ($n = 6$) or siltuximab ($n = 1$) were included in the analyses, the association of peak IL-6 fold-increase with severe CRS and neurotoxicity was still present, but less statistically robust (Figure 4, H and I). We did not observe significant differences in peak fold cytokine increases between cohorts 1 and 3, which received the same dose of CART-BCMA cells with or without Cy conditioning, respectively (Supplemental Figure 7).

We also assessed serum concentration of sBCMA, as well as its ligands BAFF and APRIL. Compared with a panel of healthy donors (HDs), enrolled subjects had significantly elevated sBCMA and reduced APRIL levels at baseline, with high variability among subjects (Figure 5A). BAFF concentrations in subjects were not significantly different from HDs. Serial assessments of serum sBCMA showed decreases following CART-BCMA infusions, which were more pronounced in responding compared with non-responding subjects (Figure 5B). sBCMA increased overall as both responders and nonresponders developed progressive disease, though the 3 long-term responders maintained low sBCMA concentrations (Supplemental Figure 8), confirming serum sBCMA concentration as a useful adjunctive biomarker for assessing myeloma disease response and progression (7).

Twenty subjects were evaluable for BCMA surface expression on MM cells by flow cytometry performed on fresh marrow aspirates prior to treatment, and all had detectable BCMA expression, though intensity varied (median MFI, 3741; range 206–24,842; see Supplemental Figure 9 for representative gating). Of 18 subjects with evaluable serial BCMA expression (Figure 5C), either at 1 month ($n = 16$), 3 months ($n = 8$), and/or 5.5 months ($n = 1$), 12 (67%) had a decline in BCMA intensity at least at 1 postinfusion time-point, including 8 of 9 responders and 4 of 9 nonrespond-
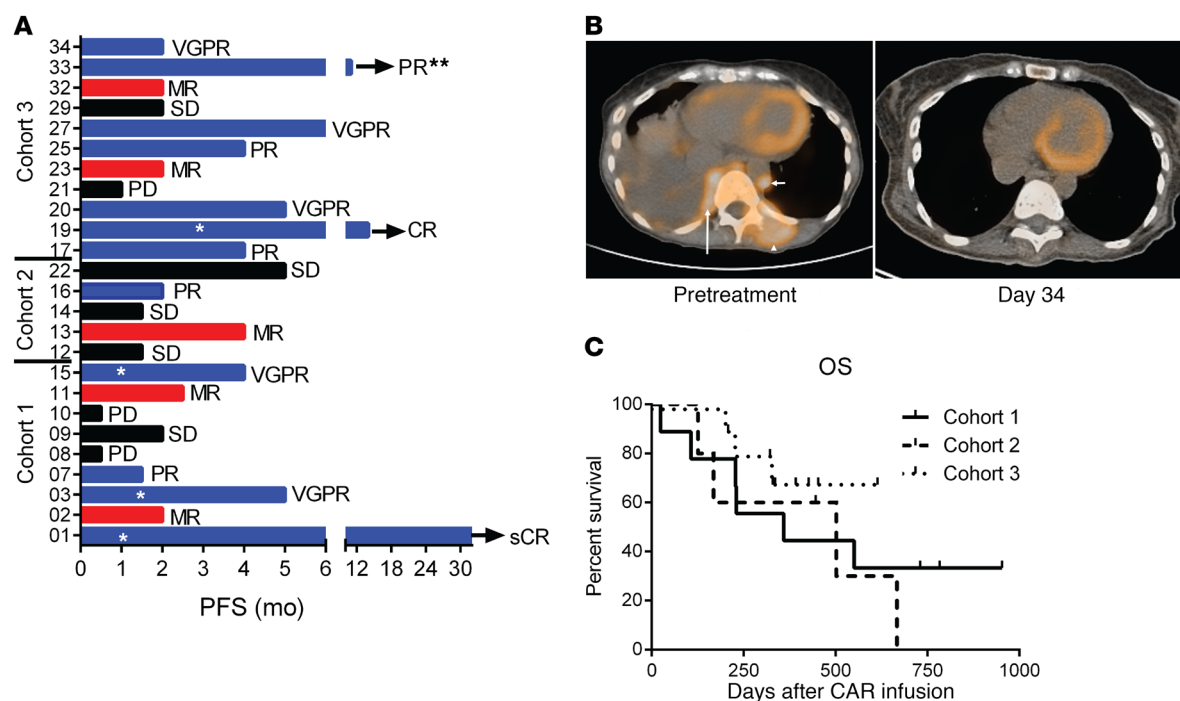
Figure 2. Clinical outcomes. (**A**) Swimmer's plot showing best response and progression-free survival (PFS) for each subject in cohort 1 (1×10^8 to 5×10^8 CART-BCMA cells alone), cohort 2 (Cy plus 1×10^7 to 5×10^7 CART-BCMA cells), and cohort 3 (Cy plus 1×10^8 to 5×10^8 CART-BCMA cells). Arrow indicates ongoing response. Blue bars represent PR or better; red bars, MR; black bars, no response (SD or PD). *Minimal residual disease (MRD), negative by flow cytometry (estimated sensitivity 1 in 10^{-5} cells). **Has negative serum and urine immunofixation and negative bone marrow biopsy but residual retroperitoneal lymph nodes (LN), known to contain myeloma by prior biopsy, that decreased in size by more than 50% and became FDG-negative on PET/CT but did not disappear. Repeat LN biopsy not performed. (**B**) PET/CT scan images for subject 03 showing resolution of extramedullary disease (arrows) and malignant pleural effusion after treatment. (**C**) Overall survival (OS) based on cohort, Kaplan-Meier plot. CR, complete response; MR, minimal response; PR, partial response; VGPR, very good partial response; PD, progressive disease; sCR, stringent complete response; SD, stable disease.

ers (Figure 5D). BCMA intensity was lowest on residual MM cells 1 month after CART-BCMA, and increased back toward baseline in most, but not all, subjects with subsequent testing. Individual subject details are provided in Supplemental Table 10. Neither progression-free nor overall survival was significantly associated with baseline BCMA MFI (Supplemental Figure 10).

Responses were significantly associated with peak expansion by qPCR (median 75,339 copies/μg DNA for ≥PR vs. 6368 copies/μg for <PR, $P = 0.0002$), as well as with persistence over the first 28 days, as measured by the area under the curve (AUC_{0-28d}) (median 561,796 copies × days/μg DNA for ≥PR vs. 52,391 copies × days/μg DNA for <PR, $P = 0.0002$) (Figure 6, A and B). Both expansion and response were more likely in the setting of more severe CRS (grade 3–4 or grade 2 requiring tocilizumab) (Figure 6, C and D). Neither expansion nor response was significantly associated with age, years from diagnosis, number of prior lines of therapy, presence of del17p or *TP53* mutation, being penta-refractory, most recent therapy prepheresis, bone marrow MM cell percentage, baseline serum sBCMA concentration, or MM cell BCMA intensity (Supplemental Figure 11, Supplemental Figure 12).

In order to explore other pretreatment characteristics potentially associated with expansion and/or response, we analyzed features of the CART-BCMA product before, during, and at the end of manufacturing. We found that a higher CD4/CD8 T cell ratio in the leukapheresis product, premanufacturing, was associated with greater in vivo CART-BCMA expansion (Figure 6E),

and to a lesser degree, response (Figure 6F), whereas absolute CD3+, CD4+, or CD8+ T cell numbers in the leukapheresis product, or CD4/CD8 ratio in the final CART-BCMA product at the end of manufacturing was not (data not shown). Fold expansion of seeded cells during manufacturing also correlated with in vivo CART-BCMA expansion (Figure 6G), suggesting that in vitro proliferative capacity may predict for in vivo activity. Finally, our previous analyses in CLL patients receiving CD19-directed CAR T cells demonstrated that deeper clinical responses were associated with a higher percentage of CD27+CD45RO−CD8+ T cells within the leukapheresis product (18), a phenotype that identifies primarily naive but also includes stem cell memory T cell populations (19). We examined CD8+ T cells within the leukapheresis products in subjects treated with CART-BCMA cells and similarly found that subjects with higher frequencies of CD27+CD45RO−CD8+ T cells were more likely to have robust in vivo expansion and clinical response (Figure 6, H and I).

Discussion

CAR T cell therapy is emerging as a promising therapeutic option for B cell malignancies, with the potential for durable disease control following a single treatment, differentiating it from other therapies that require repeated and/or continuous administration. In this report, we demonstrate the potential of CAR T cell therapy in advanced refractory myeloma, with 12 of 25 subjects (48%) achieving a partial response or better, including 7 of 11 (64%) treated
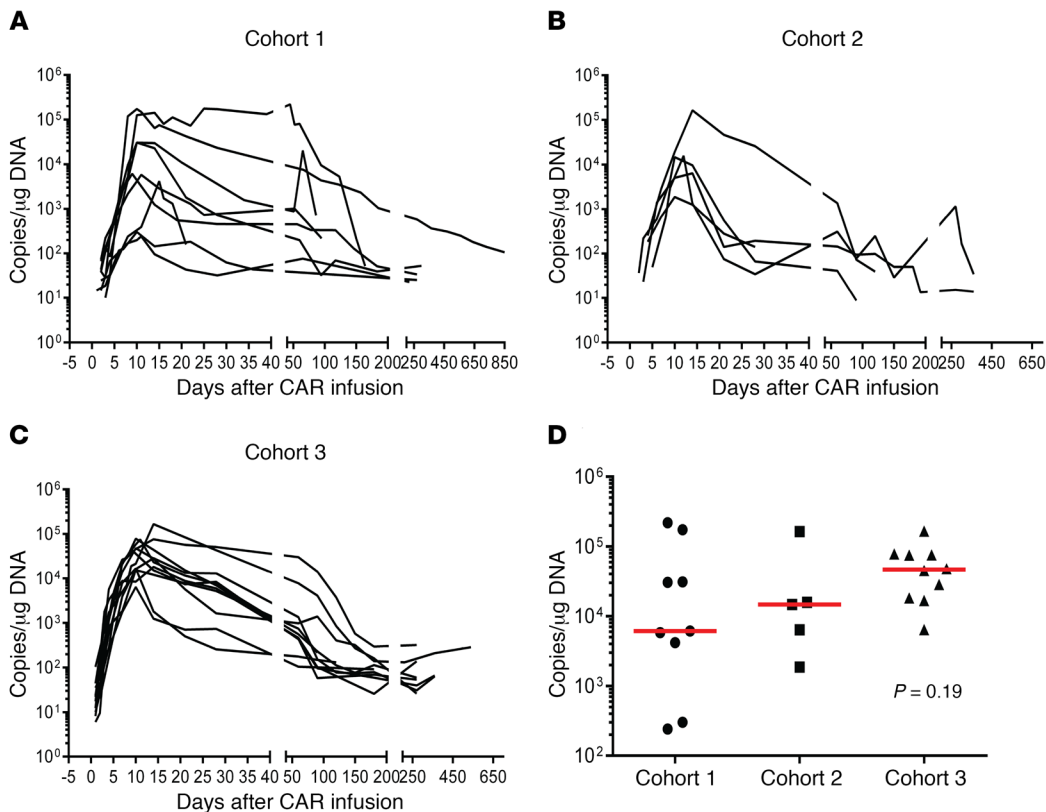
Figure 3. CART-BCMA expansion and persistence. (**A**–**C**) CART-BCMA cell levels over time in peripheral blood for each cohort are depicted, as measured by quantitative PCR for CAR sequence. (**D**) Peak CART-BCMA levels by qPCR for each subject are shown (except subject 34, for whom peak data were not available). Median peak CART-BCMA levels (red bars) were not significantly different between cohorts (Kruskal-Wallis test, $P = 0.19$).

in cohort 3 with lymphodepleting chemotherapy and the higher ($>10^8$) dose of CART-BCMA cells. Three subjects had ongoing remissions longer than 11 months after CART-BCMA therapy, including one ongoing sCR at 2.5 years. This is notable given the highly adverse biological features of the enrolled subjects' myeloma, including high tumor burden, rapidly progressing disease, and high-risk genetics, and further validates BCMA as a highly attractive target in myeloma. Importantly, this activity was seen despite the fact that our study, unlike the previously reported NCI study, did not exclude patients with low BCMA expression or high tumor burden, and used either no lymphodepletion or Cy alone, compared with Cy plus fludarabine in the prior study (12, 20). We successfully manufactured CAR T cell products from all apheresed subjects, and saw engraftment in all subjects as well, though peak levels and persistence of CAR T cells were variable.

Myeloma has long been associated with quantitative and functional deficits in T cells, particularly in more advanced, refractory disease, with inverted CD4/CD8 T cell ratios, impaired ex vivo antitumor activity, and acquisition of an exhausted or senescent phenotype (21–23). Responses correlated with degree of in vivo expansion in our study, which in turn was associated with higher premanufacturing CD4/CD8 T cell ratio, premanufacturing frequency of CD45RO⁻CD27⁺CD8⁺ T cells, and magnitude of in vitro proliferation during manufacturing. This suggests that more effective CART-BCMA products may be derived from subjects with a less differentiated, more naive and/or stem cell memory–like T cell compartment, as previously observed in a CLL trial at our center using CD19-directed CAR T cells (18). Confirmation with larger numbers and other CAR T cell products is nec-

essary, but these data suggest that pretreatment phenotypic and/or functional T cell characteristics may aid in the prediction of response to CART-BCMA therapy. They also suggest that treatment of patients earlier in the course of their disease, when T cells may be intrinsically "fitter," or modifying manufacturing techniques to generate more phenotypically favorable CAR⁺ T cells, may be more effective. Alternatively, routine harvesting and storage of T cells early in disease course (e.g., in first remission) for later use in CAR manufacturing could be considered.

Successful adoptive transfer of tumor-specific T cells, including CAR T cells, in humans has most commonly followed some form of lymphodepleting conditioning (13, 14, 24–26), which has been demonstrated to enhance T cell–mediated antitumor immunity via multiple potential mechanisms, including reduction of cellular "sinks" leading to increased availability of homeostatic cytokines, and depletion of suppressor cell populations, among others (27). Our study demonstrates that lymphodepletion is not absolutely required for robust and sustained CAR T cell expansion and clinical activity, as seen with subjects 01 and 03 in cohort 1. However, we observed short-term expansion more consistently in cohort 3, where subjects received Cy conditioning, compared with cohort 1 (Figure 3), demonstrating an effect of lymphodepletion on CAR T cell kinetics following adoptive transfer. Given that studies showing incremental increases in the intensity of the conditioning lead to enhanced engraftment and clinical outcomes (13, 24), it is possible that modifying the lymphodepletion (e.g., adding fludarabine to Cy) may further augment the activity of CART-BCMA cells, and we are testing this in ongoing trials.
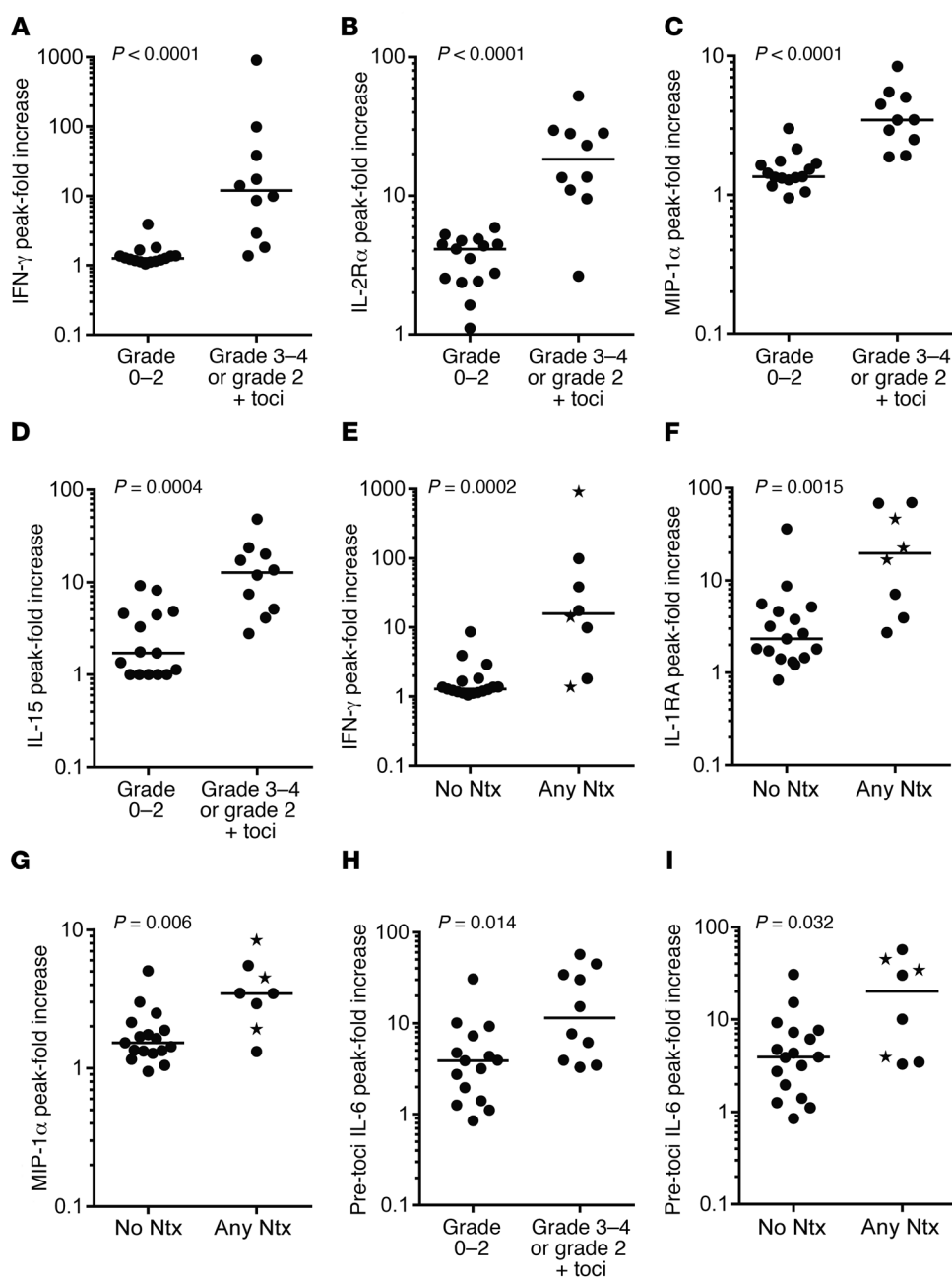
Figure 4. Serum cytokines associated with CRS severity and neurotoxicity. Serum cytokine concentrations (in pg/ml) through day 28 were measured by Luminex assay. (**A**–**D**) The median peak fold-increase over baseline for each cytokine was compared between subjects with no CRS, grade 1 CRS, or grade 2 CRS not receiving tocilizumab (CRS grade 0–2) and those with grade 3–4 CRS or grade 2 CRS receiving tocilizumab (CRS grade 3–4 or grade 2 + toci). The cytokines most significantly associated with CRS severity were (**A**) IFN-γ, (**B**) IL-2Rα, (**C**) MIP-1α, and (**D**) IL-15. (**E**–**G**) Median peak fold-increase over baseline for each cytokine was compared between subjects with no neurotoxicity (No Ntx) and those with any grade of neurotoxicity (Any Ntx). The cytokines most significantly associated with neurotoxicity were (**E**) IFN-γ, (**F**) IL-1RA, and (**G**) MIP-1α. (**H**–**I**) Peak fold-increase in IL-6 was less significantly associated with severe CRS (**H**) or neurotoxicity (**I**) when only pretocilizumab/siltuximab values were included. Stars depict subjects with grade 3–4 neurotoxicity. Exact *P* value by Mann-Whitney test is shown. Because the statistical analyses performed here are exploratory and hypothesis-generating in nature, no adjustment of the *P* values was made for multiple comparisons. Horizontal lines depict medians. IFN-γ, interferon gamma; IL-1RA, interleukin 1 receptor antagonist; IL-2Rα, interleukin 2 receptor alpha; IL-6, interleukin 6; IL-15, interleukin 15; MIP-1α, macrophage inflammatory protein 1 alpha.

An important unanswered question for BCMA-targeted CAR T cells is whether there is a threshold of BCMA expression on MM cells required for optimal recognition and killing. In the previously reported NCI trial, 52 of 85 (62%) prescreened bone marrow biopsies stained for BCMA by IHC met their prespecified threshold for eligibility, meaning more than a third of potentially eligible patients with MM would have been excluded (12). We did not require any specific level of BCMA as an eligibility requirement, and identified MM cell BCMA expression by flow cytometry in all subjects, consistent with recent data that flow cytometry is more sensitive than IHC for this purpose (28). Baseline BCMA intensity by flow cytometry did not correlate with either expansion or response in our study (Supplemental Figure 9 and Supplemental Figure 10), suggesting that excluding patients based on baseline BCMA expression is likely not necessary.

The observed dynamics of BCMA surface expression on MM cells, with the residual MM cells from several subjects in this study having significantly diminished BCMA intensity following CAR T cell therapy (Figure 5), highlights an important area for future research into resistance to CART-BCMA therapy. Down-modulation of BCMA expression was also observed in at least 1 subject in the NCI study (20), suggesting that it may be a common means of MM cell escape from BCMA-directed CAR T cell therapies. Surface BCMA expression subsequently increased in most subjects upon progression, suggesting a transcriptional or posttranslational mechanism, such as increased shedding from the cell surface. Alternatively, there may be immune selection for BCMA-dim/negative clonal variants, which are subsequently outcompeted by residual BCMA+ clones upon loss of CART-BCMA cell activity. This suggests
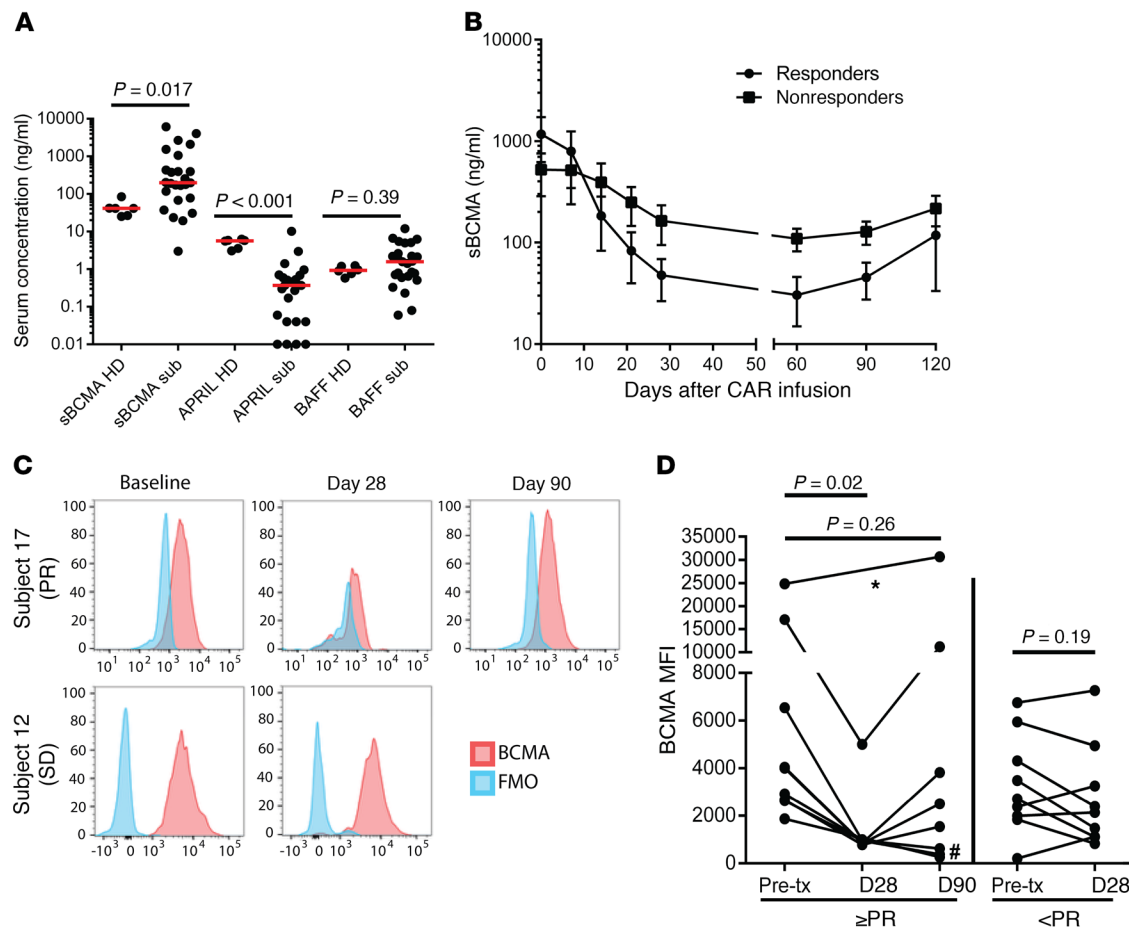
Figure 5. Soluble BCMA (sBCMA), BAFF, and APRIL concentration, and BCMA expression on MM cells before and after CART-BCMA infusions. (**A**) Baseline peripheral blood serum concentration of sBCMA and APRIL for subjects (sub) were significantly increased and decreased, respectively, compared with a panel of healthy donors (HD, n = 6) (P = 0.017, and P < 0.001, respectively, Mann-Whitney). Baseline BAFF concentrations were not significantly different. Median concentrations are depicted by red lines. (**B**) Serial sBCMA concentrations decline after CART-BCMA infusions more significantly in hematologic responders (PR/VGPR/CR/sCR) than in nonresponders (MR/SD/PD) before day 28 (P < 0.001). After day 28 the slopes of the curves are not significantly different between groups (P = 0.429). The estimation was based on a linear random intercept mixed effects model on $\log^{10}$-transform sBCMA that included 2 piecewise linear splines connected at day 28; P values were determined based on z test for the regression coefficient of interest or a linear combination of the coefficients. Mean concentration (ng/ml) + SEM are depicted. (**C**) Representative examples of BCMA expression on MM cells by flow cytometry. See Supplemental Figure 9 for gating strategy. FMO, fluorescence minus one. (**D**) BCMA mean fluorescence intensity (MFI) on MM cells over time in 18 subjects with evaluable serial bone marrow aspirates. Median MFI was significantly different between pretreatment (pre-tx) and day 28 (D28) for responders (4000 vs. 944, P = 0.02, paired t test) but not for nonresponders (2704 vs. 2140, P = 0.19). Median MFI was not significantly different between pre-tx and day 90 (D90) for responders (4000 vs. 2022, P = 0.26). *Subject 15 had no detectable MM cells at D28. #Subject 03 had no detectable MM cells at D45 (D28 not done) and too few MM cells to characterize at D90. D164 marrow is depicted at D90 time point.

that most patients progressing after CART-BCMA will remain candidates for additional BCMA-targeted therapies.

Our prior studies of CD19-specific CAR T cells in CLL and ALL suggest that durability of remission is associated with long-term persistence of CAR T cells (14, 16). In this study, however, only 1 (subject 01) of the 3 long-term responders (subjects 01, 19, 33) had detectable CART-BCMA cells by flow cytometry after day 60 (Supplemental Figure 4, Supplemental Figure 6). All 3 had detectable CAR T cells by qPCR at 3 months, but the amount of detectable BCMA CAR transgene (measured as copies/μg genomic DNA) in subjects 19 and 33 was similar to or less than several other patients who ultimately progressed between months 3 and 6 (Supplemental Table 6). Thus, in myeloma, CAR T cell persistence alone may not be enough to predict long-term durability of remission, and

other factors, such as T cell functionality, antigen expression, and/or immunosuppressive elements in the tumor microenvironment may play a role.

The primary toxicities of CAR T cells remain CRS and neurotoxicity. The frequency and severity of CRS in this study was similar to that reported in our CD19-targeted CAR T cell trials (14, 16), and was abrogated with IL-6 receptor blockade therapy. Though patient numbers are small, peak serum cytokine increases did not appear to differ significantly with or without Cy lymphodepletion, when CAR T cell dose was kept the same (cohort 1 vs. 3, Supplemental Figure 7). Interestingly, however, median peak fold-increases of IL-6 and several other cytokines (e.g., IFN-γ, IL-10, GM-CSF, IL-17) in this study were 1 to 2 orders of magnitude lower than that reported in the NCI BCMA CAR T cell study
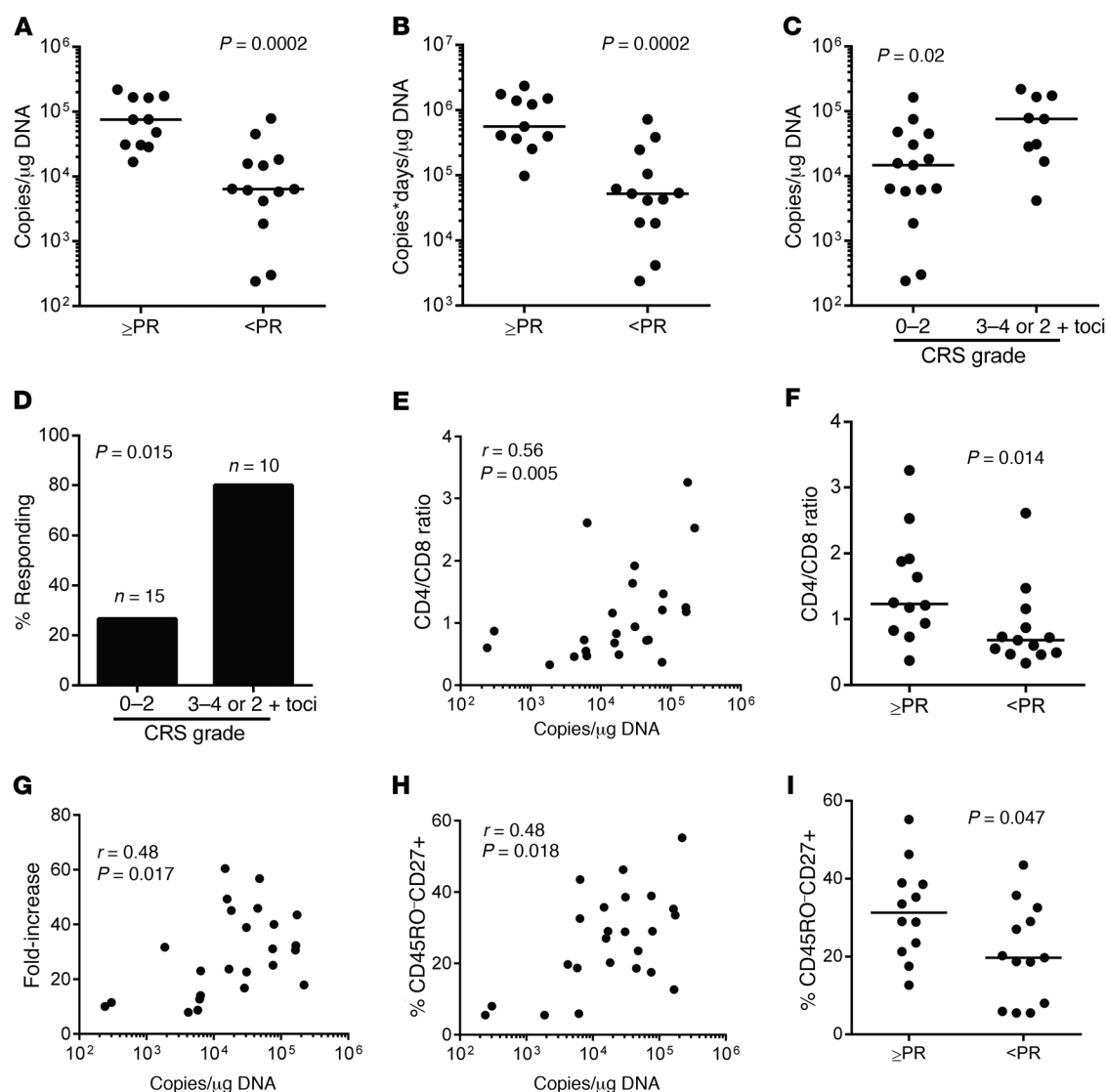
Figure 6. Predictors of in vivo CART-BCMA expansion and response. (**A**) Peak blood CART-BCMA expansion, as measured by qPCR, as well as (**B**) total CART-BCMA expansion over first 28 days (calculated as AUC), were both associated with clinical response. Greater peak CART-BCMA expansion (**C**) and response (**D**) were also associated with more severe CRS, defined as grade 3–4 or grade 2 requiring tocilizumab. A higher ratio of CD4+ to CD8+ T cells (CD4/CD8 ratio) within the leukopheresis product, as determined by flow cytometry, also correlated with both peak expansion (**E**) and response (**F**), while in vitro proliferation, measured as fold-increase of seeded cells during manufacturing, correlated only with peak expansion (**G**), but not response (P = 0.54, Mann-Whitney test, data not shown). (**H–I**) A higher proportion of CD8+ T cells within the leukapheresis product with a CD45RO−CD27+ phenotype was significantly associated with peak CART-BCMA expansion (**H**) and to a lesser degree, response (**I**). For A, B, C, F, and I, analysis was performed using Mann-Whitney test; lines represent median values. For D, analysis was by Fisher's exact test. For E, G, and H, analysis was done using Spearman correlation.

(20), despite a higher tumor burden in our study and despite the fact that our analysis of peak IL-6 levels included values obtained after tocilizumab administration, which is known to transiently further increase IL-6 levels due to binding of IL-6 receptor (17). One explanation for this difference may be the costimulatory domains used within the 2 CAR constructs, since CD28 domains, such as the one used in the NCI CAR construct, have been associated with more rapid CAR T cell proliferation and cytokine release than 4-1BB domains (29), as used in our CAR construct. However, the small number of patients and multiple differences between the studies with regard to inclusion criteria, dose, schedule, and lymphodepletion regimen preclude definitive conclusions.

Neurotoxicity has been reported in up to 50% of subjects in some CAR T cell trials (13, 30, 31), can occur concurrently with or subsequent to CRS, often does not improve with tocilizumab, and is reversible in most, but not all, cases. Neurotoxicity has been associated with early onset of CRS and rapid elevation of inflammatory cytokines both within the serum and CNS, perhaps leading to increased CNS vascular permeability (32). Consistent with this, we identified peak serum increases of IL-6, IFN-γ, and MIP-1α as most associated with neurotoxicity in this study (Figure 4). Interestingly, neurotoxicity was also associated with peak fold-increase in IL-1RA, an endogenous inhibitor of the proinflammatory effects of IL-1α and IL-1β, which have been implicated in CAR T cell–

associated neurotoxicity (33, 34). This perhaps reflects induction of an (ultimately ineffective) feedback mechanism in patients with neurotoxicity, and suggests that augmenting IL-1 blockade with the recombinant IL-1RA anakinra may have therapeutic benefit, as demonstrated in preclinical models (33, 34). Our experience demonstrating rapid reversal of a PRES-like syndrome in subject 03 suggests that cyclophosphamide may also be an option in steroid-refractory cases.

In summary, autologous T cells expressing a fully human BCMA-specific CAR could expand and induce objective responses, both with and without lymphodepleting chemotherapy, in subjects with advanced, refractory MM, and represent a promising new therapeutic approach. The toxicity profile appears similar to that seen with CD19-directed CAR T cells in B cell malignancies. Challenges include disease progression during manufacturing, potential for antigen escape due to changes in BCMA expression, and durability of responses. Subsequent studies exploring less heavily pretreated/refractory patient populations, dual-antigen–targeting CAR constructs, novel lymphodepletion regimens or manufacturing protocols, and off-the-shelf CART products may further optimize the safety and long-term efficacy of this approach.

Methods

Study design and participants. Eligible subjects had relapsed and/or refractory MM after at least 3 prior regimens, or 2 prior regimens if dual-refractory to a PI and IMID. Other key eligibility criteria at screening included ECOG (Eastern Cooperative Oncology Group) performance status of 0–2; serum creatinine no greater than 2.5 mg/dl or estimated creatinine clearance at least 30 ml/min; absolute neutrophil count at least 1000/μl and platelet count at least 50,000/μl (≥30,000/μl if bone marrow plasma cells were ≥50% of cellularity); SGOT no more than 3 times the upper limit of normal and total bilirubin no greater than 2.0 mg/dl; left ventricular ejection fraction at least 45%; lack of active autoimmune disease; and lack of central nervous system involvement with myeloma. No prespecified level of BCMA expression on MM cells was required.

This clinical trial (NCT02546167) was a phase I, single-center, open-label study. Initially, a standard 3+3 dose-escalation design was used, exploring 3 sequential cohorts: cohort 1, 1×10^8 to 5×10^8 CART-BCMA cells alone; cohort 2, Cy 1.5 g/m^2 plus 1×10^7 to 5×10^7 CART-BCMA cells; and cohort 3, Cy 1.5 g/m^2 plus 1×10^8 to 5×10^8 CART-BCMA cells. The protocol was amended to expand each cohort to 9 treated subjects, to gain more information about the safety and efficacy of CART-BCMA cells both with and without lymphodepleting conditioning and at a higher (1×10^8 to 5×10^8) and lower (1×10^7 to 5×10^7) dose. A subsequent amendment stopped enrollment in cohort 2 after 5 subjects due to suboptimal efficacy, and allowed up to 13 subjects in cohort 3; however, funding limitations ultimately ended enrollment after 11 treated cohort 3 subjects (total $n = 25$ treated).

The study was designed by the lead authors and was conducted according to principles of Good Clinical Practice. An independent data safety monitoring board reviewed adverse event data and provided oversight. Data cut-off for analysis was July 9, 2018.

Procedures. After a 2-week washout from therapy (4 weeks for monoclonal antibodies), subjects underwent steady-state leukapheresis to collect T cells for CART-BCMA manufacturing. Antimyeloma therapy could resume during manufacturing until 2 weeks prior to first CART-BCMA infusion. CART-BCMA cells were administered intravenously over 3 days (10% of dose on day 0, 30% on day 1, and 60% on day 2), as described (16). The 30% or 60% dose could be held if subjects developed signs of CRS. Cy was administered 3 days prior to first CART-BCMA infusion. Clinical and laboratory assessments were performed as shown in Figure 1.

CART-BCMA cells were manufactured in the Clinical Cell and Vaccine Production Facility at the University of Pennsylvania, as described (16, 35). Briefly, autologous T cells were collected by leukapheresis and were stimulated with paramagnetic polystyrene beads coated with anti-CD3 and anti-CD28 monoclonal antibodies (36). Cells were transduced with a lentiviral vector encoding anti-BCMA scFv linked to 4-1BB and CD3-ζ signaling domains (15), as described (37), and were expanded ex vivo for 10 to 12 days in the presence of recombinant human IL-2. Release criteria have been previously described (35). The frequency of CD3, CD4, and CD8 cells was determined by flow cytometry in the seed culture at the beginning and end of manufacturing. Expansion and population doubling of seeded cells was measured by cell counting. After manufacturing, CART-BCMA cells were cryopreserved until time of infusion.

Data on adverse events (AEs) were collected from time of first CART-BCMA infusion (or Cy administration for cohorts 2 and 3). Toxicity grade was determined according to National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0, with the exception of cytokine release syndrome, which was graded as per the University of Pennsylvania CRS Grading System (Supplemental Table 1) (16). Myeloma responses were assessed by updated International Myeloma Working Group criteria (38).

Research sample processing, freezing, and laboratory analyses were performed in the Translational and Correlative Studies Laboratory at the University of Pennsylvania, as described (14, 16, 35). CART-BCMA cells were quantified from peripheral blood or bone marrow samples by flow cytometry and quantitative PCR. Reagents and protocols for flow cytometry are described in the Supplemental Methods. Genomic DNA was isolated directly from whole blood or marrow aspirate, and qPCR analysis was performed using ABI TaqMan technology and a validated assay to detect the integrated CAR transgene sequence as per Supplemental Methods and described (35).

Serum cytokine levels were assessed on batched cryopreserved samples using the human cytokine magnetic 30-plex panel (LHC6003M) from Life Technologies, as described (14). Measurement of soluble BCMA, BAFF, and APRIL concentrations in serum was performed by ELISA using antibody sets for human BCMA (DY193), APRIL (DY884B), and BAFF (DT124-05) from R&D Systems. See Supplemental Methods for details.

Flow cytometric assessment of MM cells, including BCMA expression, on fresh bone marrow aspirate material was adapted from the EuroFlow protocol (39) as described in Supplemental Methods. Flow cytometric assessment of T cell phenotype in cryopreserved leukapheresis specimens was performed and analyzed as described (18).

Outcomes. The primary objective was to evaluate the safety of CART-BCMA in patients with relapsed/refractory myeloma. The primary endpoint was incidence of study-related grade 3 or higher AEs, including DLTs. A DLT was defined as a serious and unexpected AE whose relationship to study therapy could not be ruled out, occurring within 4 weeks of receiving protocol therapy. Hematologic toxicity

was not considered a DLT due to the refractory nature of the underlying disease and expected myelosuppression from cyclophosphamide. In addition, any death related to protocol treatment, as well as any expected grade 4 organ toxicity or grade 4 neurologic toxicity that did not resolve or improve to grade 2 or less within 4 weeks of onset, despite medical management, was also considered a DLT. Secondary objectives were to assess feasibility of manufacturing CART-BCMA cells, and clinical activity. Secondary endpoints were frequency of successful manufacturing, and clinical outcomes, including response rates, progression-free survival, and overall survival. Exploratory endpoints included CART-BCMA expansion and persistence in vivo; changes in concentration of serum cytokines and soluble BCMA, and expression of BCMA on MM cells.

Statistics. Statistical analysis of the study is primarily descriptive due to the pilot nature of the trial and small sample size of each cohort. Kaplan-Meier method was used to estimate duration of response, progression-free and overall survival, and the associated median survival times. Association between a binary endpoint (e.g., response) and a continuous factor (e.g., CAR T cell numbers) was evaluated using the Mann-Whitney test, or Kruskal-Wallis test if more than 2 groups were compared simultaneously. Association between a binary endpoint and a categorical factor (e.g., presence or absence of deletion 17p) was evaluated using Fisher's exact test. Spearman correlations were used to measure the correlations between 2 continuous variables. Significance of the Spearman correlation against the null hypothesis of no correlation was computed based on permutation. Modeling of longitudinal trajectories over time (e.g., for soluble BCMA concentrations) was done using a linear mixed effects model with random intercepts on the $\log^{10}$-transformed scale. Piecewise linear spline was used to capture the nonlinear patterns before and after day 28. Because the statistical analyses performed here are exploratory and hypothesis-generating in nature, no adjustment of the P values was made for multiple comparisons. Exact 2-sided P values are reported when applicable. A P value less than 0.05 was considered significant. Analysis was performed using Graphpad Prism version 6.0.

Study approval. The study was reviewed and approved by the University of Pennsylvania Institutional Review Board. The regulatory sponsor was the University of Pennsylvania. All subjects provided written informed consent prior to participation.

Author contributions

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