Background: BCMA-targeted CAR-T cell therapy has showed high response rates in multiple myeloma (MM); however, remission is transient in most of patients (pts). Variable and even absent BCMA expression on MM cells have been documented after single BCMA-targeted CAR-T cell treatment. CS1 (CD319, SLAMF7) plays a vital role in myeloma pathogenesis, including promoting MM cells growth, survival and adhesion. CS1 is highly expressed on tumor cells in almost all MM pts, and is also retained at significant levels at relapse. Therefore, we propose to augment BCMA targeting with CS1. Bispecific CS1-BCMA CAR-T cells are effective in targeting MM cells in preclinical studies (Biomedicines 2021, 9, 1422).

Aims: Here we report the outcomes of 13 pts with refractory or relapsed (RR) MM in our phase I clinical trial (NCT 04662099).

Methods: CS1-BCMA bispecific CAR contained a murine anti-CS1 scFv (clone 7A8D5) and a murine anti-BCMA scFv (clone 4C8) in tandem, and a 4-1BB costimulatory domain (Figure 1A). The enrolled pts must have received at least 2 prior lines of therapy, and previous BCMA- or CS1-targeted immunotherapies were allowed. Pts were subjected to lymphodepleting regimens with cyclophosphamide (250 mg/m², d-5 to d-3) and fludarabine (30 mg/m², d-5 to d-3) daily prior to the CAR-T infusion (d0). Planned dose levels were 0.75, 1.5, and 3.0x10^6 CAR+T cells/kg, and repeated infusions were allowed. Primary objectives were incidence of adverse events. Cytokine release syndrome (CRS) and neurotoxicity were graded using the ASTCT criteria, and other AEs using CTCAE v5.0. Secondary objectives were overall response rate (ORR), overall survival (OS), duration of response (DOR), and progression-free survival (PFS). Response was assessed per the IMWG criteria (2016). Other objectives included in vivo kinetics of CAR-T cells.

Results: As of February 10, 2022, 13 pts received the infusion of CS1-BCMA CAR-T cells and were included in the final analysis. Six pts (46%) carried cytogenetic abnormalities, and 3 pts had only extramedullary diseases (EMD) without detectable MM cells in bone marrow (BM). BCMA and CS1 were highly expressed on MM cells in BM (median BCMA+ 77.2% and CS1 + 96.5%) and EMD (Figure 1B). Eight pts (62%) were refractory, and median prior lines of treatment was 5.5 (range 2-10), and 7 pts (54%) received autologous stem cell transplantation.

Four pts (31%) experienced CRS, and grade 3 CRS occurred once and lasted for 5 days. Neurotoxicity was not observed. The ORR is 76.9% (10/13), including 4 stringent complete, 2 very good partial, and 4 partial response (Figure 1C). Although CAR-T cells infiltrated into the tumor tissue, response was not observed in the 3 pts with only EMD (Figure 1D). Soluble BCMA decreased remarkably in peripheral blood (PB) and BM (Figure 1E). The median follow-up and DOR were 290 days. The median OS and PFS weren’t reached, and OS and PFS at 9-month were 77.1%.

On day 14 after infusion, CAR-T cells peaked at 46482 copies/ug DNA in PB (n=13) and 282920 copies/ug DNA in BM (n=10) by droplet digital PCR; CAR-T cell expanded at 400 CAR+T cells/ul PB (n=13) and 271 CAR+T cells/ul BM (n=10) by flow cytometry. CAR was detected in vivo at a median of 5 months. Moreover, we systematically
evaluated the immune characteristics of infused products (Figure 1F). Limited by small sample size, correlation analysis wasn’t done.

**Image:**

A. Schematic creation of CS1-BCMA CAR. B. Immunofluorescence of CS1, CD138, and BCMA on tumor puncture smear for IgG at screening. C. Flowing plot of the 13 yrs in the clinical trial. D. Immunofluorescence of CAR (green) and BCMA (red) or EMD for p0, p1, and p2 after infusion. E. Change of alveolar in PD and BM one month (m) after infusion. F. CAR/CD3- in IP and marker expression on CS1-T cells.

**Summary/Conclusion:** Our study demonstrates bispecific CS1-BCMA CAR-T cells are clinical active with a good safety profile in heavily pretreated patients with MM.