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704.CELLULAR IMMUNOTHERAPIES: EARLY PHASE AND INVESTIGATIONAL THERAPIES

New Optimized Academic Anti-BCMA CAR (CARTemis-1): How Does the Manufacturing Process Impact CAR-T Cell Features?

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Introduction

CAR therapy targeting BCMA (B-cell maturation antigen) is under research as treatment for multiple myeloma (MM). However, given the lack of a plateau in most studies, it becomes imperative to pursue more effective alternatives. BCMA downmodulation, the presence of soluble BCMA or the immune dysfunction observed in MM are some limitations that may affect efficacy. Next-generation CAR-T cells are being designed exploring new approaches to overcome restrictions to CAR-T efficacy in MM. We present a novel optimized anti-BCMA CAR (CARTemis-1) and its translation to the clinic. We have specifically focused on the study of the dynamics of the immunophenotype of CARTemis-1 along the manufacturing process, analyzing how these variables might impact the quality of the final product and influence the release decision.

Materials and methods

CARTemis-1 was evaluated in preclinical *in vitro* and *in vivo* models. CARTemis-1 generation from three healthy donors was validated under GMP-conditions in CliniMACS Prodigy with IL-7/IL-15. Flow cytometry was performed to characterize the immunophenotype along the manufacturing process. Quality controls of the final CARTemis-1 product were performed according to the criteria of the Spanish Medicine Agency. Stability of the final cryopreserved CARTemis-1 cell product was also analyzed.

Results

CARTemis-1 is a second generation anti-BCMA CAR that co-expresses the truncated epidermal growth factor receptor (EGFRt) as a transduction marker and suicide gene. Two different versions of CARTemis-1 were synthesized with different spacer regions (short vs long) with the long version showing significantly greater anti-myeloma function *in vitro* compared to the short version ($p<0.001$) (Figure 1A). During the preclinical validation, CARTemis-1 demonstrated potent anti-tumor capacity *in vitro* and *in vivo*, showing no susceptibility to the presence of soluble BCMA. Expansion with IL-2 or IL-7/IL-15 was compared (n=8), obtaining increased proliferation (mean $1.59x10^7$ vs $3.07x10^7$, respectively, $p=0.002$), increased stem cell memory and naïve population (mean 37.48% vs 43.95%, respectively, $p=0.0052$), and less PD1+LAG3+ levels (1.38% vs 0.55%, respectively, $p=0.0338$) with IL-7/IL-15. The generation of CARTemis-1 was validated under GMP-conditions closely monitoring the immunophenotype dynamics from leukapheresis to the final product. Through comprehensive evaluations, the optimal time-point for product release to achieve the best-fitness product was >6 (specifically, day 8-10) with reduced levels of exhaustion markers (Figure 1B) maintaining hallmarks of T cell activation and anti-tumor potency. *In vivo* biodistribution of CARTemis-1 cells was studied in mice models without myeloma cells, finding preserved tissue structure without relevant

morphological alterations in brain, cerebellum, intestine, lung, endometrium, ovary, testicle, spleen, and liver. We performed graft-versus-host disease (GVHD) *in vivo* mice models and found no increase in GVHD incidence following CARTemis-1 inoculation. Additionally, we assessed the cryopreserved product stability, and it met all specifications and retained its functionality for up to 12 months after cryopreservation. With this positive outcome and having obtained the approval of the Spanish Medicine Agency, we plan to proceed with a phase I/II clinical trial using CARTemis-1 for patients with multiple myeloma who have experienced relapse following allogeneic transplantation (EudraCT 2021-001955-15).

Conclusions

CARTemis-1 has been rationally designed to increase anti-tumor efficacy and overcomes sBCMA inhibition. To our knowledge, this is the first study analyzing the impact of the manufacturing process in the dynamics of the CAR-T immunophenotype highlighting the importance of the immunophenotypic characterization of CAR-T cells throughout the manufacturing process to define the optimal cell culture protocol and expansion time to increase CAR-T cell product fitness.

Disclosures Reguera: *AMGEN:* Speakers Bureau; *KITE:* Speakers Bureau; *BMS:* Speakers Bureau; *Janssen:* Consultancy, Speakers Bureau. **Einsele:** *Takeda:* Honoraria, Other: Consulting or advisory role, Travel support; *GlaxoSmithKline:* Honoraria, Other: Consulting or advisory role, Travel support, Research Funding; *Sanofi:* Honoraria, Other: Consulting or advisory role, Travel support, Research Funding; *Novartis:* Honoraria, Other: Consulting or advisory role, Travel support; *Amgen:* Honoraria, Other: Consulting or advisory role, Travel support, Research Funding; *Janssen:* Honoraria, Other: Consulting or advisory role, Travel support, Research Funding; *Bristol Myers Squibb/Celgene:* Honoraria, Other: Consulting or advisory role, Travel support, Research Funding.

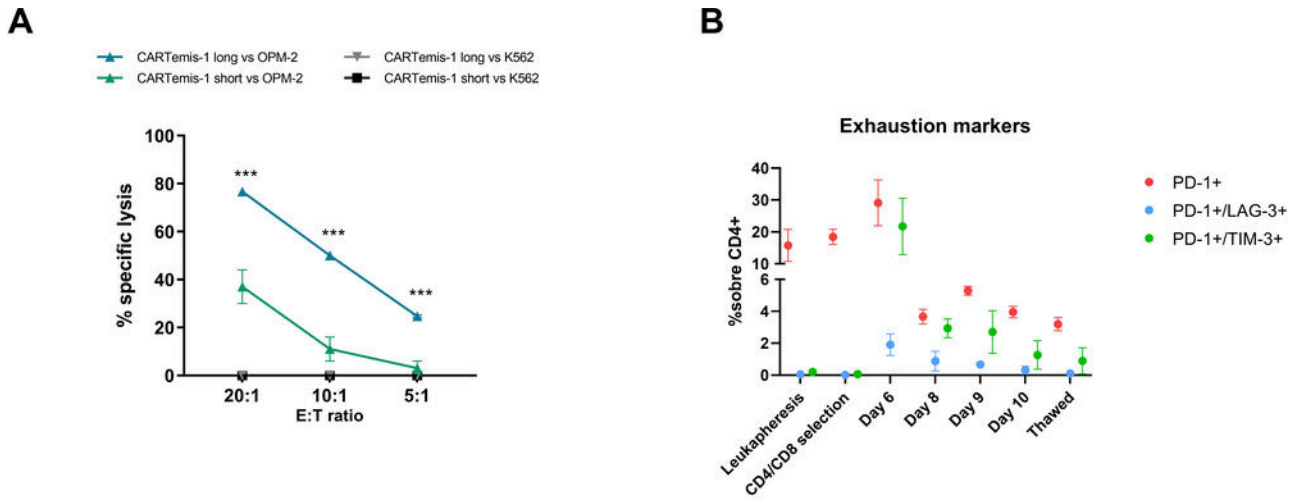

Figure 1. A) Tumor specific lysis of CARTemis-1 short and long versions. CARTemis-1 cells were co-cultured with multiple myeloma cell line (OPM-2) or BCMA- cells (K562) at different effector:target ratios (E:T ratio) to compare specific anti-tumor efficacy of short vs long version. **B) Immunophenotypic characterization of GMP-generated CARTemis-1 cells throughout the manufacturing process (from leukapheresis to final cryopreserved product).** Expression levels of exhaustion markers (PD-1, LAG-3 and TIM-3) from CD4+ CARTemis-1 cells.Depicted are mean values of three independent experiments.P-values between the indicated groups were calculated using unpaired T-test. *p<0.05 **p<0.01, ***p<0.001.

Figure 1

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