Cellular immunotherapy targeting kappa myeloma antigen for the treatment of multiple myeloma

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Abstract

Kappa (κ) Myeloma Antigen (KMA) has a higher cell density on malignant plasma cells in patients with relapsed refractory multiple myeloma (RRMM) compared to B cell maturation antigen (BCMA; 1). Here, we engineered a lentiviral vector encoding a second-generation CAR expressing a KMA reactive scFv from KappaMab fused to a 4-1BB co-stimulatory domain and CD3 zeta chain (Figure 1A). We successfully generated human anti-KMA CAR-T cells with high and stable CAR expression and a predominately memory T cell phenotype. The CAR-T cells selectively killed KMA-expressing tumor lines, secreted interferon-gamma upon target recognition, and demonstrated potent anti-tumor activity in a xenograft model. Anti-KMA CAR-T cell therapy therefore represents a novel and potent treatment, ready to enter a phase I clinical trial for patients with myeloma.

Introduction

Multiple myeloma (MM), the second most common blood cancer, is characterized by the accumulation of malignant plasma cells in the bone marrow.

Chimeric Antigen Receptor (CAR)-T cell therapy has recently entered the standard of care for RRMM, following the FDA-approval of two CAR-T cell products, ide-cel® and cilta-cel®, which target BCMA. However, despite impressive response rates, most patients relapse within 1-3 years.

KMA is a tumour specific, membrane associated protein expressed on malignant plasma cells in patients with kappa light-chain restricted (κ-type) MM. KMA is present on occasional mononuclear cells in normal tonsillar tissue and mucosal secondary lymphoid tissue (2,3). However, it is absent on normal peripheral blood B cells, lambda light chain restricted MM plasma cells, normal plasma cells and haematopoietic stem cells, making it an attractive and alternative target antigen for CAR-T cell therapy for MM (2,3). The monoclonal antibody, KappaMab (formerly MDX-1097; 3), binds to a conformational epitope on KMA, and has been assessed in phase I, IIa and IIb clinical trials in RRMM patients (4).

The purpose of this study was to perform preclinical experiments to determine if CAR T cells targeted against KMA could be efficacious as a treatment for MM patients (Figure 1B)

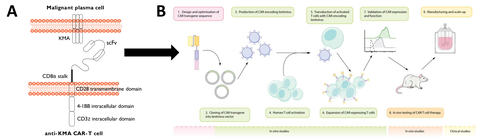

Figure 1. Schematic of of the KMA CAR (A) and workflow for project (B)

Methods and Materials

Human peripheral blood mononuclear cells (PBMCs) from 4 healthy donors were isolated using a standard density gradient and enriched for T cells using negative selection before being cryopreserved.

Transduction and expansion of CAR-T cells: Thawed PBMCs were transduced with the anti-KMA CAR transgene using a lentiviral vector. T cells were activated for 48 hrs with anti-human CD3/CD28/CD2 activator (StemCell) in optimised medium containing 5 ng/mL recombinant human IL-7 and IL-15. Day 2, activated T cells were transduced by incubation with lentivirus at a multiplicity of infection (MOI) of 5.

Flow Cytometry: Transduced T cells were assessed for transduction efficiency by flow cytometry using biotinylated kappa light chain with streptavidin-PE as secondary antibody, along with staining for CD4, CD8, CD45RA and CD62L for phenotypic analyses. Fluorescence was measured on a BD FACSymphony™ .

Cytotoxicity assay: Cytotoxicity of the anti-KMA CAR-T cells was assessed using a Calcein-AM killing assay (5). The CAR-T cells were co-cultured with KMA positive (JJN3-KMA^{mid}) or negative (OPM2) MM target cells, at various effector-target ratios for 4 hours. KMA expression on the target cell lines was assessed using a KMA-Fab'2-APC antibody, supplied by HaemaLogiX Ltd.

Cytokine analyses: A Cytometric Bead Array (CBA) was used to quantitate CAR-T cell-mediated secretion of cytokines. The supernatants used in these assays were harvested from 24 hour CAR T cell/tumour cell (JJN3 KMA^{mid} or OPM2) co-cultures.

Results – In vitro

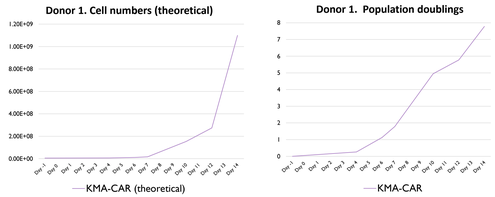

Figure 2. Expansion kinetics of the KMA-CAR T cells: For each donor, there was healthy expansion of the CAR-T cells, with theoretical cell numbers ranging from 3.5 x 10^8 - 1 x 10^9 cells, reaching 7 - 8 doublings by day 14. Data is representative of 4 donor expansions.

Results – In vitro

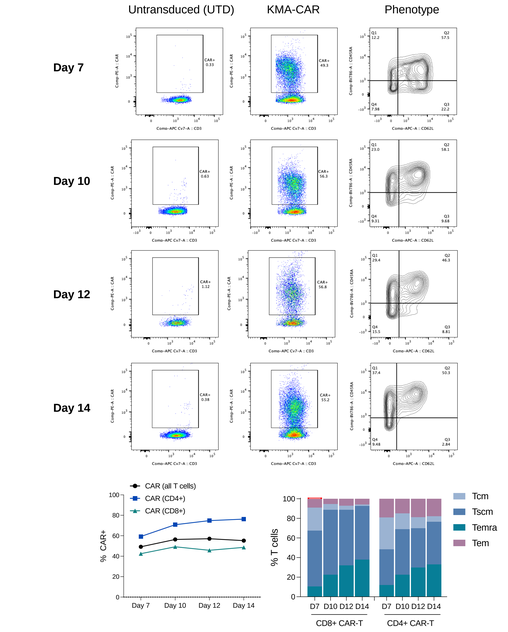

Figure 3. Time course of CAR expression and phenotype of the transduced T cells: High and stable CAR expression was achieved, with the CAR-T cells showing a predominant stem-cell memory (Tscm) phenotype. Data is representative of 4 donor products.

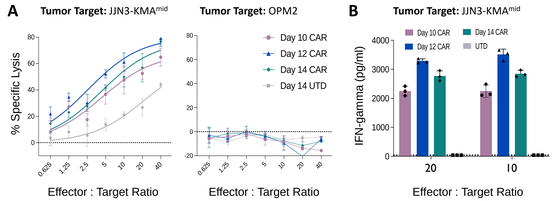

Figure 4. Functional expression of the KMA CAR was confirmed in *in vitro* cytotoxicity (A) and cytokine bead array (B) assays: Anti-KMA CAR-T cells efficiently recognized and killed KMA$^+$ target cells and produced high levels of IFN-gamma. Data is representative of 3 donor products.

Results – In vivo

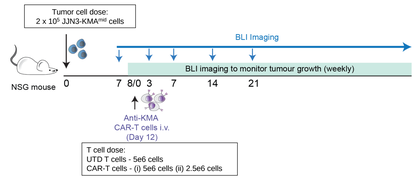

Figure 5. Schematic of treatment schedule for in vivo testing of anti-KMA-CAR-T cells

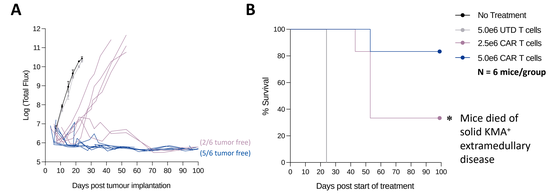

Figure 6. Impact of anti-KMA-CAR-T cells on disease burden as read out by bioluminescence imaging (A) and record of survival (B): In a separate *in vivo* study, we demonstrated that OPM2, a lambda myeloma cell line, is refractory to the anti-tumor activity of the KMA-CAR-T cells, confirming the on-target specificity of the anti-KMA-CAR-T cells *in vivo*.

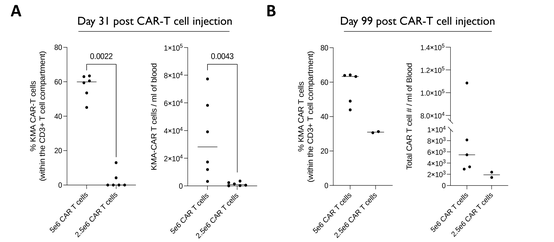

Figure 7. Analysis of KMA-CAR-T cell persistence in the peripheral blood of treated mice at days 31 (A) and 99 (B) post T cell injection.

Conclusions

These data demonstrated that novel anti-KMA CAR-T cell therapy is specific for KMA and may be highly effective at treating κMM and support progression of this cellular immunotherapy towards a Phase I clinical trial.

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Glossary

1. Tcm – Central memory T cells
2. Tscm – Stem memory T cells
3. Temra – Effector memory RA$^+$ T cells
4. Tem – Effector memory T cells
5. UTD – Untransduced T cells