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

Jessica Li, PhD a, Nicole M Haynes, PhD a,1, Katherine D Cummins, MD, PhD a,1,2,2, Kavitha Gowrishankar, PhD,1, Kenneth P Micklethwaite, MD, PhD b, Halley Hilton b, Rosanne Dunn, PhD a, Jane Oliaro, PhD a,1,2, Simon J. Harrison, MD, PhD a,1,2

1Centre of Excellence in Cellular Immunotherapy, Peter MacCallum Cancer Centre, Melbourne, Australia, 2Clinical Haematology, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, Australia, 3Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Australia, University of Sydney, NSW, Australia, 4Haemalogix Ltd, Sydney, Australia.

Abstract

Kappa (k) Myeloma Antigen (KMA) has a higher cell density compared to malignant plasma cells in patients with relapsed refractory multiple myeloma (RRMM) compared to B cell maturation antigen (BCMA). 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 (k-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 in phase I, Ia and Ib 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 18).

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. Cells were harvested at 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 human anti-CD3/CD28/CD2 (StemCell) in optimised medium containing 5 ng/ml recombinant human IL-2 and IL-7. 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 CD28 for phenotypic analysis. Fluorescence was measured on a BD FACSymphony™.

Cytotoxicity assay: Cytotoxicity of the anti-KMA-CAR-T cells was assessed using a CellTiter-AMK killing assay (5). The CAR-T cells were co-cultured with KMA positive (LNS3-KMA+™) or negative (OPM2) MM target cells, at various effector to 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 analysis: A Cytometric Bead Array (CBA) was used to quantify CAR-T cell-mediated secretion of cytokines. The supernatants used in these assays were harvested from 24 hour CAR T cell/tumourcell (LNS3 KMA+™ or OPM2) co-cultures.

Results – In vitro

Figure 3. Time course of CAR expression and phenotype of the transduced T cells: High-level CAR-D CAR expression was achieved, with the CAR-D CAR cells showing a marked increase cell viability. (Top panel). Phenotype data show maintenance of CD8+ phenotype.

Figure 4. Functional expression of the KMA-CAR was confirmed in vitro cytotoxicity (A) and cytokine bead array (B) assays: Flow cytometry and CBA confirm high levels of CAR expression. Data is representative of 3 donor products.

Conclusion

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

References


Glossary

1. Tcm – Central memory T cells
2. Tcm – Stem memory T cells
3. Tmef - Effector memory kA T cells
4. Tme - Effector memory T cells
5. UTD – Undoubtedly T cells

Contacts

Dr Rosanne Dunn
Director | Chief Scientific Officer
Haemalogix Ltd
Email rosanne@haemalogix.com

Peter MacCallum Cancer Centre
Email jane.oliaro@petermac.org

Dr Simon Harrison
Director | Chief Scientific Officer
Peter MacCallum Cancer Centre
Email simon.harrison@petermac.org

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 real time imaging (B): In a syngeneic model, we demonstrated that OPMM, a kappa myeloma cell line, is infiltrated by the anti-tumor activity of the KMA-CAR-T cells, confirming the in vivo 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 69 (B) post T cell injection.