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/CD154 (IonomC/Inter whisper) in medium containing 5 % RPMI patients (2). 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 CD69 for phenotypic analyses. Fluorescence was measured on a BD FACSymphony.

Cytotoxicity assay: Cytotoxicity of the anti-KMA CAR T cells was assessed using a Cell-Tek-AM killing assay (3). The CAR-T cells were co-cultured with KMA-pulsed (ULN-13-CELLA) or negative (OPM2) MM target cells, at various effector-to-target ratios for 4 hours. A 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 (ULN-13-CELLA or OPM2) co-cultures.

These data demonstrated that:
- Novel anti-KMA CAR-T cells specifically target KMA positive plasma cells
- Anti-KMA CAR-T cells are highly effective at treating xMM in a mouse model
- We are developing a Phase 1, FITH clinical trial of anti-KMA CAR-T cells for the treatment of relapsed and refractory multiple myeloma via the Centre of Excellence for Cellular Immunotherapy at the Peter MacCallum Cancer Centre, Melbourne, Australia.