

CAR T-cells targeting the kappa myeloma antigen for the treatment of multiple myeloma

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INTRODUCTION

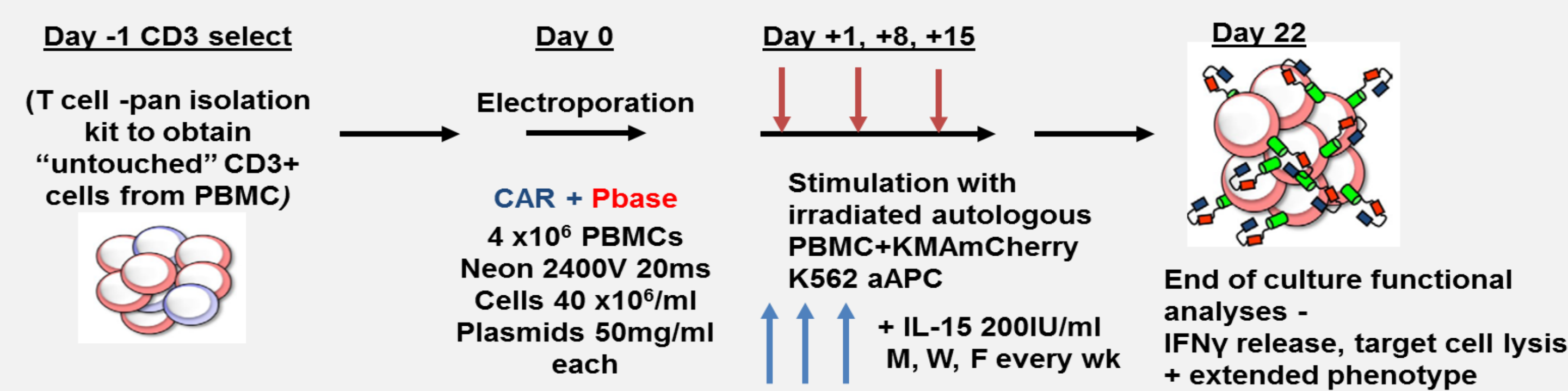
Multiple Myeloma (MM) is a malignancy of clonal plasma cells in the bone marrow. Despite recent advances in the treatment and management of MM, the majority of patients will ultimately relapse and die from their disease within 3-5 years.

Chimeric antigen receptor (CAR) T-cells for haematological malignancies are revolutionising immunotherapy for cancer, after dramatic response rates were obtained with CAR19 against B-cell leukaemia and lymphomas.

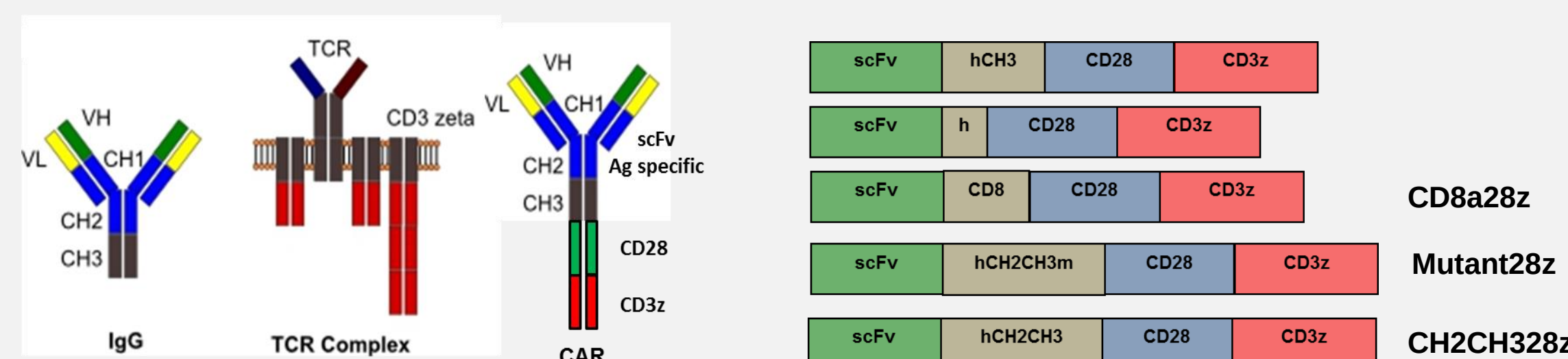
CAR T-cells for MM – There have been recent reports of phenomenal success with CARs targeting BCMA; other preclinical studies reported targeting CS1/SLAMF7, CD44v6, CD138, CD38, CD70.

KappaMab (MDX-1097) is a chimeric monoclonal antibody currently being assessed, in a Phase IIb clinical trial for the treatment of kappa light-chain restricted (κ-type) MM. (Trial ID - ACTRN12616001164482). MDX-1097 binds to a conformational epitope on **kappa myeloma antigen (KMA)**, a tumour-specific membrane associated protein expressed on malignant plasma cells in patients with κ-type MM (and light chains not associated with Immunoglobulin heavy chains).

METHODOLOGY



CD3+ cells were isolated by MACS separation and co-electroporated with CAR piggyBac transposon DNA and piggyBac transposase DNA using the Neon system (Invitrogen). Transfected CAR T-cells were preferentially expanded by stimulation at days 1, 8 and 15 post-transfection with irradiated PBMC, irradiated KMA expressing K562 cell line (aAPC) and IL-15. Phenotyping and functional characterisation of CAR T-cells were carried out at end of culture.



Second generation KMA CAR constructs used in the study with the various spacer regions- CH2CH3 (from IgG1) or mutant CH2CH3 (mutated at the Fcγ binding region) or from CD8α. Six constructs were analysed in detail in this study.

scFv from Kappa Mab (MDX-1097) was fused to CD28 or 41BB co-stimulatory domain and activating ζ subunit of CD3 to generate KMA CAR

RESULTS – KMA CAR T-cells expand in response to KMA

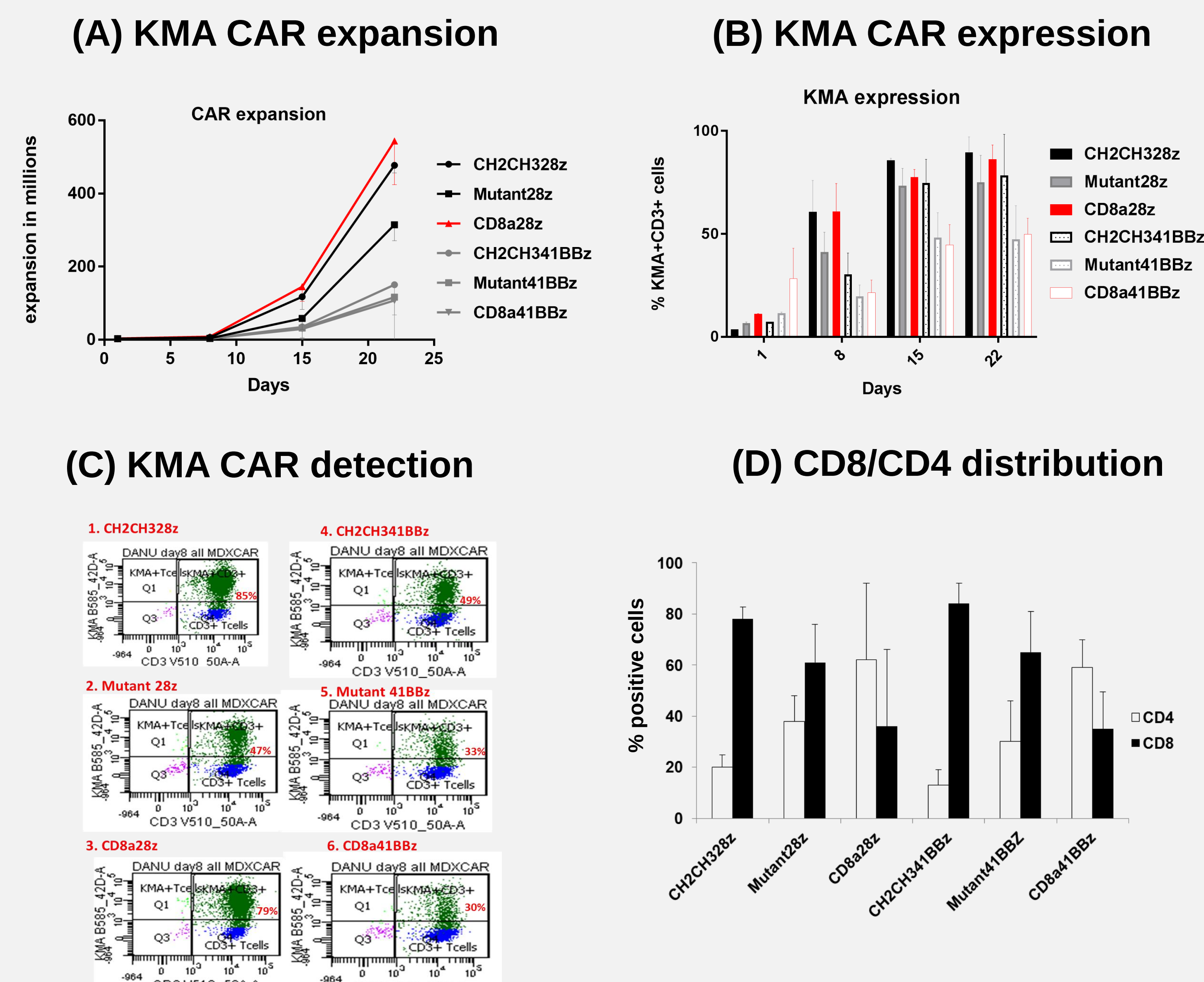


Fig1(A): Average expansion of CAR T-cells from 3 donor PBMCs over 3 weeks showed a range between 150 to 500 fold expansion upon exposure to KMA antigen (CD8α spacer containing CARs in red) (**B&C**): Expression of CAR was detected by staining with biotinylated KMA followed by Streptavidin-PE. % of CAR+ T-cells is plotted (n=3). Flow cytometry dot plots from a representative day 8 culture are shown (**D**) Average CD4/CD8 distribution within the final CAR T-cell culture showing a preponderance of CD8+ T-cells over CD4+ cells in a majority of cultures (n=3).

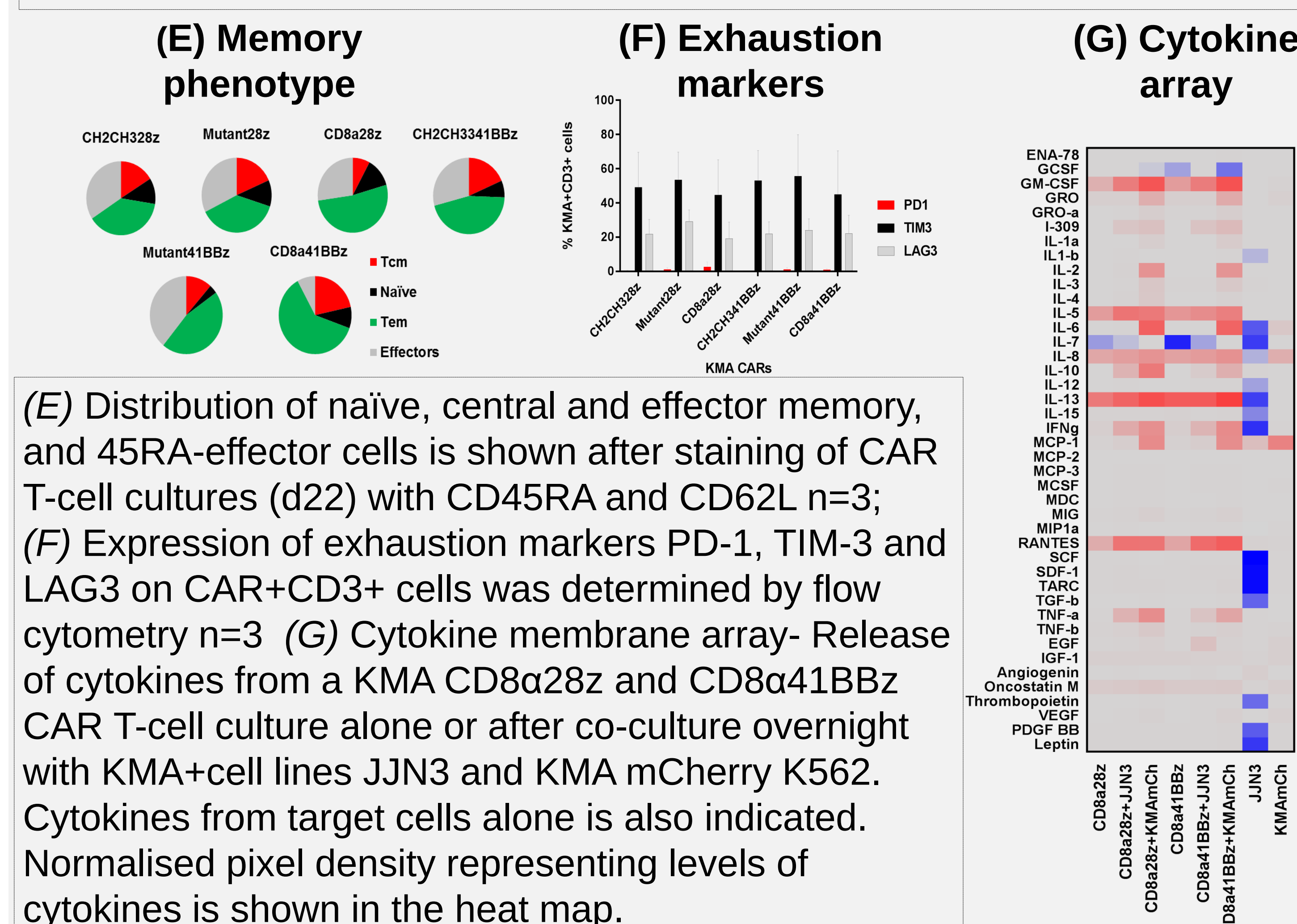


Fig2 (A) Average IFNγ and TNFα release from the CAR T-cells only upon co-culture with KMA+ cell lines JJN3 and Pfeiffer and not with KMA-ve CD19+ cell line Nalm6; n=3 (**B**) Cellular cytotoxicity as measured by lysis and ⁵¹Cr release from KMA+ target cell lines JJN3 and KMA mCherry K562 and not Nalm6 after 4h of co-culture from 3 donor cultures.

KMA CAR T-cells are specific in their functional response

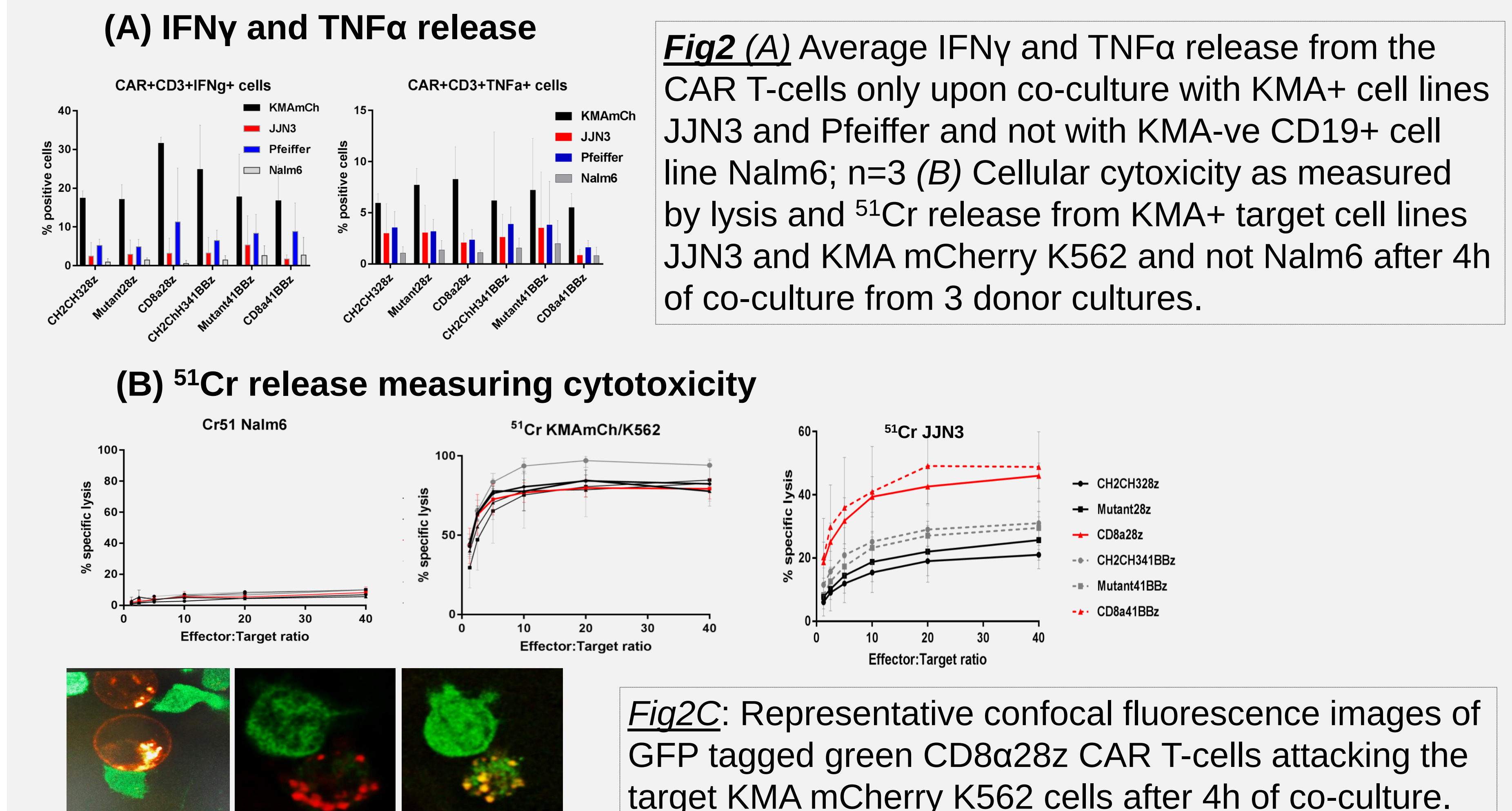
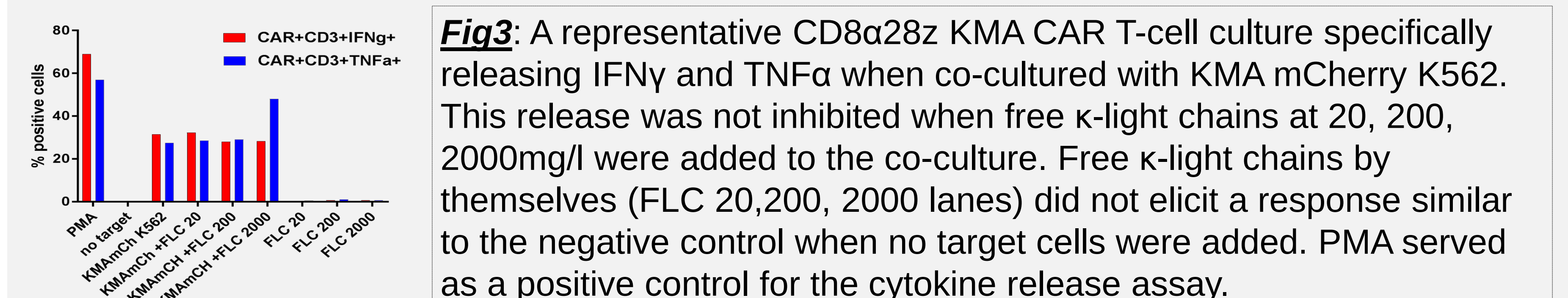


Fig2C: Representative confocal fluorescence images of GFP tagged green CD8α28z CAR T-cells attacking the target KMA mCherry K562 cells after 4h of co-culture.

CAR T-cell function is not inhibited by free κ-light chain



SUMMARY

- CAR T-cells targeting KMA are highly specific and effective**
- KMA CAR T-cells containing the CD8α spacer expanded the best *in vitro* and were the most efficacious against KMA+ cells**
- 28z co-stimulatory domain containing CAR T-cells expanded better *in vitro* than the 41BB co-stimulatory domain containing CAR T-cells**
- Approximately 30% of the CAR+T-cells were memory T-cells with negligible expression of PD-1 and expected to persist and function *in vivo*; However, expression of TIM-3 and LAG-3 was significant
- CAR T-cell function is not inhibited by the presence of free kappa light chains
- In vivo* testing in Rag2-/- mouse models and primary tumour cells currently underway**

SIGNIFICANCE

- Myeloma is the next haematological malignancy after leukaemia that is showing phenomenal response to CAR T-cell therapy.
- KMA is a highly specific antigen present on malignant cells and not on normal plasma cells or normal haematopoietic stem cells**, making it an ideal targetable antigen.