Optimal, 'Off-the-shelf', CAR-iNKT Cell Platform-based Immunotherapy for Multiple Myeloma

Kanagaraju Ponnusamy[1], *Lyra Randzavola*[2], *Hongwei Ren*[2], *Klesti Karaxhuku*[1], *Bryan Lye*[2], *Dimitrios Leonardos*[1,3], *Reza Nadafi*[4], *Irene Sarkar*[4], *Ilia Leontari*[1], *Farah Sabrin*[2], *Mehmood Zaidi*[2], *Marco Bua*[5], *Maria Atta*[1], *Alexia Katsarou*[1], *Irene Roberts*[6,7], *Aristeidis Chaidos*[1,5] *and Anastasios Karadimitris*[1,5]

[1]Hugh & Josseline Langmuir Centre for Myeloma Research Centre for Haematology, Department of Immunology and Inflammation, Imperial College London, London, United Kingdom. [2]Centre for Haematology, Department of Immunology and Inflammation, Imperial College London, London, United Kingdom.[3]University of Ioannina Medical School, Ioannina, Greece.[4]Lumicks, Amsterdam, Netherlands. [5]Department of Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom. [6]Department of Paediatrics, University of Oxford, Oxford, United Kingdom.[7]Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom.

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Imperial College London

Hugh and Josseline Langmuir CENTRE FOR MYELOMA RESEARCH

BACKGROUND

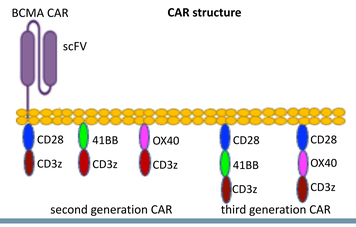
Multiple myeloma (MM) is an incurable cancer of plasma cells[1,2]. Autologous anti-BCMA chimeric antigen receptor (CAR)-T immunotherapy is a licensed treatment for MM.

The co-stimulatory molecule domain of a CAR structure is critical for its anti-cancer activity. Interestingly, both licensed anti-BCMA CAR-T products contain 4-1BB as the co-stimulatory domain of a 2nd generation CAR.

iNKT cells offer an alternative platform to conventional T cells for CAR-based immunotherapy[3,4,5].

iNKT cells are CD1d-restricted, glycolipid-reactive T cells characterised by an invariant TCRVa24-Ja18 chain nearly always pairing with a diverse TCRVβ11 chain[6]. Since iNKT cells do not cause acute graft-versus-host-disease (aGVHD) they can be used as an 'off-the-shelf' immunotherapy platform[7].

Here we compare and contrast co-stimulatory molecules in five different second (CD28z, 41BBz, OX40z) and third (CD28z-41BBz and CD28z-OX40z) generation CARs in the context of anti-BCMA CAR-iNKT immunotherapy for MM.



METHODS

iNKT cells were purified from healthy donor PBMCs followed by anti-CD3-CD28-mediated activation and transduction to express BCMA CAR. CAR levels were detected using either L-protein or sBCMA. Cells were expanded in the presence of IL-15 in R10 media and stimulated with C1R-CD1d cells pulsed with alpha-Galcer. Proliferation was assessed by Incucyte Zoom imaging and trypan blue-based cell counting.

In vitro cytotoxicity was performed by co-incubating iNKT cells and target cells with indicated effector : target ratios.

Avidity was measured by seeding iNKT cells on MM1.S cell monolayer followed by low-bound cells removal by acoustic force using the Lumicks instrument.

Whole transcriptome of iNKT cells was performed and analysed using standard approaches.

For *in vivo* assays, 7E6 Luc-dsRed-expressing MM1.S cells were injected intravenously (i.v) into 6-8 weeks old NSG mice followed by treatment with 1E6 BCMA CAR iNKT cells i.v on day 7, post-tumor cells injection. Tumor engraftment and burden levels were assessed by serial bioluminescence (BLI).

RESULTS

Fig 1a. iNKT cell selection & purity

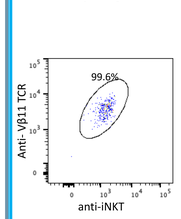


Fig 1b. Transduction efficiency of iNKT cells

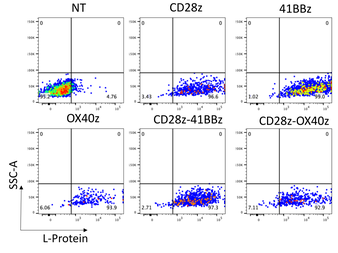


Fig 1c. BCMA and CD1d co-expresión on MM cells

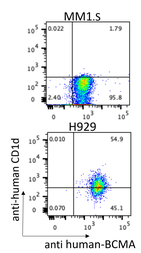


Fig 1d. Effect of co-stimulatory molecules on myeloma cell killing by BCMA CAR iNKT

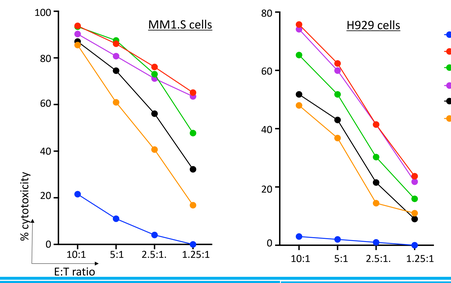


Fig 2. BCMA CAR iNKT eliminates MM/plasma cell leukemia cells

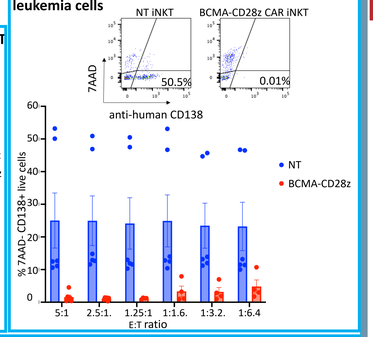


Fig 3a. Cell proliferation with IL-15

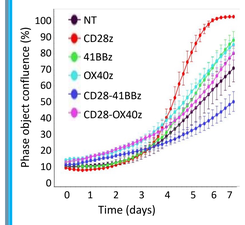


Fig 3b. Cell proliferation after alpha-Galcer stimulation

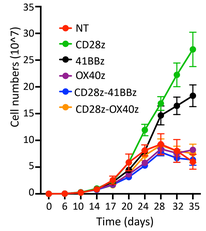


Fig 4. BCMA-CD28z CAR iNKT shows higher avidity under acoustic force

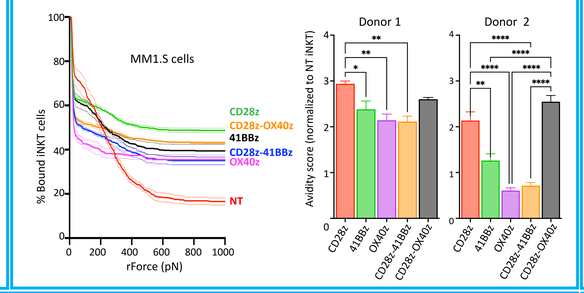


Fig 5a. BCMA-CD28z CAR iNKT cells controls myeloma disease efficiently in NSG mice

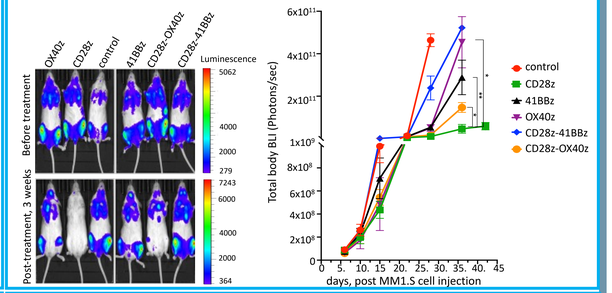


Fig 5b. BCMA-CD28z CAR iNKT prolongs survival

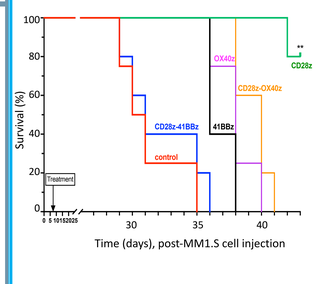


Fig 5c. MM1.S cells in bone marrow

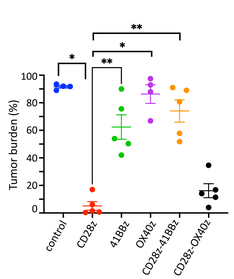


Fig 5d. iNKT cell frequency in BM of BCMA CAR iNKT-treated mice

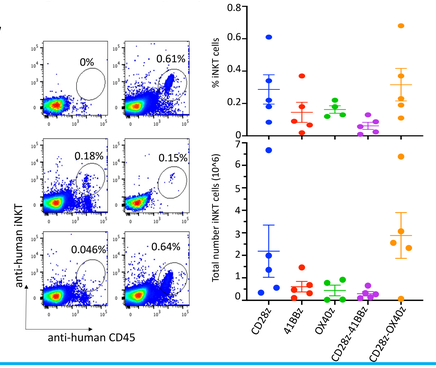
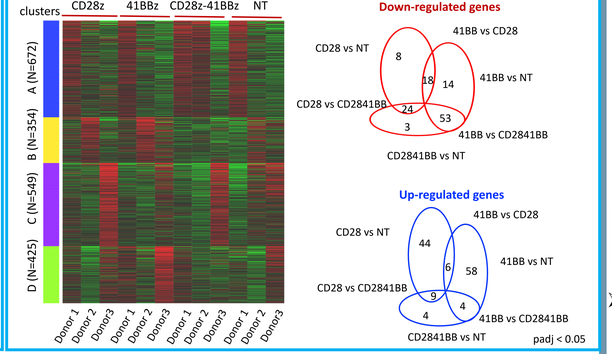


Fig 6. Differential gene regulation of BCMA CAR iNKT cells



SUMMARY

- Highly pure (>99%) iNKT cells were isolated from PBMCs
- >90% transduction were achieved for all BCMA CARs
- BCMA CARs with different co-stimulatory molecules vary in their *in vitro* cytotoxic activity against MM and show donor-dependency
- CD28z BCMA CAR induces the highest CAR iNKT cell proliferation and expansion *in vitro*
- BCMA-CD28z CAR is associated with highest avidity of CAR-iNKT cells
- Highest levels of *in vivo* expansion was observed for CD28z and CD28z-OX4Oz CAR-iNKT cells
- In line with avidity assays, BCMA-CD28z CAR-iNKT cells exert the highest anti-myeloma activity *in vivo*
- Comparative transcriptome analysis reveals only a small number of genes differentially expressed between different CARs
- Differentially expressed genes involved in cell adhesion and immunological synapse are currently under investigation.

CONCLUSIONS

- Proliferation and cell avidity but not cytotoxicity predict *in vivo* anti-myeloma activity of CAR-iNKT cells
- Unlike CAR-T cells, future clinical development of CAR-iNKT cells for MM would include CD28z as the preferred co-stimulatory domain

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CONTACT INFORMATION

k.ponnusamy@imperial.ac.uk a.karadimitris@imperial.ac.uk