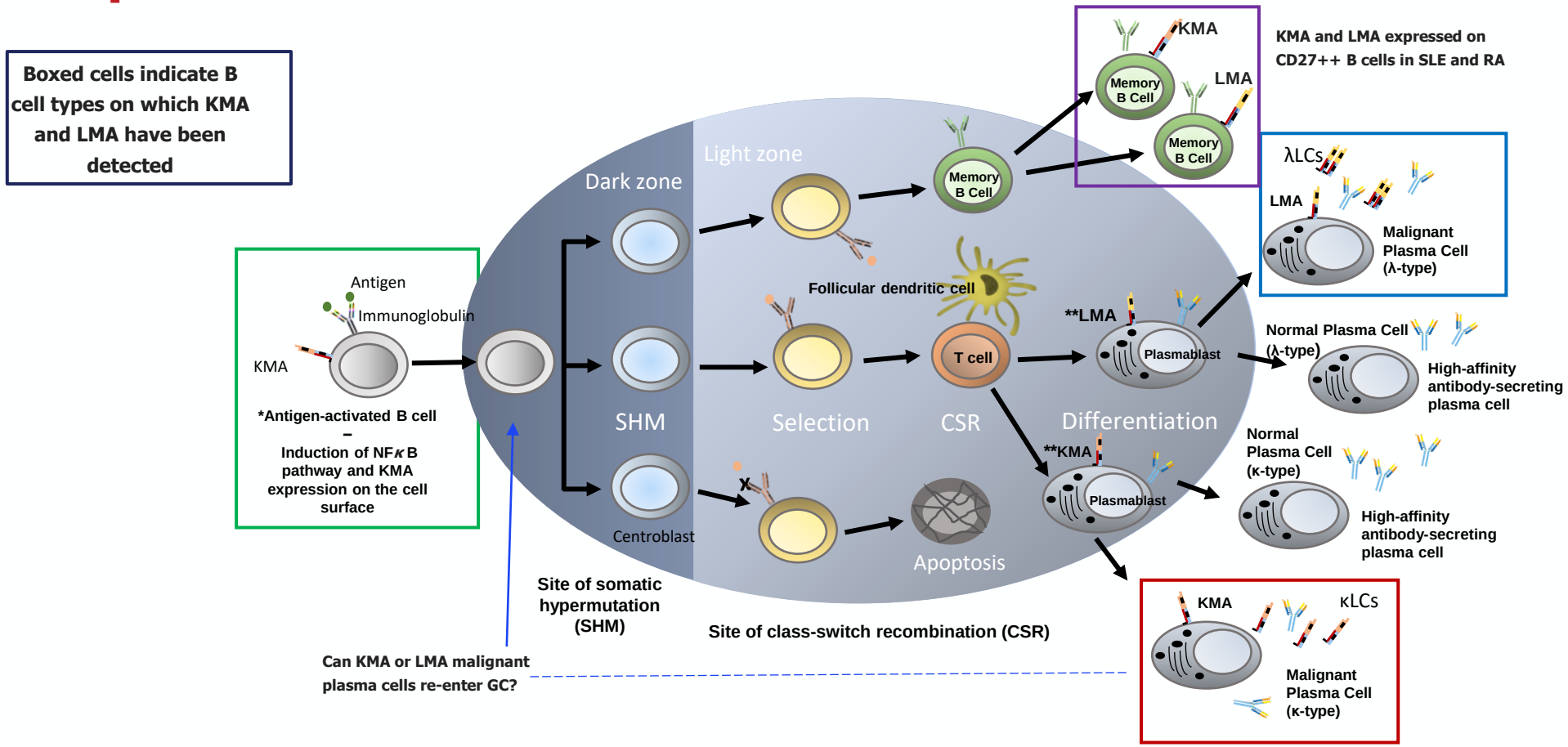
Specificity of KMA.CAR T cells against a novel B cell target called kappa myeloma antigen (KMA)

IMAPAC – Cell and Gene Therapy, World Asia 2023

HaemaLogiX

B cell development in the germinal centre: genesis of KMA and LMA expression



"The germinal centre (GC) of lymphoid organs is the main structure where antigen-activated B cells diversify their immunoglobulin genes by somatic hypermutation (SHM) to generate high-affinity antibodies" *(Klein and Dalla-Favera. Nat Rev Immunol. 2008; 8:22-23).*

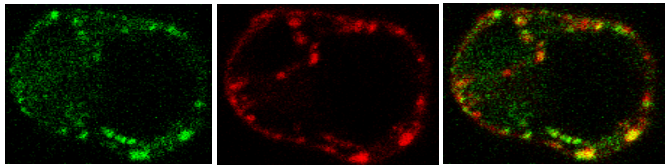
*NFkB binds to the enhancer element of the kappa light chain gene and initiates expression and *Vκ* to *Jκ* recombination *(Schlissel and Baltimore (1989) Cell)*

**KMA and LMA expressed during CSR and T cell induced selection and differentiation

KMA: a novel antigen on the surface of kappa restricted myeloma cells

- **KappaMab (formerly MDX-1097) binds specifically to KMA a cell surface antigen found on:**
  - Kappa restricted myeloma cells and cell lines, other malignant B cells,
  - SLE and RA peripheral blood B cells,
  - a small population of plasmablasts in normal tonsillar, salivary gland and secondary lymphoid tissues[1-6]
- **KMA** is **not** detected on normal B cells, lambda myeloma cells or other immune cells and KappaMab does not bind to intact Igk[1-6]
- **KMA** is expressed on plasma cells at all stages of myeloma disease from the premalignant stage (MGUS) through to relapsed refractory MM and on bone marrow plasma cells in plasmacytomas and amyloidosis[7,8]
- **The range of KMA antigen density is greater than BCMA on myeloma cells and they are not always co-expressed [7,8]**

KMA is associated with lipids in the cell membrane of myeloma cells[1]

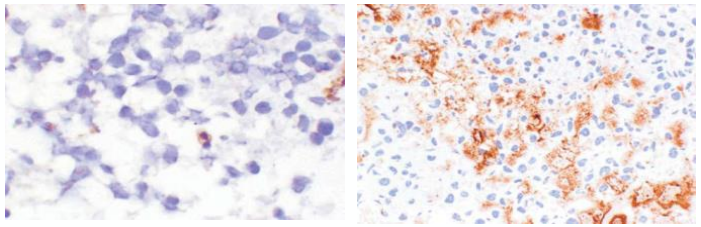


KMA is expressed on the myeloma cell membrane

Anti-CD59 is associated with lipid rafts

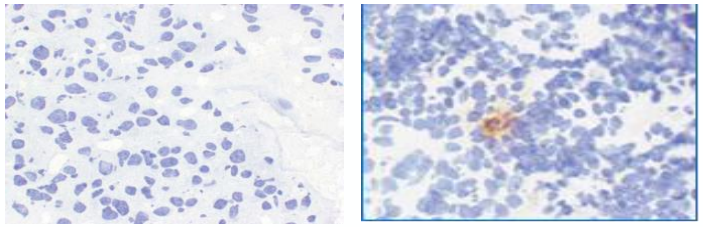
Merged images show co-localisation of KMA and CD59 in lipid rafts

KappaMab only binds to malignant plasma cells in the bone marrow from kappa light chain restricted multiple myeloma[5]



Normal Bone Marrow

Kappa myeloma bone marrow



Lambda myeloma bone marrow

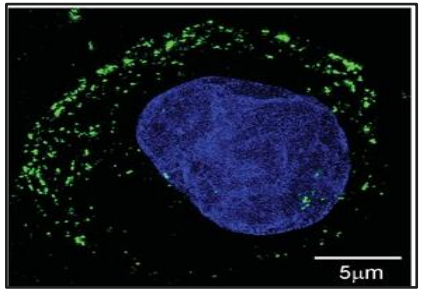
Occasional mononuclear cells in salivary gland

[1]Asvadi et al. (2015) *BJH*; [2]Boux et al. (1983) *J Exp Med*; [3]Walker et al 1985 *Plenum Pub Corp*; [4]Goodnow and Raison (1985) *J Immunol*; [5]Charles River Laboratories, Pathology Associates (PAI), Maryland, USA). [6]Raison and Boux 1985 *Mol Immunol*; [7]Sartor et al. (2021) *Blood*,138, S1:1595; [8]Sartor et al. (2022) *Blood*, 140, S1:4211-4212

LMA: a novel antigen on the surface of lambda restricted myeloma cells

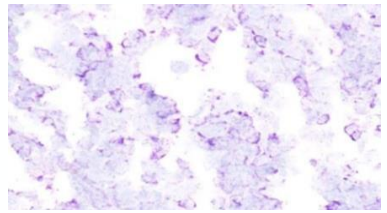
- **LambdaMabs (10B3 and 7F11) are specific to LMA which is expressed on:**
  - Lambda restricted myeloma and amyloidosis plasma cells,
  - SLE and RA peripheral blood B cells,
  - a small population of plasmablasts in normal tonsillar, salivary gland and mucosal secondary lymphoid tissues[1-4].
- **LMA** is **not** detected on normal B cells, kappa myeloma cells or other immune cells and LambdaMabs do not bind to intact immunoglobulin, Igλ[2]
- **LMA** is expressed on malignant plasma cells at all stages of myeloma disease (MGUS through relapsed refractory MM) and on bone marrow plasma cells in amyloidosis and plasmacytomas[1,3]
- **The range of LMA antigen density is greater than BCMA on myeloma cells and they are not always co-expressed [1,3]**

LMA is associated with lipids in the cell membrane

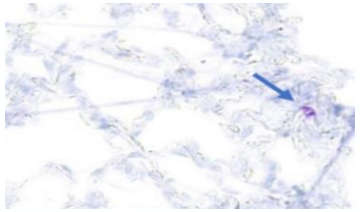


3D-structured illumination microscopy image of a lambda myeloma cell stained with DAPI (blue) in the nucleus and LMab Alexa Fluor® 488 (green) on the cell membrane

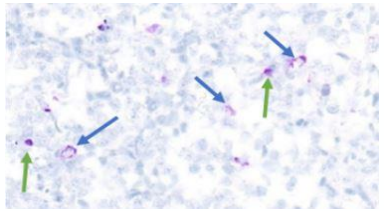
LambdaMabs stain myeloma plasma cells and occasional mononuclear cells in secondary lymphoid tissue[3]



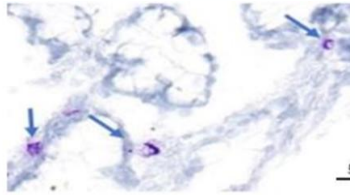
Lambda myeloma in the lung (plasmacytoma)



Mononuclear cells in normal lung



Mononuclear cells in tonsil



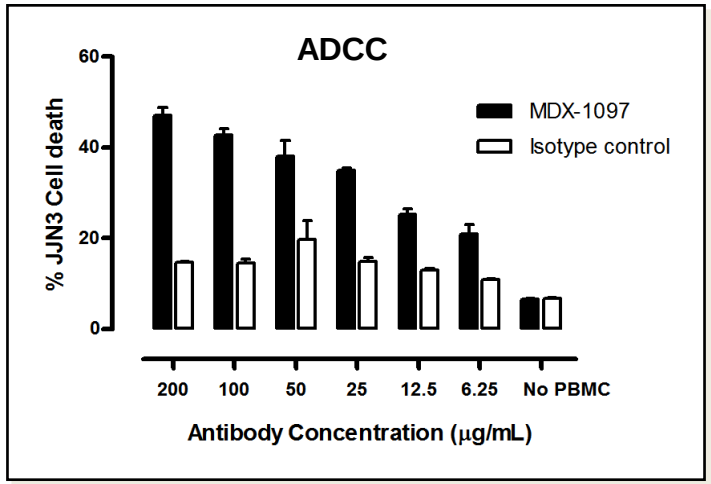
Mononuclear cells in colon

[1]Sartor et al. (2021) *Blood*,138, S1:1595 : [2]Asvadi et al (2013) *Haematologica*;98(s1); P756: [3] TPL Path Labs GmbH Sasbacher Str. 10 D-79111, Freiburg, Germany
[4]Sartor et al. (2022) *Blood*, 140, S1:4211-4212

HaemaLogiX

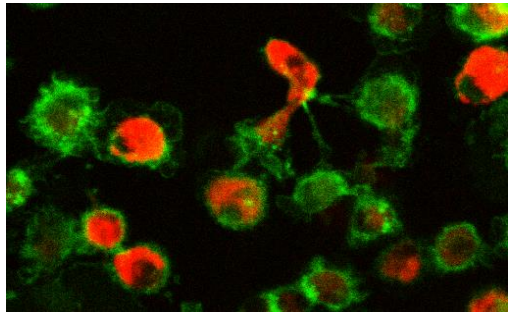
KappaMab: Mechanisms of action

- **IMiDs increase KMA or LMA expression** on myeloma cells and increase KappaMab antibody dependent cellular cytotoxicity (ADCC)[1,2]
- KappaMab also induces antibody dependent cellular phagocytosis (ADCP) in myeloma cells[3]
- KMA is not internalised upon antibody binding[4]
- In a phase I clinical trial, KappaMab **decreased** Interferon-γ induced CXCR3 binding ligands CXCL9 and CXCL10 that are associated with leukocyte trafficking[5]
  - **Increased** CXCL9 and CXCL10 are involved in aberrant trafficking and fate of immune effector cells in myeloma
  - **Increased** serum levels associated with poor overall survival in myeloma[6-9]



ADCP

Macrophages (green) engulf the myeloma cells coated with KappaMab (red) and destroy them

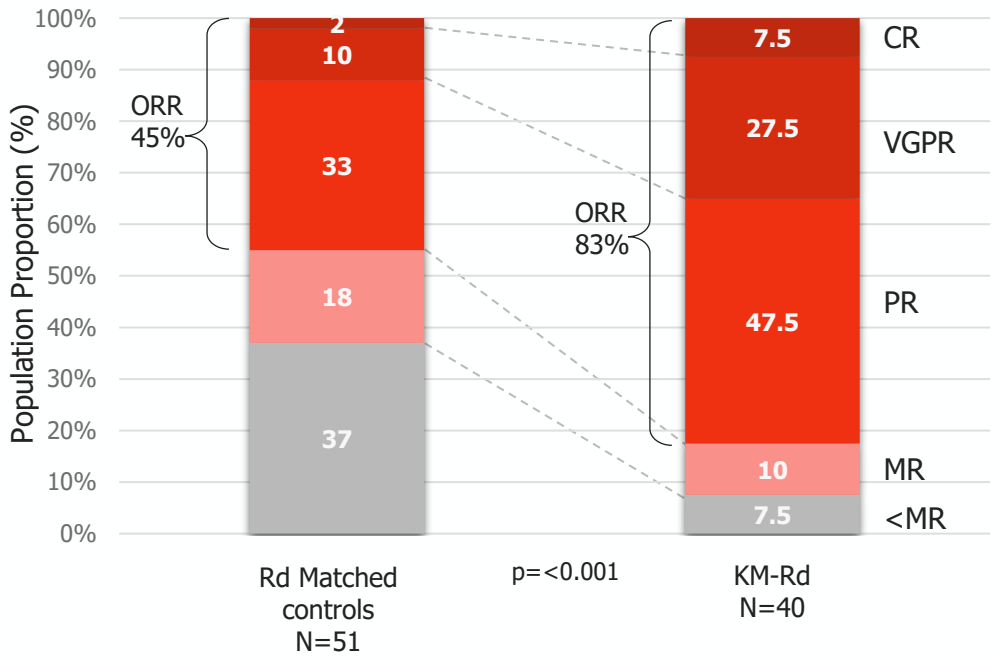


[1]Asvadi et al. (2015) *BJH*; [2]Cuddihy et al. (2012), *Blood* 120(21): 4012 : [3]Wong et al. (2009) *Blood*, 114(22): 1846; [4]Boux et al. (1984) *Eur J Immunol* 14:216-222; [5] Spencer et al. (2019) *BCJ*; [6]Feyler et al.(2009) *BJH*; [7]Giulianai et al. (2006) *Haematologica*; [8]Ponzetta et al. (2015) *Cancer Res*; [9]Bolomsky et al. (2016) *Leukemia and Lymphoma*

Lead asset KappaMab - Phase IIb results

KappaMab (10mg/kg) boosts efficacy of Revlimid and dexamethasone (Rd)

- Patients had **relapsed, refractory** myeloma and disease was progressing
- KappaMab **improved the depth of response**, **increased Overall Response Rate (ORR)** compared to the matched Case Control patient group from the Australian patient registry
- The median Overall Survival has not been reached as 2 patients remain on therapy
- There were **no haematological toxicities associated with KappaMab** and the safety profile was similar to that of len/dex in the literature



Patients were resistant/refractory (1-3 lines)

Failed an IMiD (~50%),

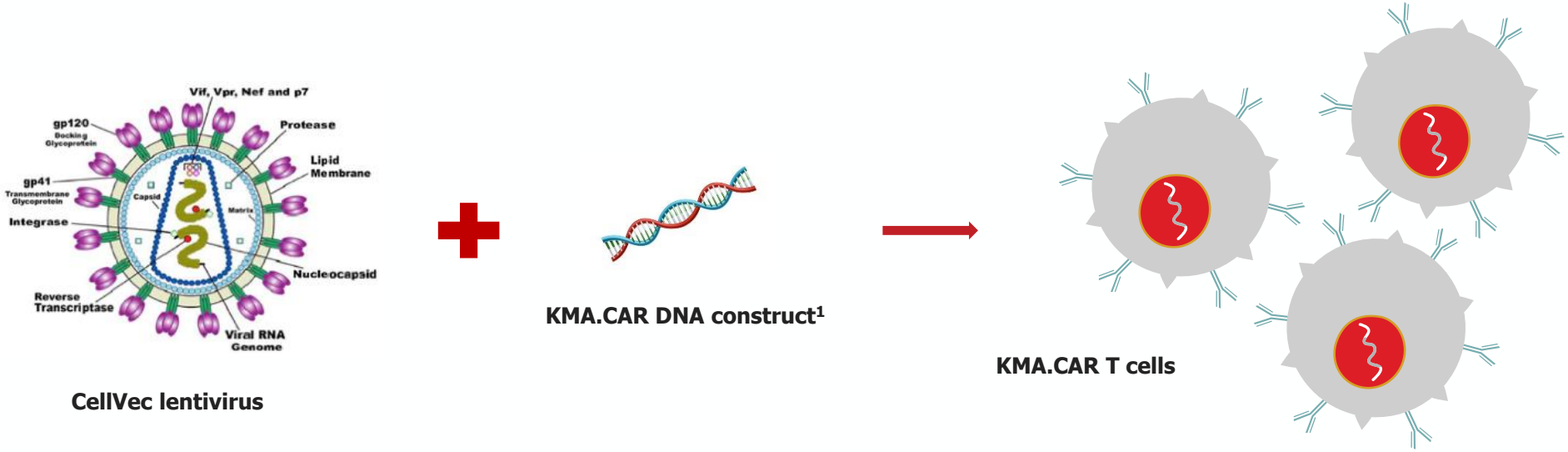
Failed PI (~90%)

Failed an autologous stem cell transplant (~50%)

Spencer, A, et al. Br J Haematol. 2023 Aug;2023(4):801-811

KMA.CAR T cell optimisation

Schematic of lentivirus – DNA construct transduction – KMA.CAR T cells

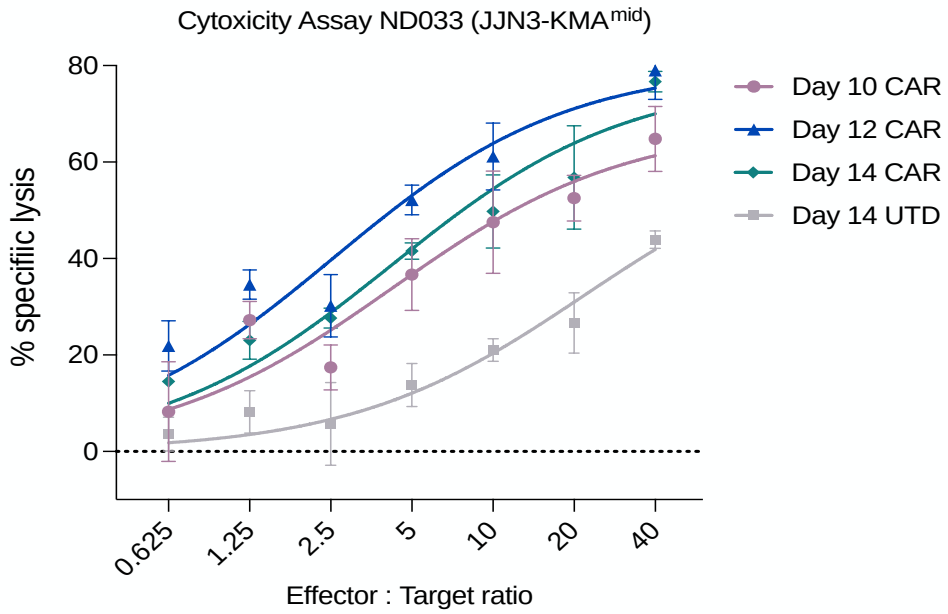


- **CellVec** optimised the EF1 alpha promoter
- Demonstrated efficient CAR expression upon vector genome integration
- **KMA.CAR T cell preclinical and *in vivo* studies were conducted by the Centre of Excellence in Cellular Immunotherapy at Peter Mac (CoE_CI) in collaboration with HaemaLogiX**

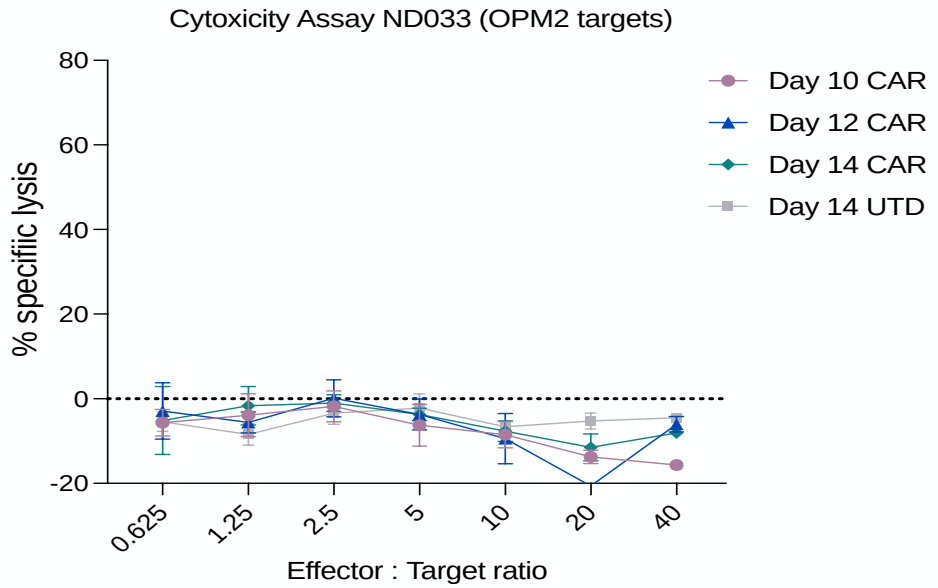
[1]https://www.freepik.com/free-vector/realistic-vector-icon-dna-medical-concept-element_35188118.htm">Image by user15245033 on Freepik

Functional expression and specificity of the KMA.CAR was confirmed by *in vitro* cytotoxicity experiments

KMA positive JJN3 - kappa myeloma cell line



KMA negative OPM2 - lambda myeloma cell line



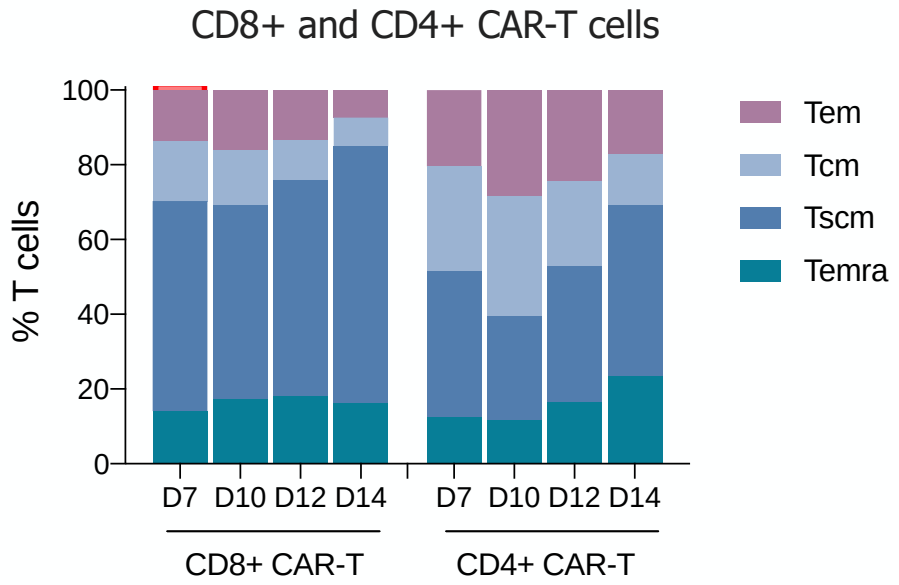
UTD=untransduced

Cytotoxicity of anti-KMA CAR-T cells assessed by Calcein-release assay over 6-hour co-culture with KMA+ JJN3 cells or KMA- OPM2 cells at indicated effector:target ratio

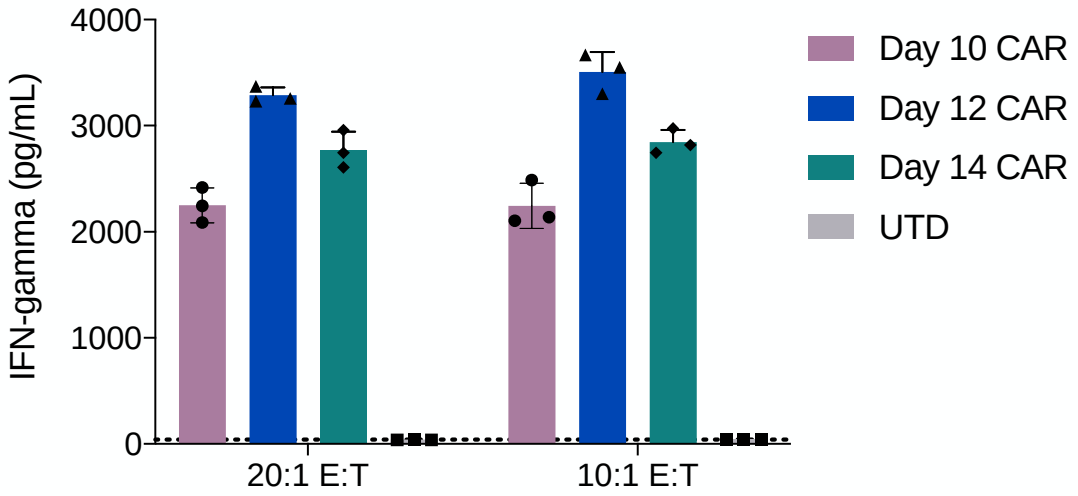
Li J et al. *Cancer Res*. 2023; 83 (7_Supplement): 4074 (CoE_CI)

Anti-KMA CAR-T cells display a predominant Tscm phenotype

Phenotype of anti-KMA CAR-T cells assessed by flow cytometry over 14 days in culture



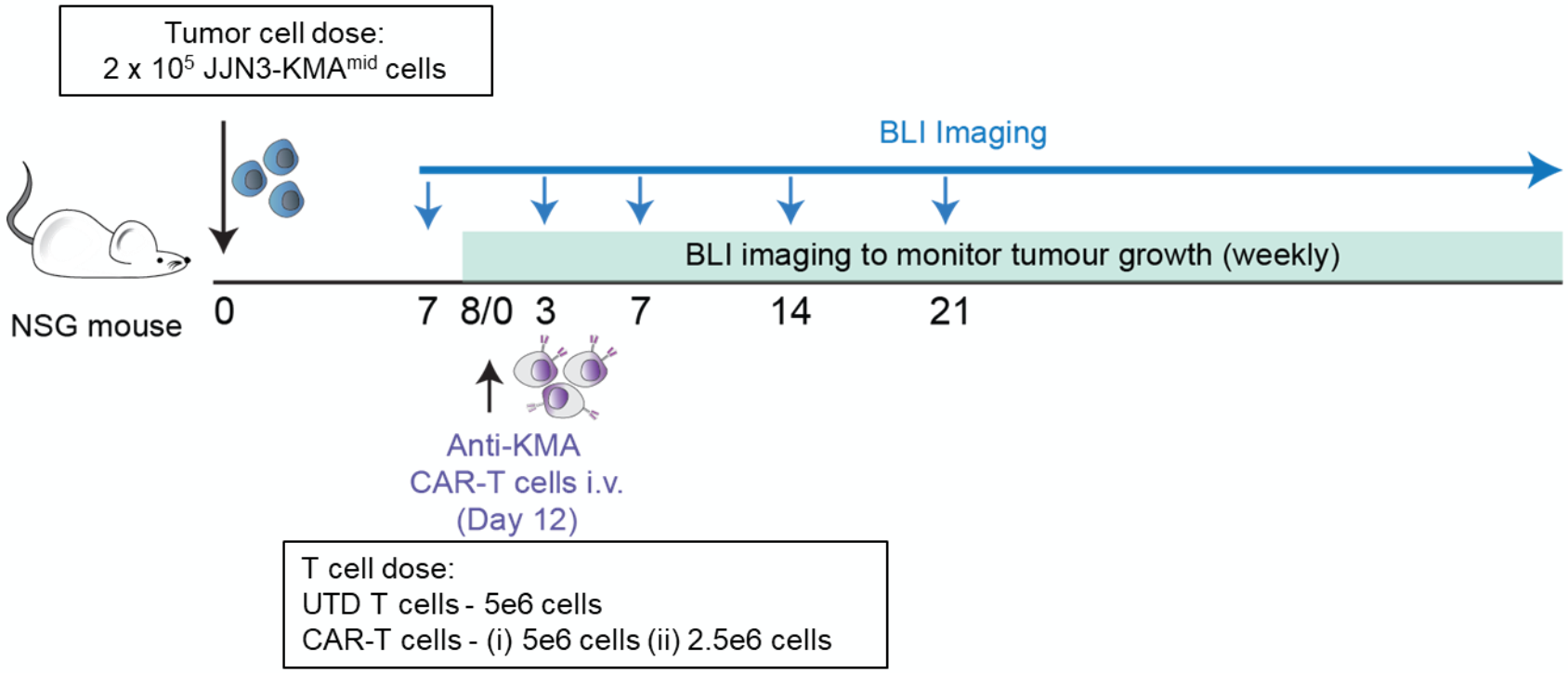
Interferon-gamma release by anti-KMA CAR-T cells assessed by cytokine bead array over 6-hour co-culture with KMA+ JJN3 cells at indicated effector:target ratio (n=3)



Tem – Effector memory T cells
Tcm – Central memory T cells
Tscm – Stem memory T cells
Temra – Effector memory RA$^+$ T cells

Li J et al. *Cancer Res*. 2023; 83 (7_Supplement): 4074 (CoE_CI)

In Vivo KMA.CAR T cell therapy – study design

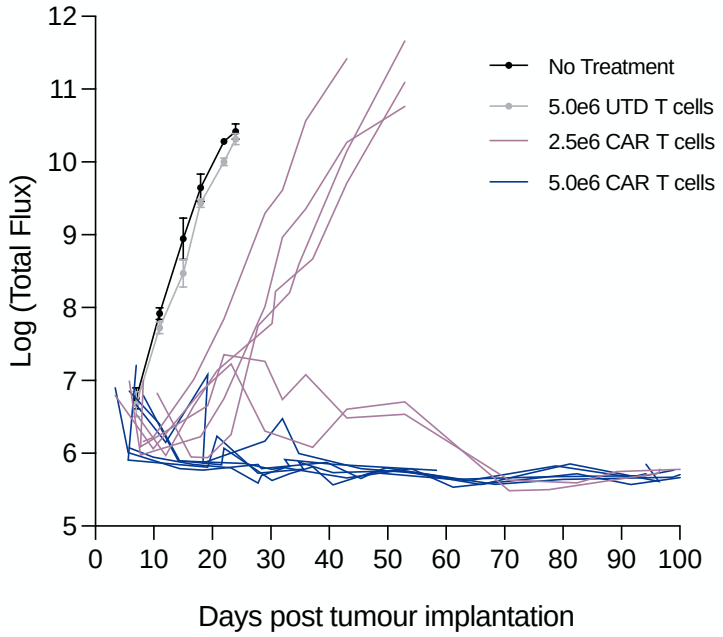


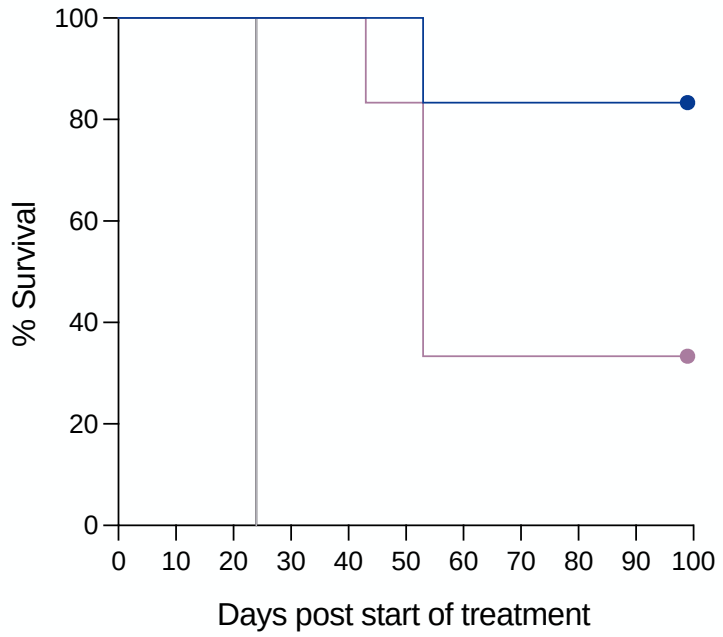
Schematic of treatment schedule for in vivo testing of anti-KMA-CAR-T cells

Li J et al. *Cancer Res*. 2023; 83 (7_Supplement): 4074 (CoE_CI)

In Vivo KMA.CAR T cell therapy - animal survival

The experiment went to Day 110 with no further deaths in the cohort given 5.0e6 CAR T cells

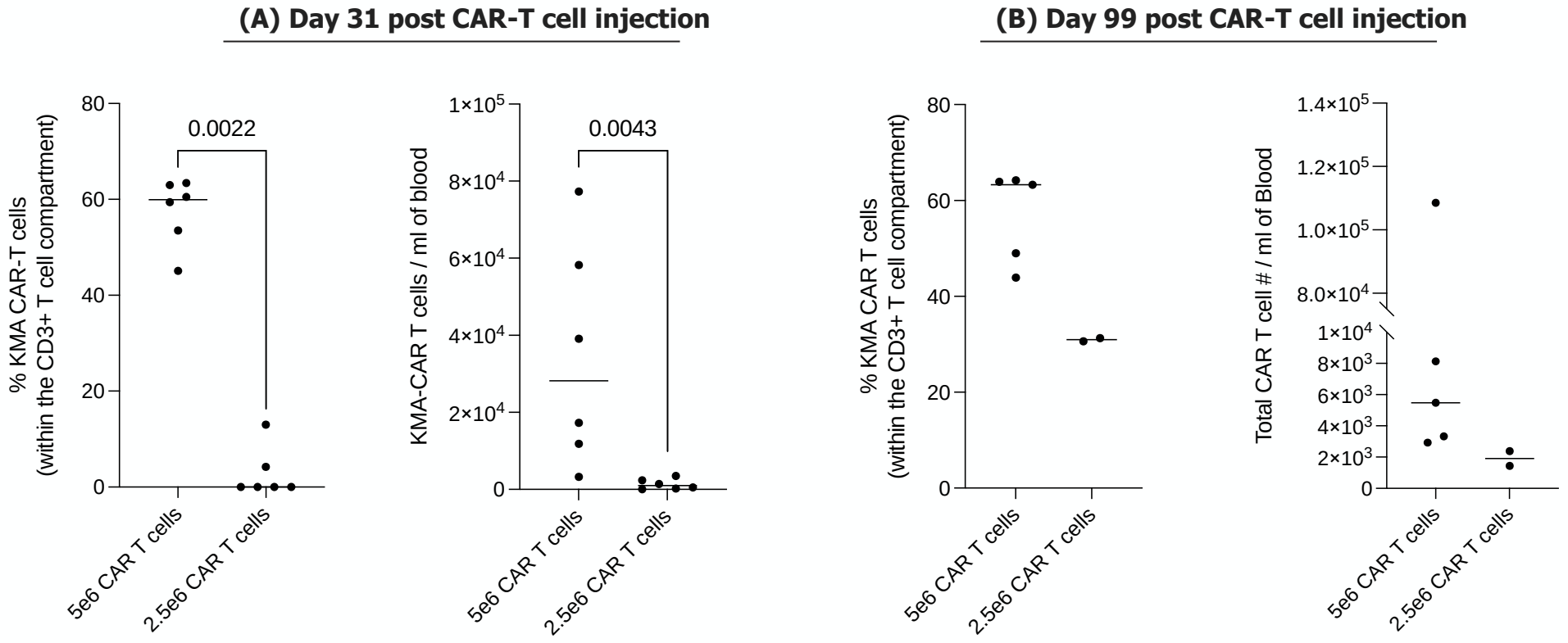




- This study demonstrated that the KMA-CAR can evoke potent, long-term antigen specific anti-tumour responses *in vivo*

Li J et al. *Cancer Res*. 2023; 83 (7_Supplement): 4074 (CoE_CI)

In Vivo Therapy – KMA.CAR T Cell persistence

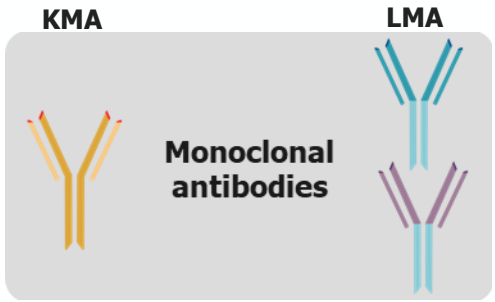


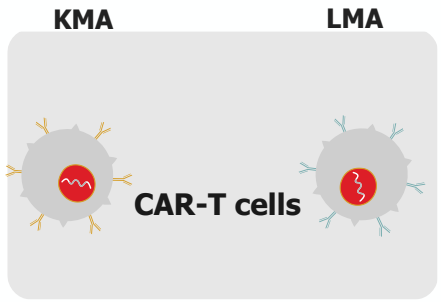
Analysis of KMA-CAR-T cell persistence in the peripheral blood of treated mice at days 31 (A) and 99 (B) post T cell injection

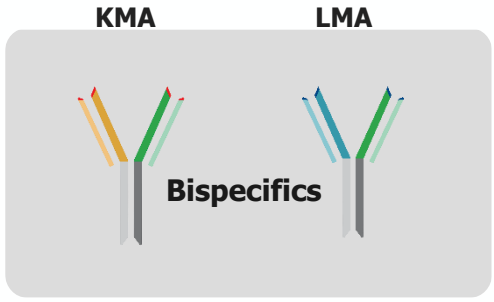
Li J et al. *Cancer Res*. 2023; 83 (7_Supplement): 4074 (CoE_CI)

HaemaLogiX Ltd immunotherapy assets

- The completed preclinical studies demonstrated that the KMA-CAR T cell can evoke potent, long-term antigen specific and anti-tumour responses *in vivo*
- A phase I KMA.CAR T cell in myeloma patients with RRMM has been initiated with the Centre of Excellence in Cellular Immunotherapy at Peter Mac in collaboration with HaemaLogiX
- HLX future development includes LMA.CAR T cells and KMA and LMA bispecifics







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