Specificity of KMA.CAR T cells against a novel B cell target called kappa myeloma antigen (KMA)
B cell development in the germinal centre: genesis of KMA and LMA expression

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

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

“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).

Can KMA or LMA malignant plasma cells re-enter GC?
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 \(^7\),\(^8\)

---

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\textsuperscript{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, Ig\textsubscript{\lambda}\textsuperscript{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\textsuperscript{1,3}.

- The range of LMA antigen density is greater than BCMA on myeloma cells and they are not always co-expressed \textsuperscript{1,3}.

\textsuperscript{1}Sartor et al. (2021) Blood, 138, S1:1595; \textsuperscript{2}Asvadi et al (2013) Haematologica;98(s1); P756: \textsuperscript{3} TPL Path Labs GmbH Sasbacher Str. 10 D-79111, Freiburg, Germany
\textsuperscript{4}Sartor et al. (2022) Blood, 140, S1:4211-4212
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-\(\gamma\) 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\(^6-9\)

---

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%)

**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. 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

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)

CD8+ and CD4+ CAR-T cells

<table>
<thead>
<tr>
<th>% T cells</th>
<th>D7</th>
<th>D10</th>
<th>D12</th>
<th>D14</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8+ CAR-T</td>
<td>100</td>
<td>80</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>CD4+ CAR-T</td>
<td>100</td>
<td>80</td>
<td>60</td>
<td>40</td>
</tr>
</tbody>
</table>

Tem – Effector memory T cells
Tcm – Central memory T cells
Tscm – Stem memory T cells
Temra – Effector memory RA1 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

(A) Day 31 post CAR-T cell injection

(B) Day 99 post CAR-T cell injection

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 \textit{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.
Acknowledgments

Centre of Excellence in Cellular Immunotherapy,

**Peter Mac**
Professor Simon Harrison
Associate Professor Jane Oliaro
Dr Jessica Li
Dr Nicole Haynes
Dr Katherine Cummins

**CellVec**
Dr Lucas Chan
Dr Fabio Michelet

**Westmead Institute of Medical Research (WIMR)**
Dr Ken Micklethwaite
Dr Kavitha Gowrishankar