PET Imaging of Cytotoxic Human T Cells Using an ^{89}Zr-Labeled Anti-CD8 Minibody

Tove Olafsen, Michael Torgov, Green Zhang, Jason Romero, Charles Zamilpa, Filippo Marchioni, Karen Jiang, Jean Gudas and Daulet Satpayev

ImaginAb Inc., Inglewood, CA

Introduction

Major challenges to advancing the new wave of cancer immunotherapies are selecting patients who will respond to single immunotherapies, identifying those who need more tailored combination regimens, and then, determining early during treatment whether the therapy is effective. "ImmunoPET" imaging of tumor infiltrating T cells with radiolabeled antibody fragments can provide a specific and sensitive modality to achieve this goal. For this purpose we have developed ^{89}Zr-Df-IAB22M2C, an anti-CD8 minibody (Mb) conjugated with desferrioxamine (Df) and radiolabeled with Zirconium-89 (^{89}Zr) for imaging human CD8+ T cells in humans.

IAB22M2C is a Mb comprised of humanized VL and VH sequences from a murine anti-human CD8α antibody assembled into scFv and fused to the human IgG1 CH3 domain via a proprietary hinge sequence H (**Figure 1**). IAB22FL is a respective full length human IgG1 comprised of the same VL and VH sequences. IAB22M2C minibody was conjugated with Df on lysine residues to generate the respective conjugated Mb, Df-IAB22M2C (**Figure 1**).

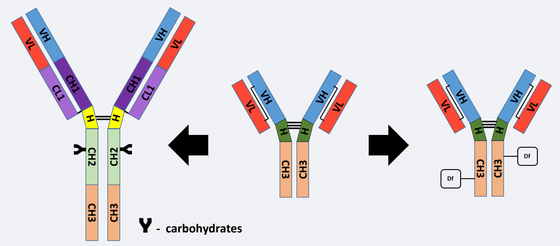

Figure 1. Structure of IAB22M2C Mb (center), respective Df- conjugate (right) and full length hIgG1 (left)

IAB22FL – full length huIgG1 (~150 kDa) | IAB22M2C Minibody (~80 kDa) | Df-IAB22M2C Conjugated Mb ~(80 kDa)

Objectives

- Engineer a Mb fragment with high affinity to huCD8α (IAB22M2C) and characterize respective conjugated forms *in vitro* and *in vivo*
- Demonstrate feasibility of CD8+ T-cell imaging in graft vs. host disease model (GVHD) in NSG mice
  - Use the probe to visualize CD8+ T-cells in spleen and other organs
  - Determine biodistribution and preliminary PK parameters

Results

Binding of IAB22M2C and Df-IAB22M2C. Humanization and engineering of the original murine antibody's VH and VL sequences into a Mb format yielded the high affinity Mb, IAB22M2C (**Figure 2A**). Conjugation of Df- chelator molecule to IAB22M2C at CMR=2 does not affect binding of the conjugate as shown in **Figure 2B.**

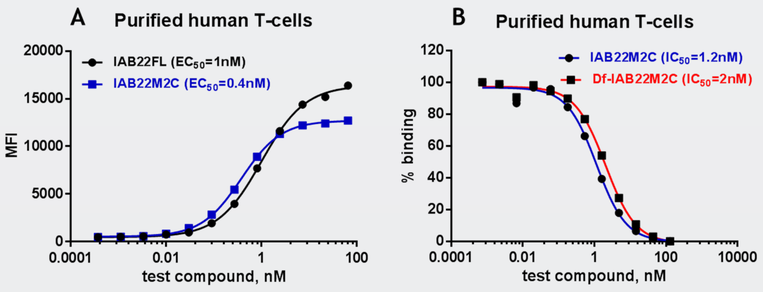

Figure 2. Both IAB22M2C and IAB22FL retain high affinity to CD8α expressed on human T cells (A). Conjugation to Df does not impact binding to T-cells (B).

***In vitro* T-cell assays**. Df-IAB22M2C conjugate was tested in a panel of assays to ascertain that its binding to CD8α did not result in T-cell activation. Df-IAB22M2C was tested for CD69 upregulation (Figure 3), proliferation (Figure 4), and cytokine release (Figure 5).

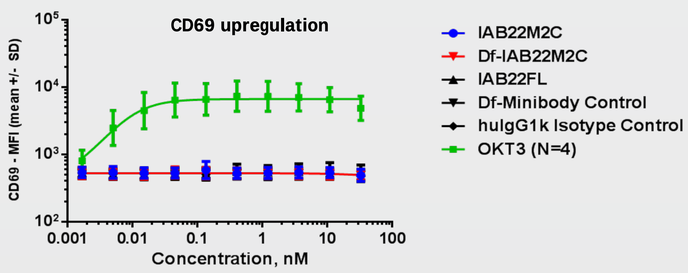

Figure 3. CD69 expression upregulation in PBMCs (T-cell gate) in 18hr assay

Proliferation assay: PBMC culture

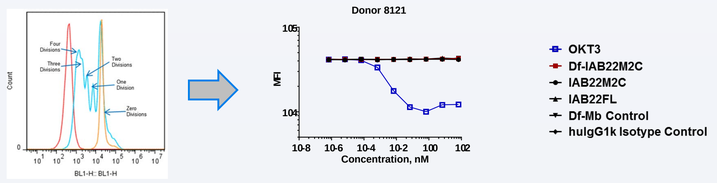

Figure 4. CFSE labeled PBMCs (single donor) do not show a proliferative response in a 4-day assay. OKT3 antibody elicited robust response (left panel). Cellular MFI reduction indicative of cell divisions is plotted against concentration (right panel). No MFI reduction observed for Df-IAB22M2C. Total of 5 donors analyzed with similar results.

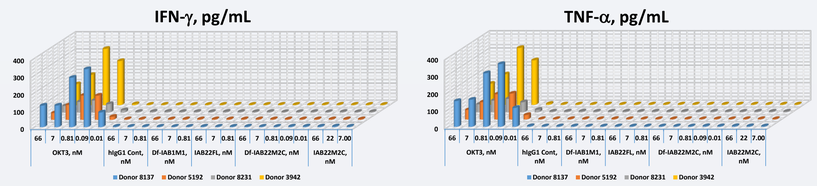

Figure 5. Cytokine release from PBMCs (4 healthy donors, 18 hrs). Supernatants were analyzed using LegendPlex® bead assay (IL-2, IL-6, IL-10, IFN-γ and TNF-α). OKT3 stimulated robust release of all 5 cytokines (IFN-γ and TNF-α are shown). Df-IAB22M2C and all controls did not show any detectable elevation of any of cytokines in supernatants.

In Vivo evaluation of Mb fragments in Hu-CD34$^+$ NSG™ mouse model. Newborn NSG mice engrafted with human CD34+ progenitor cells at birth were used to evaluate effect of Df-IAB22M2C, IAB22M2C and IAB22FL on circulating and splenic T cells (**Figure 6**).

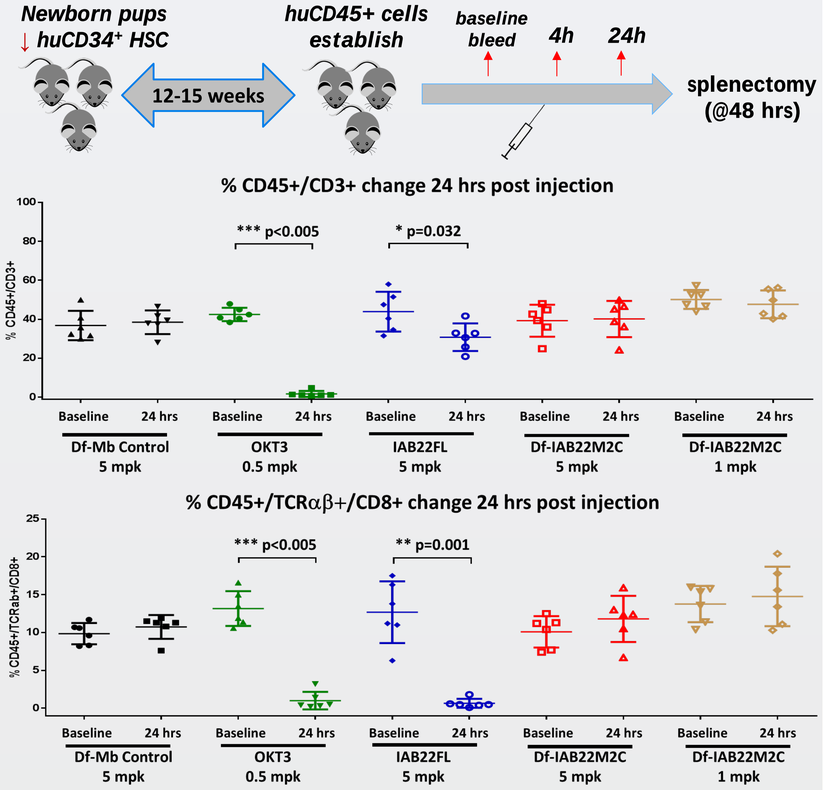

Figure 6. Enumeration of CD45/CD3+ (upper panel) and CD45+/CD8+ (lower panel) at baseline and 24 hrs post administration of test compounds at indicated doses.

OKT3 depleted all CD3+ cells in 24 hrs. IAB22FL selectively depleted CD8+ T-cells sparing the CD4+ population. Conjugated Df-IAB22M2C minibody did not modulate circulating or splenic (data not shown) CD8+ T-cell. In contrast, OKT3 induced cytokine release (IL-2, IL-6, IL-10, IFN-γ, TNF-α) that was detectable at 4 and 24 hrs. None of the other tested compounds resulted in cytokine release in Hu-CD34$^+$ NSG™ mice (data not shown).

CONCLUSIONS

- Both IAB22M2C and the respective conjugate, Df-IAB22M2C, bind to human CD8α with high affinity
- Df-IAB22M2C appeared to be "inert" with respect to CD8+ T-cell activation and depletion *in vitro* and in "humanized" Hu-CD34$^+$ NSG™ mice
- ^{89}Zr-Df-IAB22M2C detects engrafted human CD8+ T-cells in NSG-mice and can be used to monitor dynamic changes in the course of GVHD
- ^{89}Zr-Df-IAB22M2C properties justify further exploration of its utility in the clinic to visualize CD8+ T-cells in humans

PET imaging, biodistribution and PK studies.

Df-IAB22M2C was radiolabeled with ^{89}Zr to specific activities ranging 70-100 µCi/µg, and used to visualize CD8+ T-cells in NSG mice engrafted with human PBMCs (**Figure 7**)

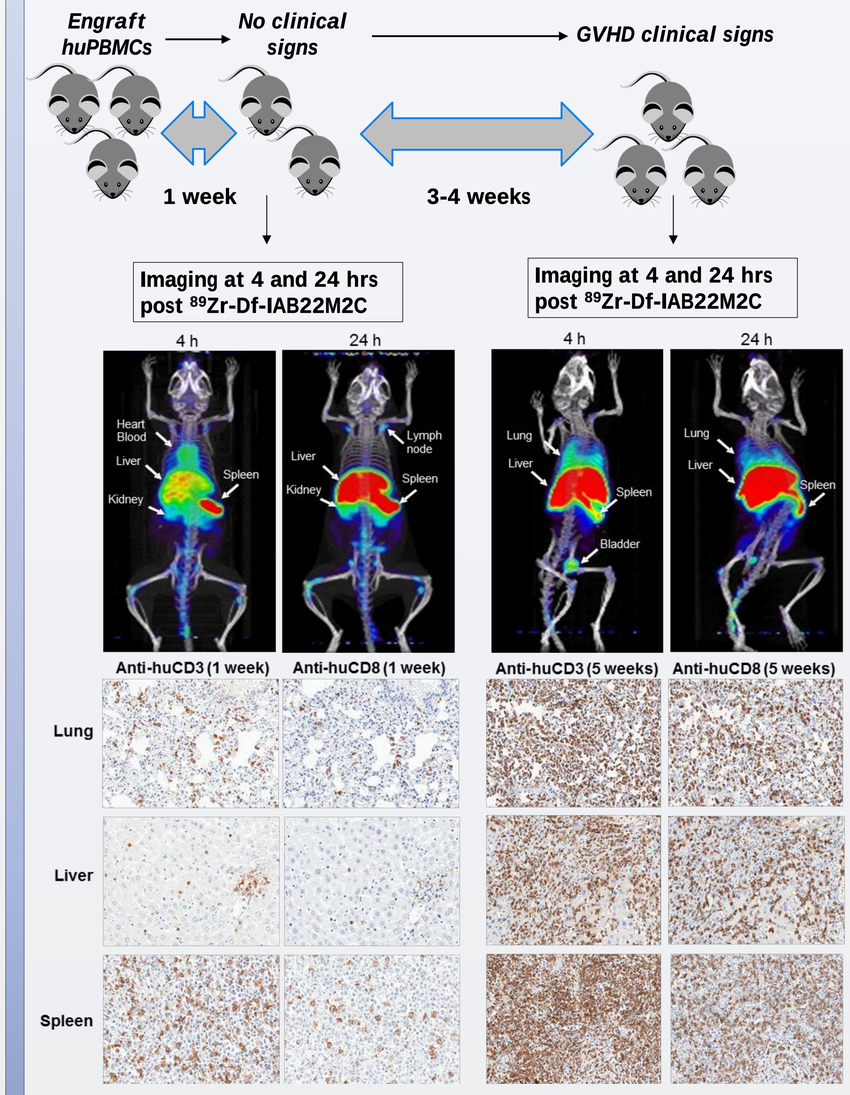

Figure 7. Visualization of infiltrating T-cells in the graft vs host disease model at different stages of disease (upper panel). Lung, liver and spleens of representative animals were formalin fixed and stained for hCD3 and hCD8 to identify T-cell subpopulations (lower panel)

Biodistribution (24 hrs) of ^{89}Zr-Df-IAB22M2C in animals at 1 week and 5 weeks post huPBMCs engraftment confirmed findings from imaging and IHC. Whole blood PK profile is favorable and supports same-day imaging feasibility in the clinical setting (**Figure 8**).

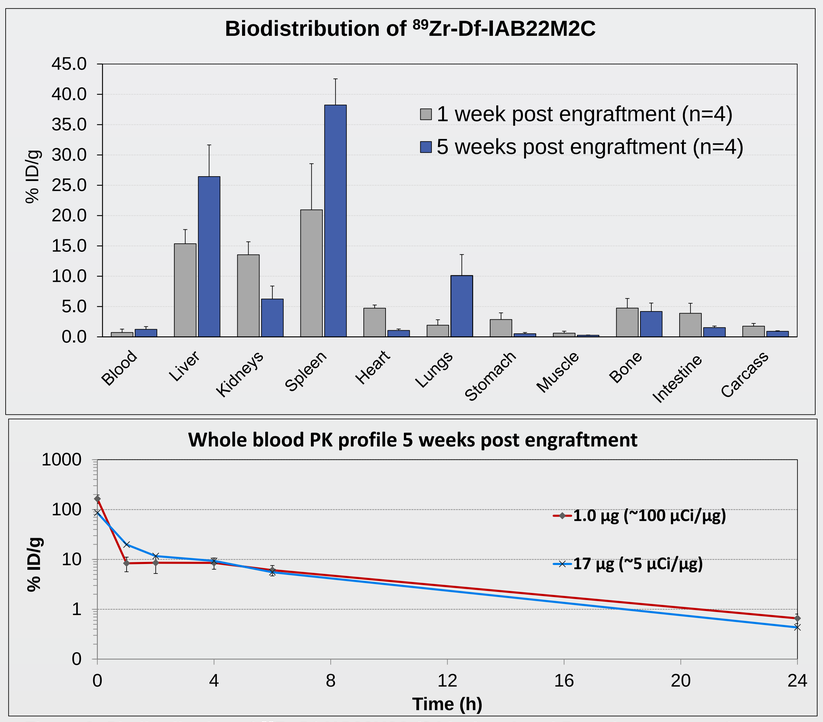

Figure 8. Biodistribution of ^{89}Zr-Df-IAB22M2C (24 hrs) at 1 and 5 weeks post engraftment (upper panel). Whole blood PK profile at 2 doses in animals at 5 weeks post engrafment.

RESEARCH POSTER PRESENTATION DESIGN © 2015
www.PosterPresentations.com