Development of an ImmunoPET Tracer for Imaging Human CD8+ T Cells

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Abstract

Background: Infiltration of CD8+ T cells into human tumors is associated with an increased disease-specific survival in many cancers. A better understanding of T cell trafficking and characterization of the subset of CD8+ T cells with the highest localized activity would improve the ability to schedule and select patients for chemo- and immuno-therapy regimens. To facilitate this objective, we have engineered a humanized anti-CD8 antibody fragment and used it to image human CD8+ T cells in a tumor xenograft and humanized mouse model.

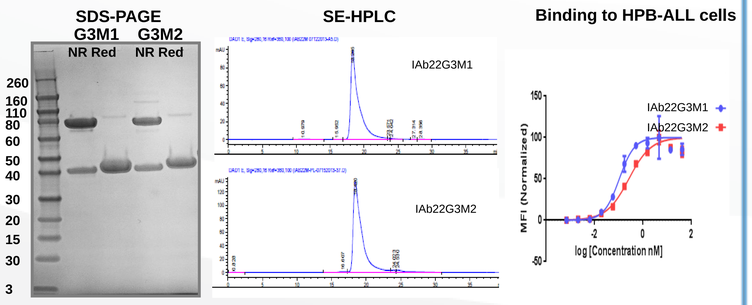
Methods: The V_H and V_L sequences of a murine anti-human CD8 antibody were cloned by RT-PCR, humanized by CDR grafting onto a human germline framework and engineered into minibody (Mb) fragments (scFv-CH3 dimer) of 80 kDa in size . Multiple humanized variants were evaluated and the lead Mb candidate was selected based on ELISA, flow cytometry and SPR binding properties. The lead Mb, IAb22G3M1 was transiently expressed in Expi293 cells and purified by Protein L chromatography. PET imaging was performed with desferrioxamine (Df) conjugated IAb22G3M1 radiolabeled with ^{89}Zr ($T_{1/2}$ 3.3 d). SCID mice bearing subcutaneous HPB-ALL (CD8+ve) or Daudi (CD8-ve) xenografts were serially imaged at 4, 24 and 41 h after i.v. administration of ^{89}Zr-IAb22G3M2 and tissues harvested and counted to determine the biodistribution at the time of sacrifice. The Mb was also evaluated in NOD-SCID-Gamma (NSG) mice that were engrafted with 20 million human PBMCs. In this latter study IAb22G3M1 was radiolabeled with ^{64}Cu ($T_{1/2}$ 12.7 hrs) following NODAGA conjugation. Mice were imaged at 4 and 7 hrs followed by tissue collection for biodistribution. NSG mice that were not grafted with PBMCs served as experimental controls.

Results: Purification of IAb22G3M1 yielded a product that migrated at the expected MW of 80 kDa (SDS-PAGE) with very low (<1%) HMW aggregate (SEC). Cell based binding to human CD8-expressing T cells demonstrated a relative affinity of <0.1 nM. Following conjugation and radiolabeling, immunoreactivity was preserved. PET imaging of HPB-ALL xenografts showed specific tumor targeting at 24 and 41 h. The uptake in the CD8 positive HPB-ALL tumors was ~6-fold higher (10.0±2.4%ID/g) than in the negative Daudi tumors (1.6±0.4%ID/g). Positive tumor to blood ratio was 13.5 for the positive vs. 3.5 for the negative tumor. In mice engrafted with human PBMCs, spleen uptake was 35.8±9.2%ID/g compared to 16.8±3.2%ID/g in control mice. The apparent splenic antigen sink in PBMC grafted mice likely explains the rapid blood clearance and the different tissue uptakes observed between the two groups.

Conclusion: We have successfully generated a functional anti-human CD8 imaging agent that can be used to detect and monitor T cell trafficking and expansion *in vivo*. Pre-clinical PET imaging studies suggest that IAb22G3M1 is a promising tracer for detecting CD8 positive immune cells *in vivo* in suitable models paving the way for clinical translation.

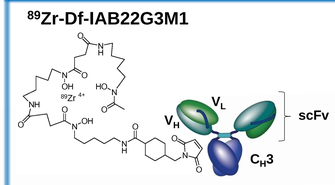
Expression, Purification an Binding

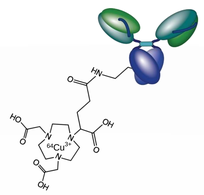
Two variants of humanized anti-human CD8 IAB22M were generated and expressed in Expi293 cells. The protein was subjected to a 2-step purification using capture Select FcXL affinity chromatography followed by cationic exchange chromatography. The purified Mbs were analyzed by SDS-PAGE, size exclusion chromatography (SE-HPLC) and flow cytometry (FACS).

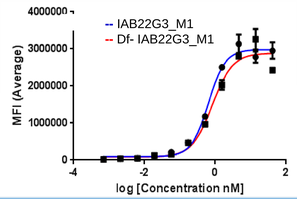


- **The minibodies migrated at the expected MW (80 kDa), were 98% pure and had high affinity for CD8 (EC_{50} is 0.15 nM for IAb22G3M1 and 0.19 nM for IAb22G3M2.**

Conjugation, Radiolabeling and Binding



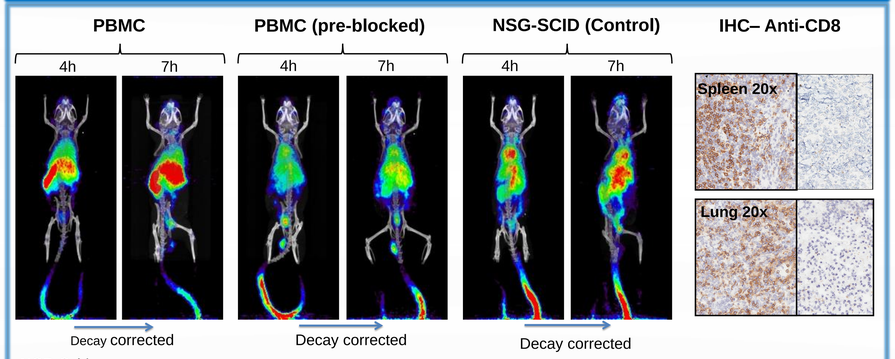




IAb22G3M1 was desferrioxamine (Df)) -conjugated to cysteine residues in the hinge region and radiolabeled with ^{89}Zr ($t_{1/2}$ 3.3 days) or NODAGA-conjugated to lysine residues and radiolabeled with ^{64}Cu ($t_{1/2}$ 12.7 hrs). IAB22G3M1 showed high affinity binding to cell-surface CD8 (EC_{50} 0.64 nM) that was retained following conjugation to Df (EC_{50} 0.83 nM).

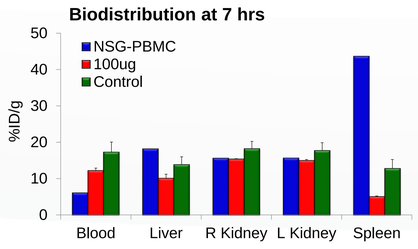
	^{89}Zr-Df-IAB22G3M1	^{64}Cu-NODAGA-IAB22G3M1
Radiochemical purity (%)	>99%	97%
Specific activity (µCi/µg)	5.2	3.2
Radiolabeling efficiency	99%	84%
Immunoreactivity (CD8 + cells)	>92%	62%

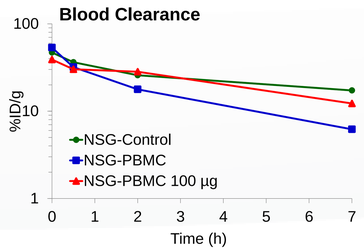
PET Imaging of Humanized Mice



Gamma SCID (NSG) mice engrafted with 20 million human peripheral blood mononuclear cells (PBMCs) in PBS by i.v. administration were imaged with ^{64}Cu-NODAGA-IAB22G3M1. Fused MIP PET/CT images comparing tumor targeting of ^{64}Cu-NODAGA-IAB22G3M in NSG-mice 4 and 7 hrs p.i Mice were injected with approximately 50 µCi (15 µg protein) each. Presence of infiltrating CD8 cells in spleen and lungs were confirmed by IHC staining on frozen tissue sections.

- **Spleen uptake in huPBMC engrafted mice but not in mice treated with unlabeled Mb**
- **Specificity was confirmed by IHC staining for infiltrating CD8 T cells**



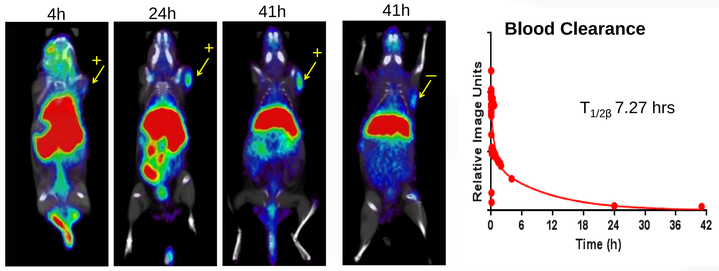


After the final scan mice were sacrificed and organs harvested and counted with the blood in a gamma counter. The radioactive uptakes were decay corrected and the percent injected dose per gram (%ID/g) was determined.

- **Splenic radioactive uptake is specific in huPBMC engrafted mice**
- **Spleen acts as an antigen sink accelerating PK**

PET Imaging of Xenografted Mice

NOD-SCID mice were inoculated with ~10 million HPB-ALL (human T cell leukemia). About 3 weeks later mice were imaged serially with ^{89}Zr-labeled Df-IAB22G3M1.



Coronal PET/CT images of one mouse with HPB-ALL (+) tumor at 4, 24 and 41 hrs and one mouse with a Daudi (-) tumor at 41. Radioactive uptake was clearly seen in the (+) tumor at 24 and 41 hrs. The %ID/g in the (+) tumor was 10.0(±1.4) and 1.6(±1.2) in the (-) tumor. Positive tumor to blood ratio was 13.5 (negative to blood 3.5). The blood activity curve was generated from drawing regions of interest (ROIs) over the left ventricle

- **^{89}Zr-Df-IAB22G3M1 localizes to CD8 expressing tumor xenografts and has a half life of approx. 7.3 hours**

Humanized mice were treated with 2 consecutive doses (100 µg) of parental mAb, Mb or Cys-Db and spleen and blood were harvested on Day 3. PBMCs were isolated, stained for CD8 with a non-competing mAb and analyzed by flow cytometry.

- **CD8 antibody fragments do not deplete CD8 positive T cells *in vivo***

Summary and Conclusion

- A humanized anti-CD8 Mb (IAB22G3M1) was generated that retained high affinity and specificity for human CD8 positive T cells
- Radiolabeled IAB22G3M1 specifically targeted human CD8 positive T cell lymphomas and human CD8 expressing T cells resident in mouse spleens
- A more rapid blood clearance was observed in human PBMC engrafted mice indicating that spleen acts as antigen sink
- IHC staining verified the presence of infiltrating CD8 positive cells in the spleen and lungs of humanized mice
- The terminal half-life in blood was 7.27 hrs as expected for a Mb fragment
- Preliminary data demonstrates that IAb22G3M1 is a promising tracer for detecting human CD8 positive immune cells *in vivo* in suitable models, paving the way for clinical translation