Anti-CD8 immunoPET detection of CD8$^+$ tumor infiltrating lymphocytes

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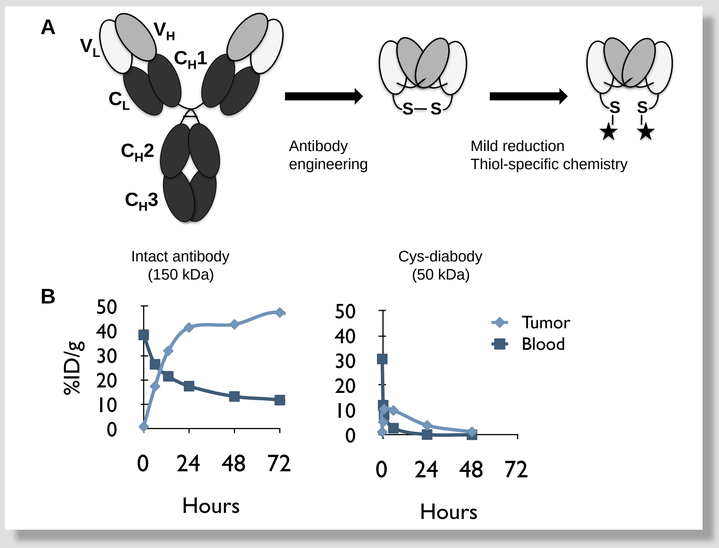
Introduction

With the recent success of various immunotherapies the ability to non-invasively determine immune response post-therapy has become a challenging problem to solve. In the context of tumor immunology, it has become apparent that the tumor microenvironment, specifically CD8$^+$ tumor infiltrating lymphocytes (TILs), is a predictor of patient outcome. ImmunoPET detection of CD8$^+$ TILs in preclinical models can potentially provide the ability to monitor the efficacy of novel immunotherapies being developed for clinical application. As a means to non-invasively detect CD8 expression in vivo[1], the depleting parental antibody YTS169 was reformatted to a cys-diabody fragment (cDb; ~50 kDa scFv dimer) with a C-terminal cysteine for the site-specific conjugation of thiol reactive fluorophores or radiometal chelators. The YTS169 cDb binds CD8α in all mice strains. Engineering to the cDb format serves two purposes: firstly, the cDb lacks the full Fc fragment and will, therefore, not deplete CD8$^+$ cells in vivo, and secondly the cDb has optimal pharmacokinetics for radionuclide imaging because the lack of a full Fc domain decreases the blood half-life. As depicted below, the cDb construct exhibits rapid clearance that allows for high tumor-to-blood ratios at earlier times post-injection when compared to the intact antibody. Although the overall tumor uptake is less, it is the blood clearance that enhances the cDb's immunoPET imaging properties.

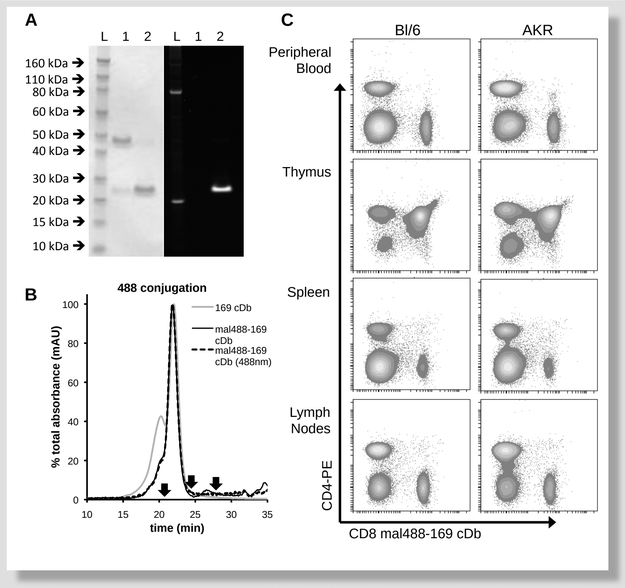


In this study, syngeneic CT26 colon cancer xenografts were established in Balb/c mice that were treated with an agonistic anti-CD137 antibody. CD137 (4-1BB) is a member of the tumor necrosis factor family expressed on activated T cells and other immune cells. The agonistic anti-CD137 antibody has been shown to cause rapid regression of CT26 tumors[2] and is currently being tested in the clinic on patients with advanced cancers.

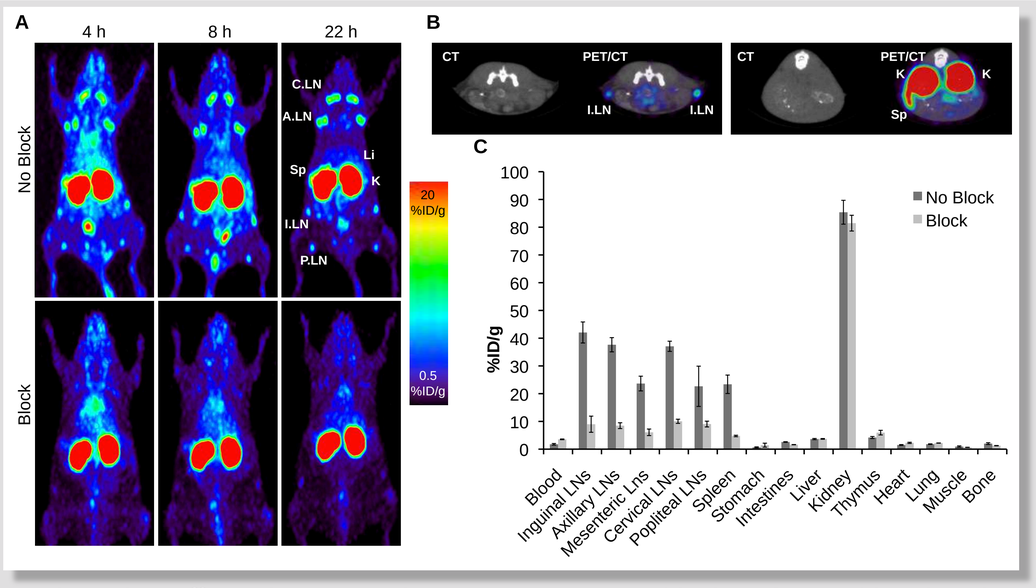
Conclusions

The anti-CD8 YTS169 cys-diabody can be site-specifically conjugated to maleimide-DFO for subsequent ^{89}Zr radiolabeling. ^{89}Zr-malDFO-169 cDb targets CD8 expressed in the spleen and liver of wild type mice, as demonstrated by in vivo CD8 blocking studies. Using CT26 colon carcinoma xenografts in syngeneic Balb/c mice and anti-CD137 immunotherapy, we were able to demonstrate intratumoral targeting using the anti-CD8 cys-diabody. Tumor uptake could be blocked in vivo and uptake was less in mice that received no anti-CD137 therapy. Importantly, anti-CD8 immunoPET signal in the tumor was validated with both quantitatively by flow cytometry and qualitatively by anti-CD8 IHC for the detection of increased numbers of CD8$^+$ tumor infiltrating lymphocytes. The work shown here demonstrates the feasibility of detecting intratumoral CD8$^+$ T cells in preclinical models and therefore provide efficacy of novel immunotherapies being developed for future clinical trials.

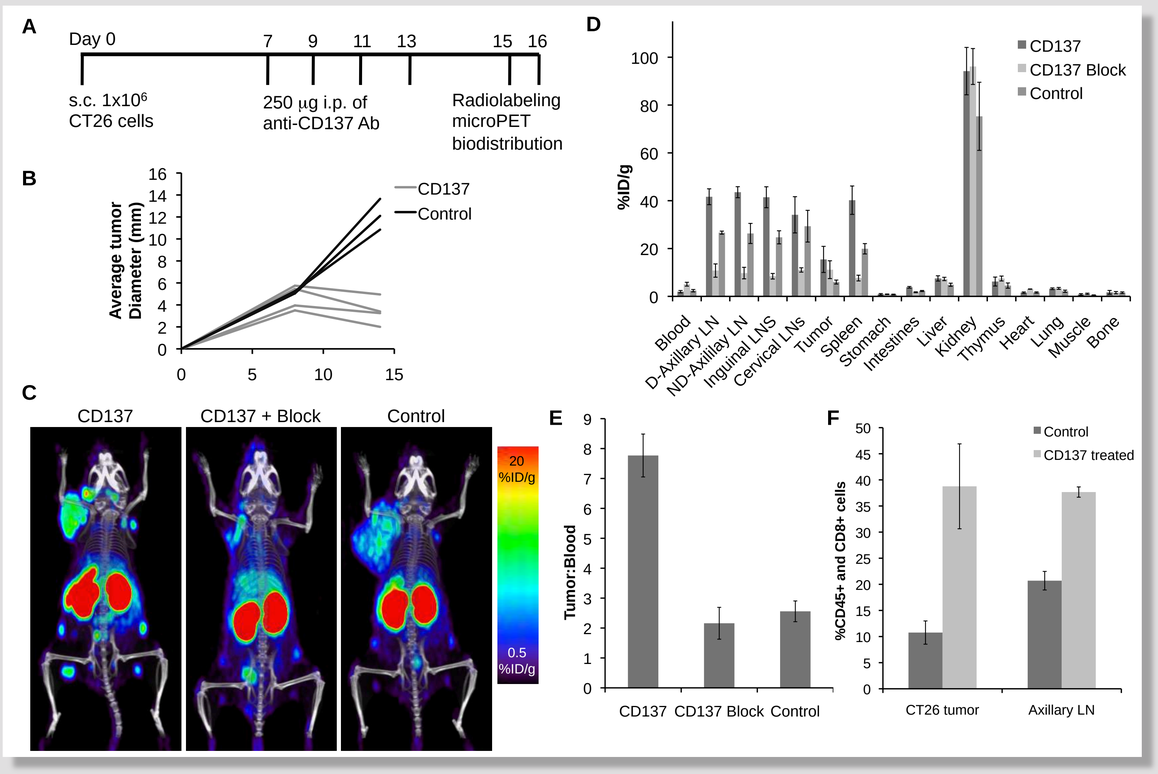
Results



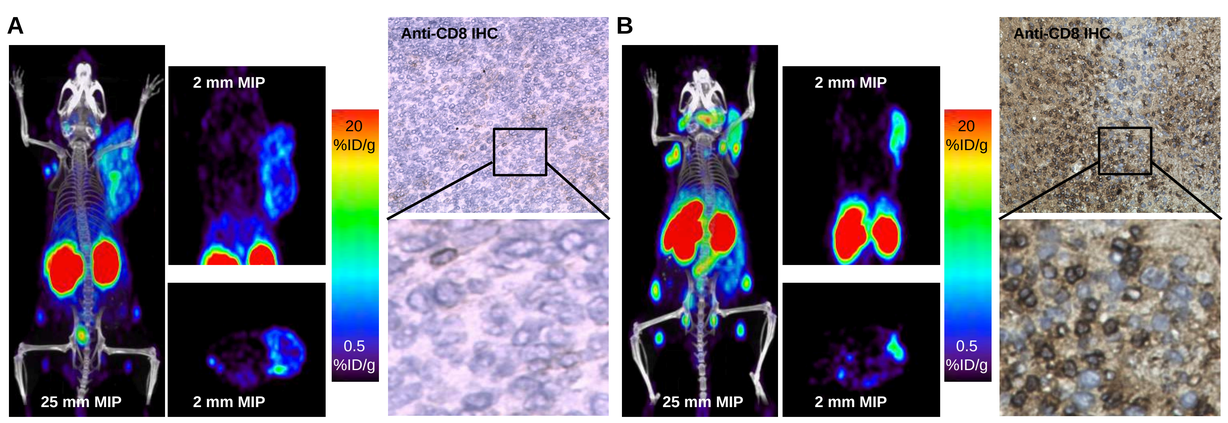
Anti-CD8 YTS169 cys-diabody characterization. (A) SDS/PAGE gel (left panel) of purified 169 cDb (Lane 1) is reduced and conjugated to maleimide-AlexaFluor488 (mal488; Lane 2). The UV image (right panel) of the same gel shows mal488 conjugated to the 169 cDb. **(B)** Size exclusion chromatography demonstrates the conjugation to mal488 has not disrupted the diabody confirmation. Reference arrows indicate albumin (66 kDa) at 20.8 min, carbonic anhydrase (29 kDa) at 24.7 min, and cytochrome C (12.4 kDa) at 27.4 min. **(D)** Flow cytometry using the mal488-169 cDb of single cell suspensions from the blood, thymus, spleen, and lymph nodes of C57BL/6 (Lyt2.2$^+$; left column) and AKR (Lyt2.1$^+$; right column) mice.



The anti-CD8 ^{89}Zr-malDFO-169 cDb targets CD8 expressed in the spleen and lymph nodes of wild type AKR mice. (A) ^{89}Zr-malDFO-169 cDb was injected into wild type AKR mice (top row) and CD8-blocked (bolus 3 mg/kg GK1.4 cDb; bottom row) AKR mice and imaged at 4, 8, and 22 h post-injection. Coronal images are represented as 25 mm maximum intensity projections (MIPs). Abbreviations: C.LN – cervical lymph node, A.LN – axillary lymph node, Li – liver, Sp – Spleen, K – Kidney, I.LN – inguinal lymph node, and P.LN – popliteal lymph node. **(B)** Transverse CT and PET/CT images confirm localization of ^{89}Zr-malDFO-169 cDb in the lymph nodes and spleen of wild type mice. Transverse image are represented as 2mm MIPs. **(C)** Ex vivo biodistribution at 22 h post-injection of ^{89}Zr-malDFO-169 cDb from wild type and CD8-blocked AKR mice demonstrates CD8-specific uptake in the lymphoid organs.



ImmunoPET detection of tumor infiltrating CD8$^+$ T cells using the CT26/anti-CD137 antibody immunotherapy model. (A) Subcutaneous CT26 colon carcinoma xenografts are grown in syngeneic Balb/c mice for 7 days prior to anti-CD137 therapy (12.5 mg/kg anti-CD137 Ab injected intraperitoneally every other day for four treatments). ^{89}Zr-malDFO-169 cDb was injected on day 15 and immunoPET imaging and subsequent ex vivo biodistribution were acquired 22 hours later. **(B)** Representative tumor growth curves of individual mice. **(C)** ImmunoPET images acquired 22 hours post-injection of ^{89}Zr-malDFO-169 cDb in mice bearing CT26 tumors plus anti-CD137 therapy, tumor bearing mice plus antiCD137 therapy and 3 mg/kg anti-CD8 blocking injection, and tumor bearing mice with no anti-CD137 therapy. **(D)** Ex vivo biodistribution acquired 22 h post-injection of ^{89}Zr-malDFO-169 cDb. **(E)** Tumor-to-blood ratios of mice bearing CT26 tumors plus anti-CD137 therapy, tumor bearing mice plus anti-CD137 therapy and 3 mg/kg CD8 blocking injection, and tumor bearing mice with no anti-CD137 therapy. **(F)** Flow cytometry analysis of CD45$^+$ and CD8$^+$ cells in the both the tumor and draining lymph nodes of Balb/c mice with subcutaneous CT26 xenografts with and without anti-CD137 immunotherapy.



Anti-CD8 immunoPET and IHC analysis of CT26 tumors treated with or without anti-CD137 Ab immunotherapy. ImmunoPET and corresponding immunohistochemistry of control **(A)** and anti-CD137 treated **(B)** mice bearing CT26 tumors. Coronal immunoPET images are displayed as both 2 and 25 mm MIPs and transverse image is a 2 mm MIP to demonstrate localization of in the center of the CT26 xenograft. Anti-CD8 IHC confirms the localization of CD8$^+$ tumor infiltrating lymphocytes in mice bearing CT26 tumors that have undergone anti-CD137 therapy.

References

1) Tavaré, R., et al. PNAS 2014, 111;1108-1113.
2) Escuin-Ordinas, H., et al. ImmunoTherapy of Cancer 2013, 1:14.

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