Targeted Delivery of Human Exosomes via Bispecific Antibody Platform

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Background

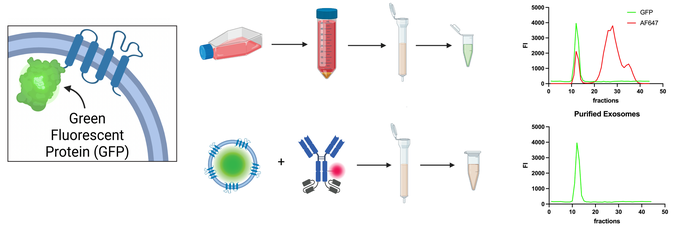
Exosomes are phospholipid membrane-bound nanovesicles produced by living cells to mediate intercellular communication. Exosomes possess an innate ability to deliver macromolecular cargo across biological barriers and evade lysosomal degradation. For these reasons, exosomes have been investigated as potential drug nanocarriers.

Exosomes show little evidence of natural tropism. Therefore, it would be beneficial to engineer them to display affinity ligands so that off-target delivery may be reduced. Current engineering methods include genetically engineering parent cells or chemical conjugation, which both present unique disadvantages. To address these limitations, we designed bispecific antibodies that can direct exosomes to target tissues.

Methods

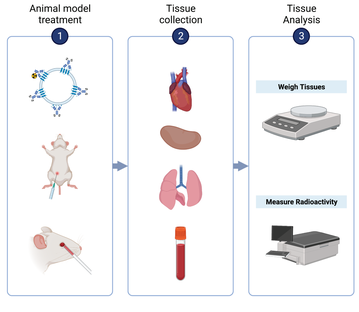
1. Exosome purification and ExTAb decoration

GFP-CD63 exosomes fluoresce green and AF647 labeled ExTAbs fluoresce red; colocalization of green and red fluorescence indicate purified and decorated exosomes.



2. Biodistribution of exosomes with I-125 labeled antibodies

Radioactive exosomes were injected and blood was drawn at 5, 30, 60 minutes. Organs were harvested and weighed at 60 minutes. Counts were measured to determine %ID/g.



ExTAb Structure and Design

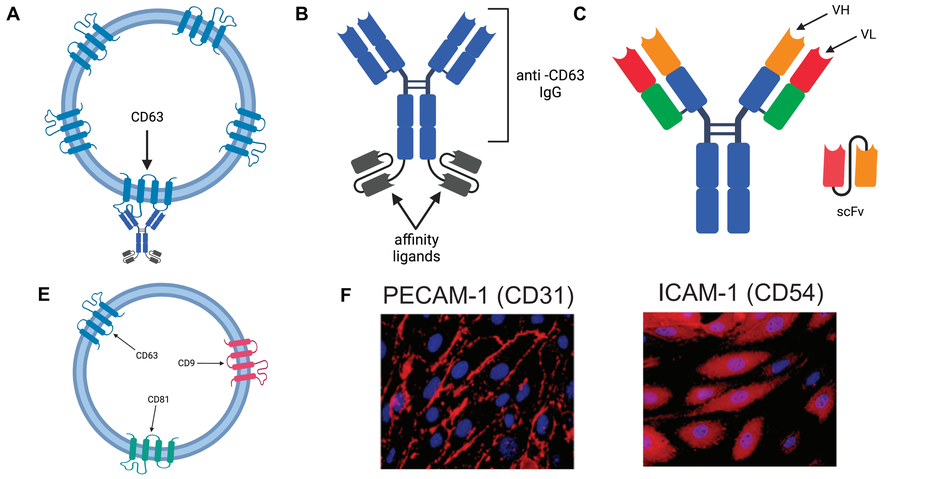


Figure 1. A) Tetraspanin protein CD63 was used as exosome marker. **B)** Exosome targeting 2x2 bispecific antibody (ExTAb) comprised of anti-CD63 IgG to bind exosomes and one affinity ligand fused to the C-terminus of each heavy chain. **C)** Single-chain variable fragment (scFv) affinity ligand consists of a light and heavy chain variable domain joined by a flexible linker. **E)** IgG portion of ExTAb can be swapped to bind other exosome markers such as CD81 and CD9. **F)** scFv portion of ExTAb can also be swapped to deliver to multiple different target cell types. For our proof-of-concept ExTAbs, we used anti-PECAM-1 (CD31) and anti-ICAM-1 (CD54) scFvs to deliver exosomes to the vascular endothelium due to its accessibility from the systemic circulation.

ExTAb Binding and Exosome Delivery

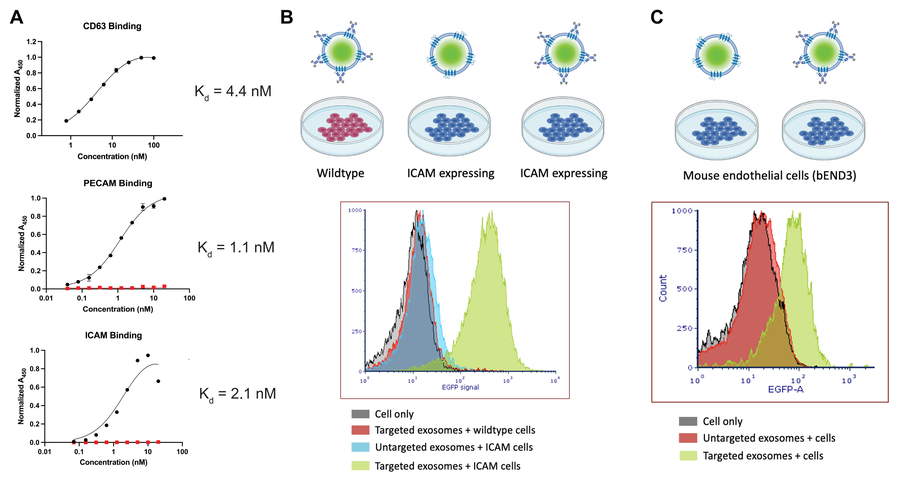


Figure 2. A) ExTAb binding affinity to targets was measured by ELISA. Dissociation constant for CD63, PECAM-1, and ICAM-1 binding was 4.4 nM, 1.1 nM, and 2.1 nM respectively. Black curve shows specific binding and red curve shows non-specific binding. **B)** *In vitro* exosome delivery was evaluated through flow cytometry. **i.** ICAM-1 targeted delivery was modeled on engineered ICAM-expressing cells. Targeted exosomes incubated with wildtype ICAM-1 non-expressing cells and untargeted exosomes incubated with ICAM-expressing cells showed very little change in GFP delivery compared to cells alone. Targeted exosomes incubated with ICAM-1 expressing cells had a significant shift in GFP fluorescence. **ii.** PECAM-1 targeted delivery was modeled on mouse endothelial cells. Untargeted exosomes showed no difference in GFP fluorescence in the cells, but PECAM-1 targeted exosomes led to an increase in fluorescence. Decrease in shift could be due to overexpression of ICAM-1 in engineered cells.

In Vivo Biodistribution

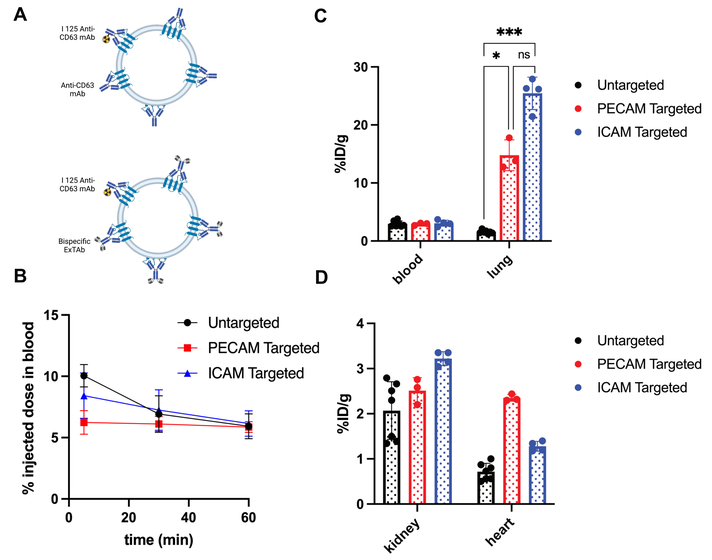


Figure 3. A) Exosomes were coated with 10% I-125 labeled anti-CD63 mAb and 90% anti-CD63 mAb (untargeted) or 90% ExTAb (targeted). **B)** Blood pharmacokinetics is comparable across untargeted and targeted exosomes, with a majority cleared before 5 minute timepoint. **C)** A significant increase in uptake was observed in the lung, a highly vascularized organ, with targeted exosomes. ICAM-1 targeted exosomes show slightly increased uptake compared to PECAM-1 targeted exosomes. **D)** The kidney and heart also show changes in uptake with targeting to the endothelium. ICAM-1 targeting causes highest uptake in kidneys while PECAM-1 targeting leads to highest uptake in heart.

Future Directions

Continued work on this project will evaluate if *in vivo* delivery is cell-specific rather than just organ specific. Additionally, we will use a CRE-reporter system to determine if our targeted exosomes can delivery functional protein to target cells. Since this platform is versatile, we will continue to explore other markers as targets for the scFv affinity ligands in our ExTAbs.

References & Acknowledgements

C. F. Greineder, A.-M. Chacko, S. Zaytsev, B. J. Zern, R. Carnemolla, E. D. Hood, J. Han, B.-S. Ding, C. T. Esmon, and V. R. Muzykantov, "Vascular immunotargeting to endothelial determinant ICAM-1 enables optimal partnering of recombinant scfv-thrombomodulin fusion with endogenous cofactor," PLoS ONE, vol. 8, no. 11, 2013.

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