Targeting DNA-Lipid Nanoparticles to Pulmonary Endothelial Cells Enhances *In Vivo* Transgene Expression

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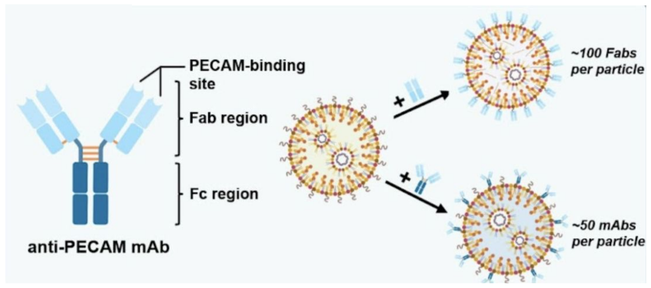
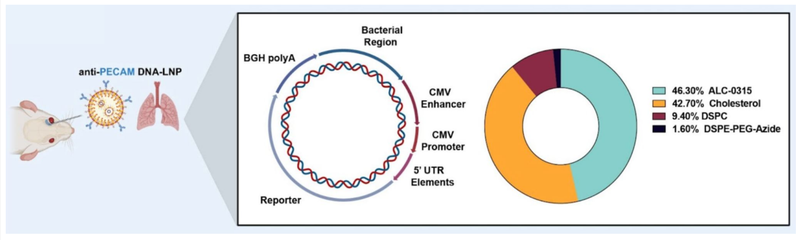
Abstract

- DNA-lipid nanoparticles (DNA-LNPs) loaded with inhibitors of the cGAS-STING pathway enable safe and effective delivery of DNA *in vivo*. However, unmodified LNPs primarily accumulate in the liver.
- One approach to redirect the delivery of LNPs is to attach affinity ligands, such as antibodies, to their surface. Here, we show that conjugating monoclonal antibodies (mAbs) and fragment antigen-binding regions (Fabs) against PECAM (Platelet Endothelial Cell Adhesion Molecule 1) to LNPs creates a drug delivery system that:
  1) Redirects DNA-LNP delivery to the lungs, and more specifically, pulmonary endothelial cells.
  2) Induces transgene expression in the lungs/pulmonary endothelial cells with increasing specificity over time.
  3) Improves the magnitude, longevity, and organ-type specificity of the transgene expression by swapping out mAbs with Fabs as the targeting moiety.

Methods





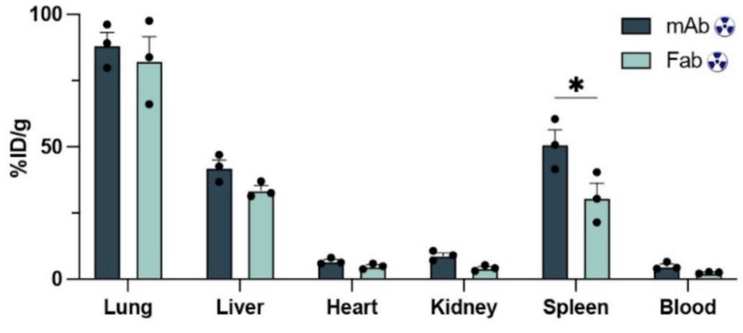


Figure 1. αPECAM DNA-LNPs specifically target the lungs
Illustrations of the structure and conjugation of αPECAM monoclonal antibodies (mAbs) vs fragment antigen-binding regions (Fabs) to DNA-LNPs. Biodistribution of ^{125}I-labeled Fab and mAb αPECAM DNA-LNPs reveal similar lung accumulation by both particles, and decreased uptake of Fab DNA-LNPs in the spleen.

Results

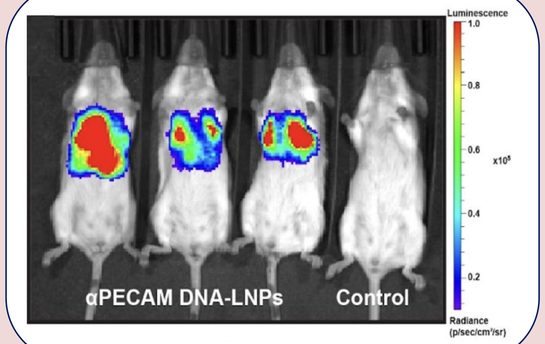


Figure 2. *In vivo* expression of PECAM-targeted DNA-LNPs in the thoracic cavity
Representative bioluminescence (IVIS) image of mice 1d after treatment with 5μg of luciferase pDNA loaded into αPECAM mAb DNA-LNPs alongside a naive, luciferin-treated control

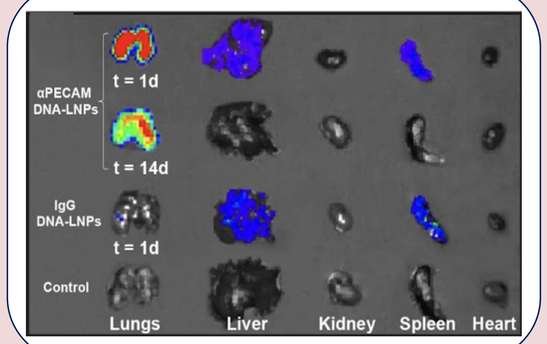


Figure 3. αPECAM DNA-LNPs express in the lungs
Representative *ex vivo* IVIS image of major thoracic and abdominal organs from mice treated with αPECAM mAb DNA-LNPs or untargeted IgG DNA-LNPs next to control organs from a naive, luciferin-treated mouse.

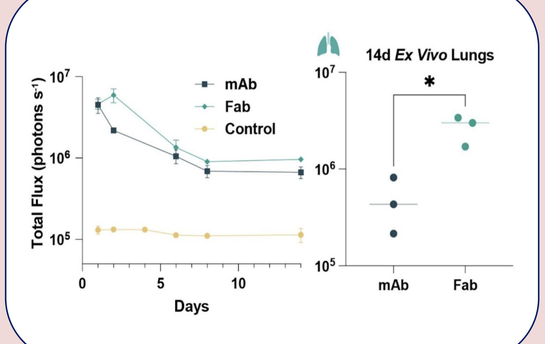


Figure 4. Conjugating Fabs to DNA-LNPs immensely increases both lung specificity and expression
Comparison of luminescence shows superiority of αPECAM Fab DNA-LNPs compared to mAb DNA-LNPs *in vivo and ex vivo*.

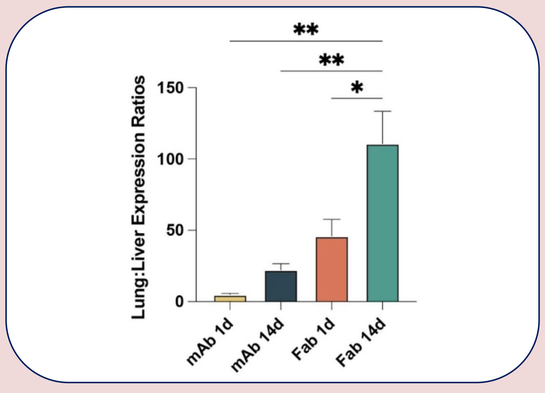


Figure 5. αPECAM Fab DNA-LNPs have superior lung specificity
Lung-to-liver expression ratios quantified at 1d and 14d via *ex vivo* luminescence shows increasing lung specificity by αPECAM Fab DNA-LNPs over time, and overall higher ratios compared to αPECAM mAb DNA-LNPs.

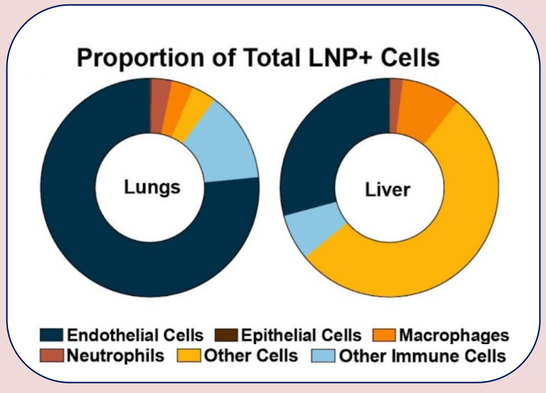


Figure 6. αPECAM Fab DNA-LNPs are predominantly internalized by pulmonary endothelial cells
Flow cytometry analysis tracking the proportion of cells positive for fluorescent αPECAM-Fab DNA-LNPs, staining for CD45, CD64, Ly6G, CD31, and EpCAM.

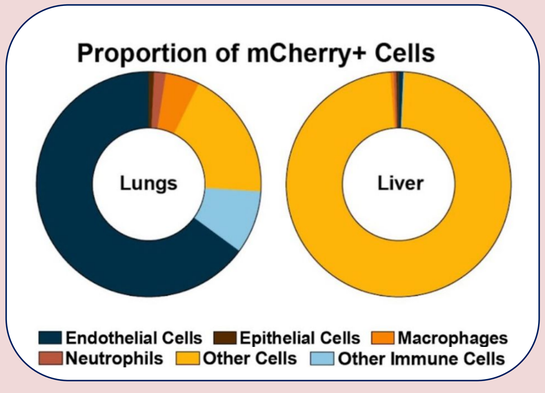


Figure 7. αPECAM Fab DNA-LNP lung expression is driven by endothelial cells
Flow cytometry analysis tracking the proportion of cells positive for expression of mCherry pDNA (encoding a red fluorescent protein), staining for CD45, CD64, Ly6G, CD31, and EpCAM.

Conclusions

- Conjugating αPECAM targeting moieties to DNA-LNPs directs ~85% of their injected dose per gram to the lungs after 30 minutes.
- αPECAM DNA-LNPs induce high transgene expression in the lungs that persists for weeks and significantly increases in lung-specificity with time. Replacing mAbs with Fabs dramatically improves this specificity.
- Single-cell analysis confirms that the vast majority of expression in the lungs was confined to target endothelial cells, which are the most quiescent compared to all other transfected cell types.
- **Future Directions:** We will test our αPECAM DNA-LNPs as a therapy for Pulmonary Hypertension in a relevant mouse model. Likewise, we will use alternative targeting moieties to redirect the delivery of DNA-LNPs to other organs and cell types.

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