

Introduction and Objectives

Type 1 Diabetes Mellitus is caused by loss of β -cells from the Islets of Langerhans in the pancreas, leading to insulin insufficiency. Type 2 Diabetes Mellitus is largely caused by insulin resistance, but also leads to a decrease in β -cell numbers.

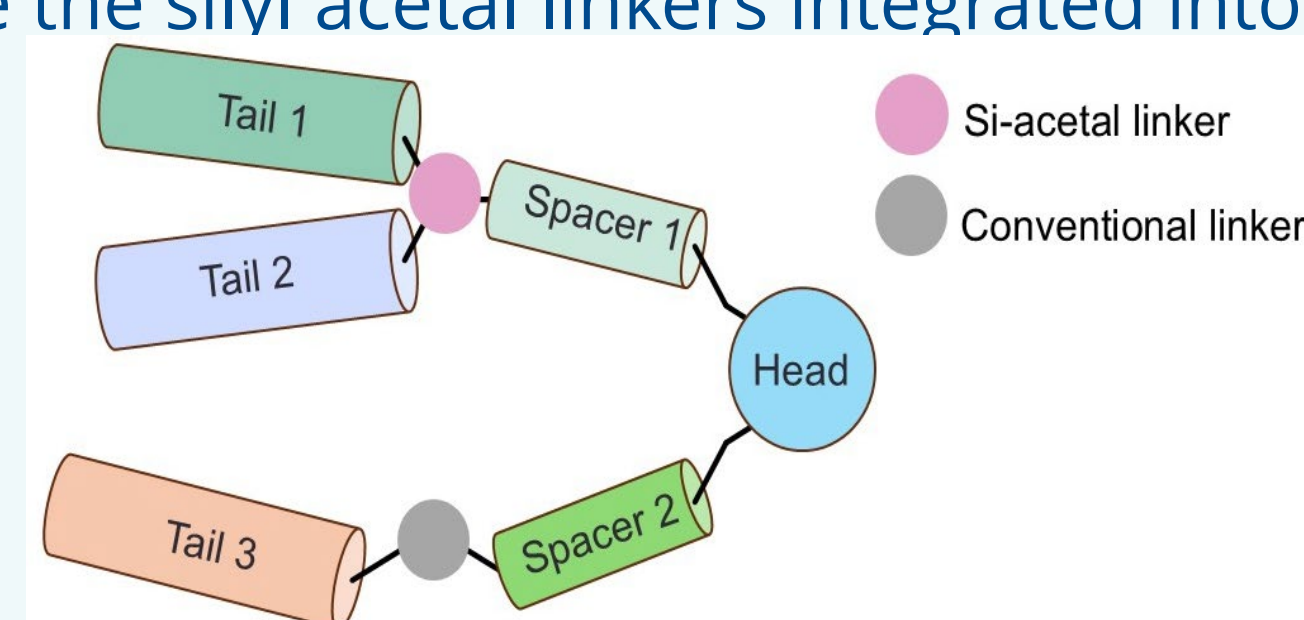
Adeno-Associated Virus (AAV) vectors that lead to expression of pancreatic transcription factors Pdx1 and MafA have been used in vivo to transdifferentiate pancreatic α -cells to β -like cells that express insulin, which can lead to control of glucose levels in diabetic animal models. However, AAV vectors can only be administered once, due to immune response to the AAV. Using a lipid nanoparticle (LNP) instead of AAV to deliver the same transcription factors to pancreatic α -cells would lead to a product that could be repeatedly administered. Our research aims to identify a LNP that can deliver a plasmid payload containing pancreatic transcription factors to α -cells. Initial experiments have utilized mRNA payloads.

Methods

Mouse pancreatic Islets of Langerhans were isolated using collagenase injected into the common bile duct, and plated in 96-well tissue culture plates. Nine LNPs prepared with LipexSil[®] lipids were used for transfection of isolated mouse islet cells as was SM-102, a lipid used in commercial vaccines. For the primary screening, cells were treated with 300 ng GFP mRNA containing LNP formulations (DSPC-Cholesterol or DOPE-Cholesterol or DSPC- β -Sitosterol phospholipid-core lipid combination). After 24 hours, nuclei were stained with DAPI and cells were immunostained for GFP, insulin, and glucagon. Transfection efficiency measured by fluorescence intensity was scored as low, medium, or high. IC₅₀ values for ALX-184 and ALX-248 were evaluated using human T-cells and HepG2 cells. In order to evaluate in vivo effects, LNP made using ALX-184 with a Luciferase mRNA payload was injected into the mouse pancreas through the common bile duct, with the bile duct itself clamped, at a dose of 1 μ g/mouse and after 24 hours luciferase activity in the pancreas and other organs was measured. Further evaluation of in vivo effects used an LNP made using ALX-184 with a GFP mRNA payload. After 24 hours, the mouse was sacrificed, Islets of Langerhans were isolated and evaluated for GFP expression.

Ionizable lipid

The LipexSil[®] platform is built on innovation through the introduction of a unique silyl acetal linker architecture embedded within the lipid structure. The synthesis of LipexSil[®] lipids utilizes patented borane catalysts, known as SiBoRed[®], enabling selective hydrosilylation of various esters to produce the silyl acetal linkers integrated into the ionizable lipids.



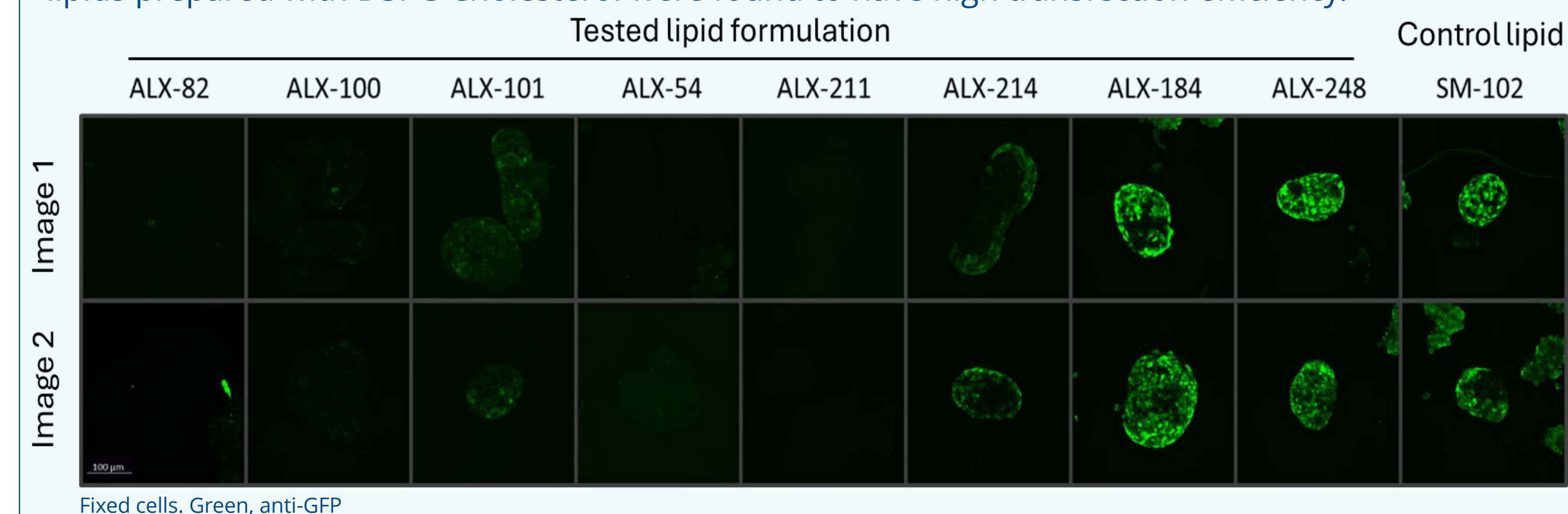
LNP composition: Ionizable lipid : DSPC (or DOPE) : Cholesterol (or β -Sitosterol) : DMG-PEG2000 = 50:10:38.5:1.5 mol% and N/P=6

Mixing: Microfluidic device, 3:1 aq.:org. phase, 12 mL/min (TFR)

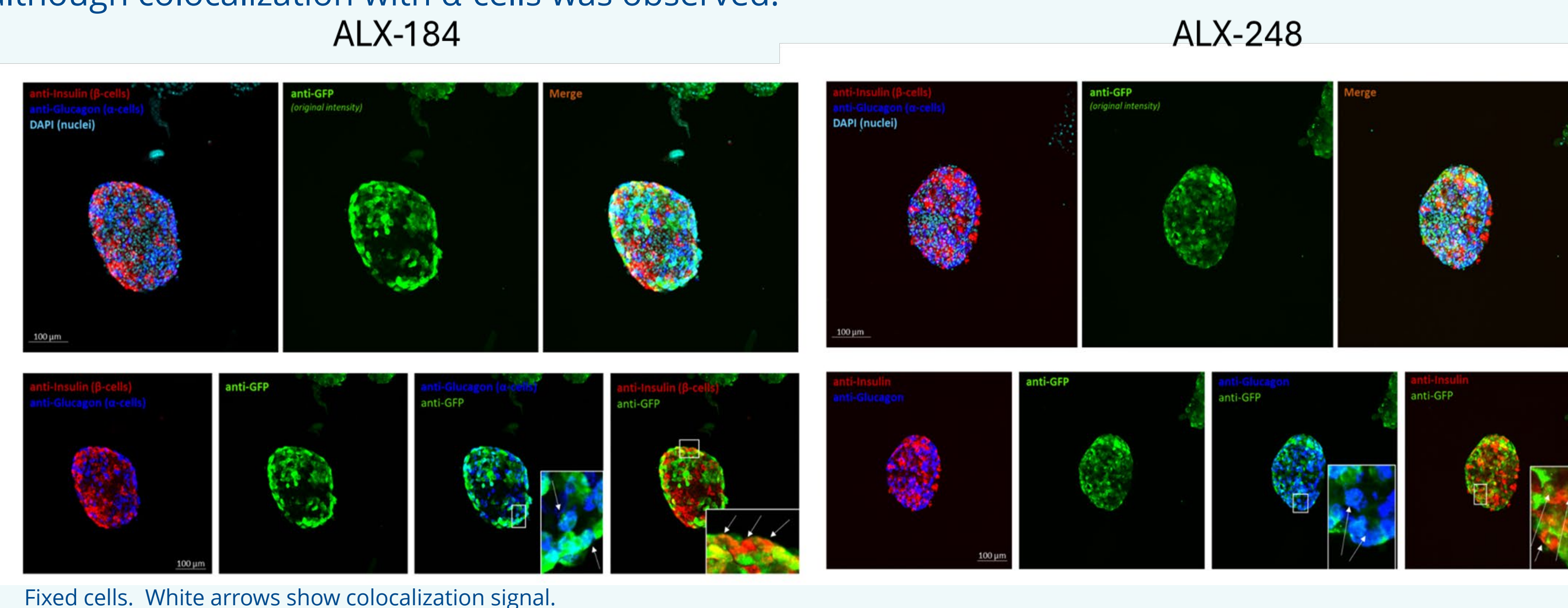
Characterization: DLS measurement: size, PDI and zeta potential and Ribogreen assay: EE%

Results

Significant differences in transfection efficiency were observed. Two LNPs of ALX-184 and ALX-248, lipids prepared with DSPC-Cholesterol were found to have high transfection efficiency.



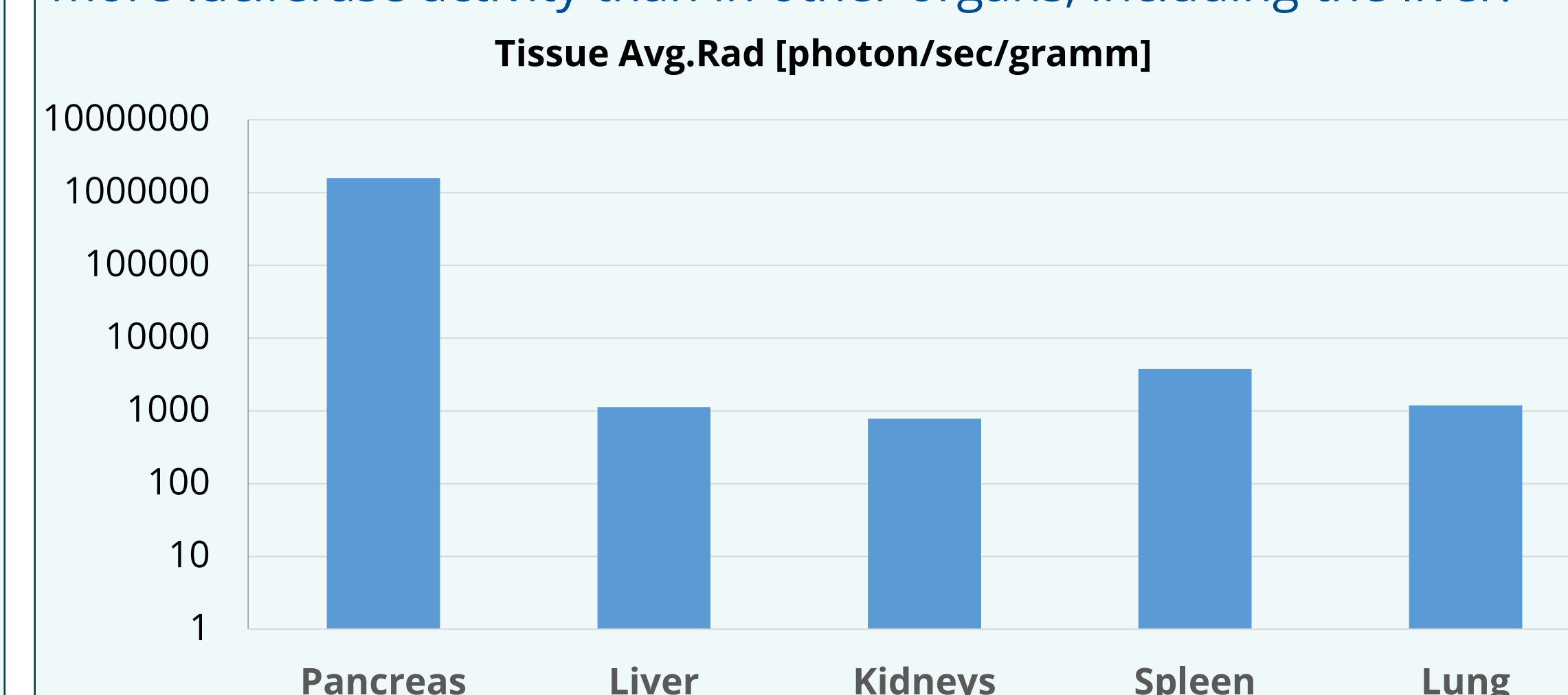
mRNA containing LNPs were used to transfect the islets and stained with anti-GFP. Anti-insulin, anti-glucagon to determine localization of the signal. Transfection was not specific to either α - or β - cells, although colocalization with α -cells was observed.



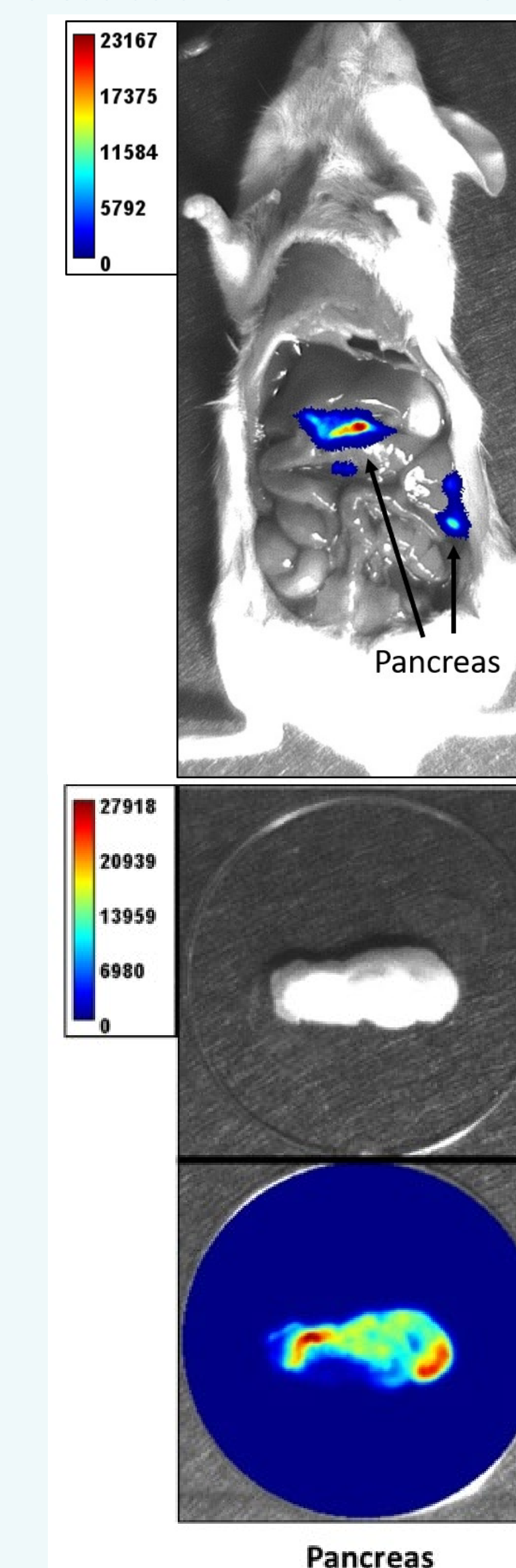
IC₅₀ values for ALX-184 and ALX-248 lipids were higher than the IC₅₀ for SM-102 (commercially available LNP)

ALX code	IC ₅₀ on human T cells (μ M)	IC ₅₀ on HepG2 cells (μ M)
ALX-184	>800	626
ALX-248	>800	>800
SM-102	309	151

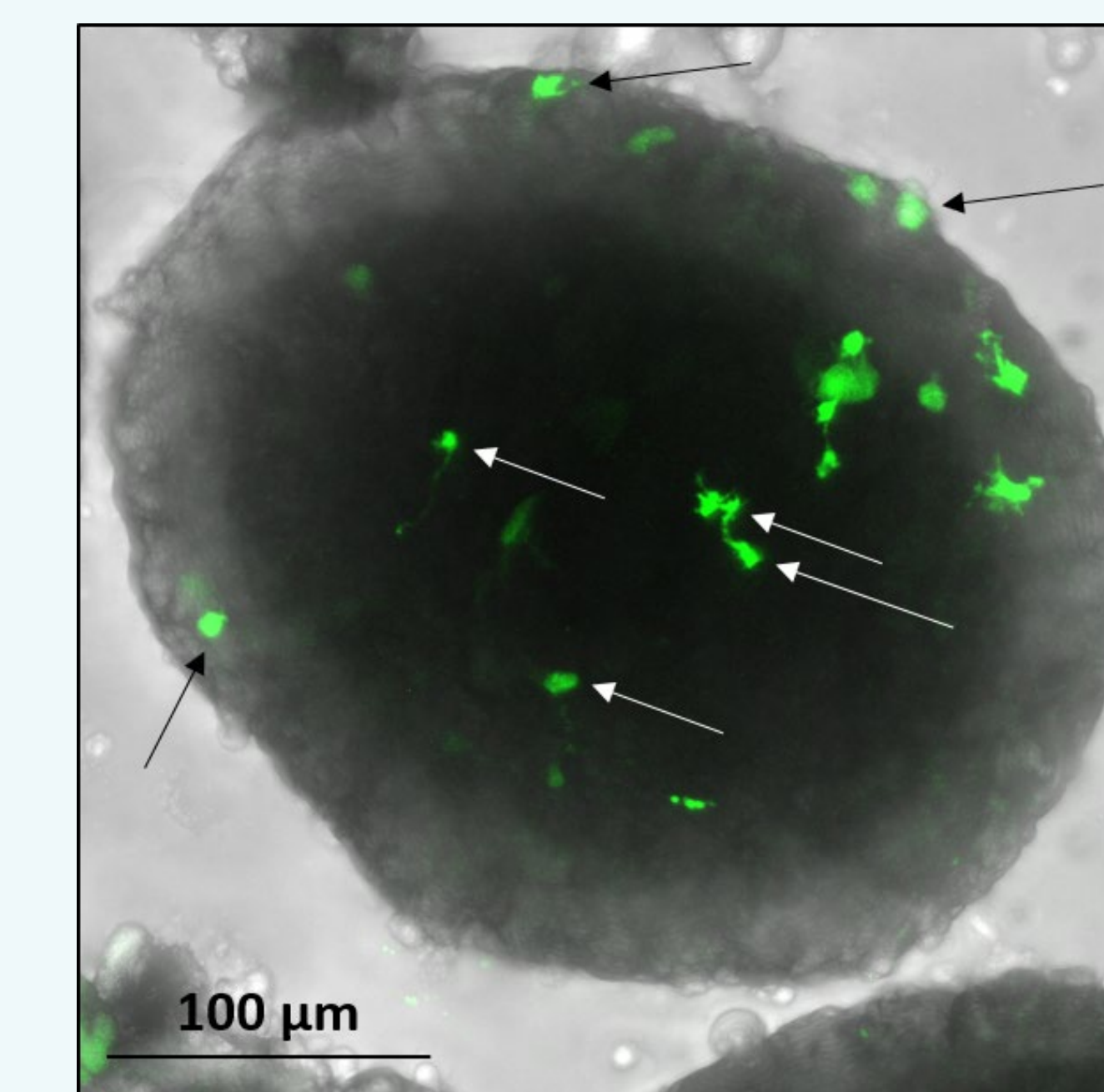
Injection of an LNP made using ALX-184 with a Luciferase payload led to widespread transduction in the pancreas, with 100-fold more luciferase activity than in other organs, including the liver.



Ductal injection of an LNP made using ALX-184 with a Luciferase payload shows activity in the pancreas as shown in the whole animal and resected pancreas.



Following injection of ALX 184-based LNP nanoparticles (LNPs) containing a GFP payload into the mouse common bile duct, isolated islets of Langerhans were evaluated for GFP expression. GFP was successfully detected in both the inner and outer cells of the islets.



Live cells. Confocal microscopy. Black arrows show cells on the periphery of the islet. White arrows show cell on the inside of the islet.

Conclusions

- Nine LNPs prepared with LipexSil[®] lipids with a GFP mRNA payload were evaluated for transfection efficiency in isolated mouse Islets of Langerhans
- Two specific LNPs were highly efficient in transfecting α - and β -cells in isolated mouse Islets of Langerhans
 - Both lipids of the selected LNPs had improved IC₅₀ values compared to a reference lipid
- LNP made using ALX-184 with a Luciferase mRNA payload injected into the mouse common bile duct efficiently transfected pancreatic cells, and thus efficiently crossed the basement membrane separating the pancreatic duct from pancreatic cells
 - Luciferase activity in the pancreas was >100-fold more than in other organs
- LNP made using ALX-184 with a GFP mRNA payload injected into the mouse common bile duct efficiently transfected mouse Islet of Langerhans cells
- Future work will evaluate pDNA as the LNP payload in vitro and in vivo