Genetically Modified Transfusable Donor Platelets using mRNA-Lipid Nanoparticles

Christian Kastrup

Professor
Departments of Surgery, Biochemistry, Biomedical Engineering, and Pharmacology and Toxicology
Medical College of Wisconsin

Senior Investigator
Versiti Blood Research Institute

Affiliate Professor
Michael Smith Laboratories
University of British Columbia

ckastrup@versiti.org

Important Required Disclosures

I am a co-founder, director and shareholder in:

NanoVation Therapeutics, Inc.

- Provides lipids and LNP for partner companies

SeraGene Therapeutics, Inc.

- RNA therapies for blood disorders

CoMotion Drug Delivery Systems, Inc.

- Device engineering for controlling traumatic hemorrhage

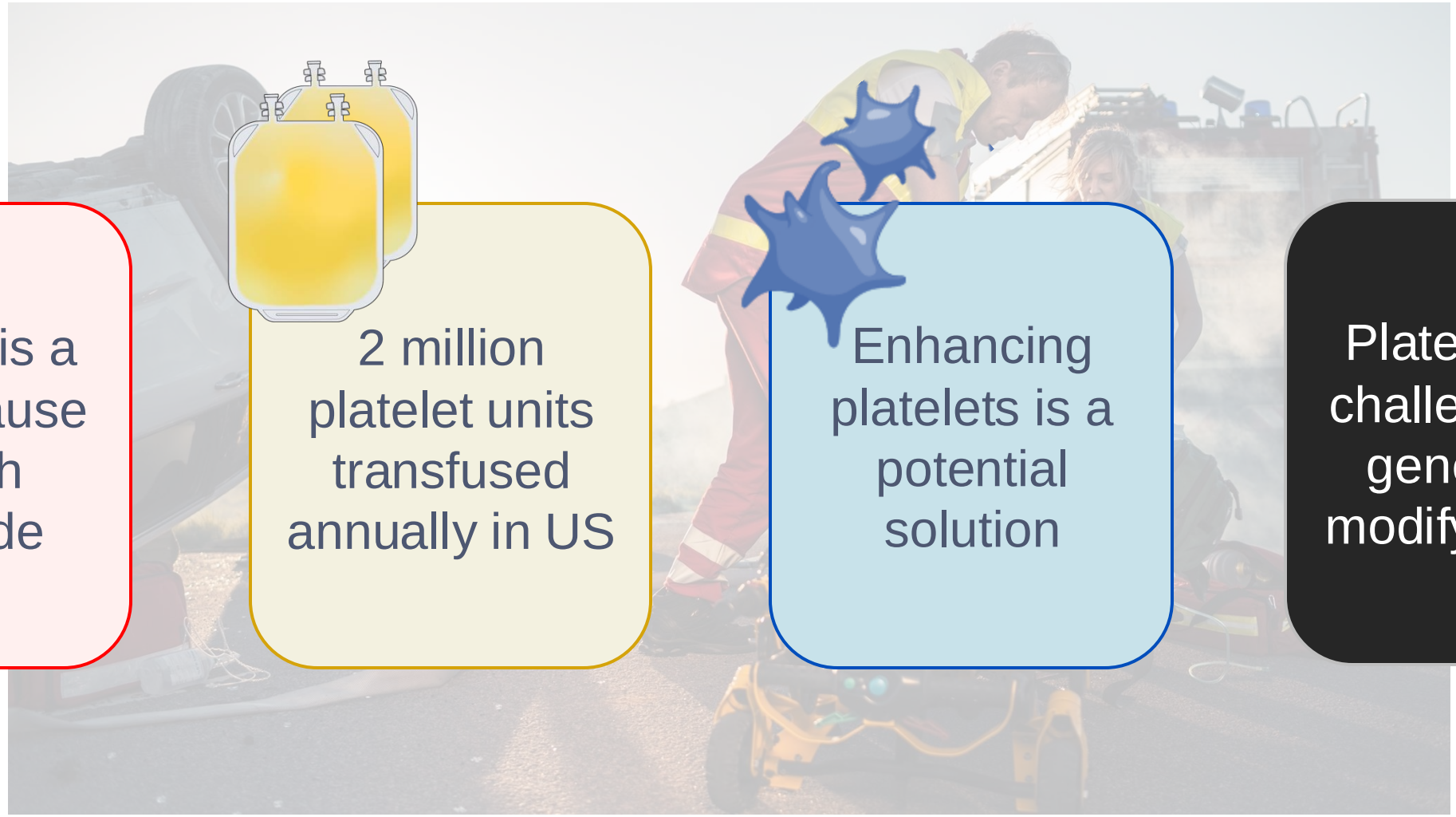
Consulting and/or Contract research :

- **Moderna**
- **CSL Behring**
- **Alnylam**
- **Bayer**
- **Acuitas**
- **Novo Nordisk**

Bleeding... It's a problem.

Bleeding... It's a problem.

Bleeding… It's a problem.



Bleeding is a leading cause of death worldwide

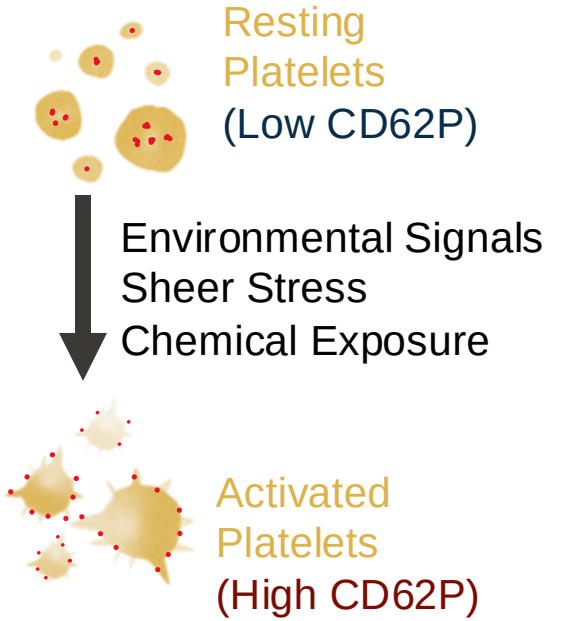
2 million platelet units transfused annually in US

Enhancing platelets is a potential solution

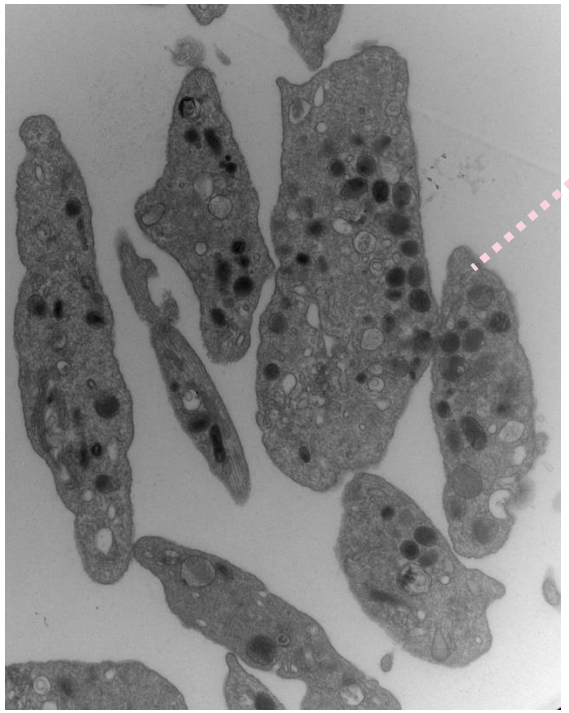
Platelets are challenging to genetically modify *ex vivo*

There are barriers to genetically modifying platelets

Platelets are highly sensitive to their environment



Platelets lack DNA



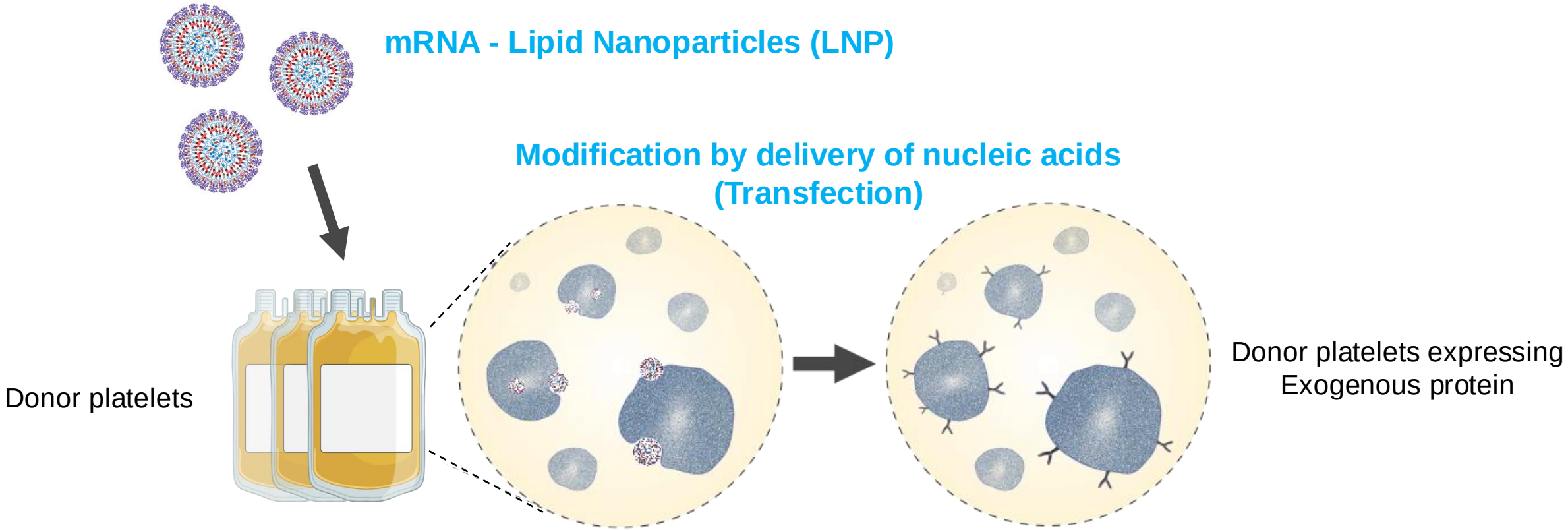
Platelets contain all the translational machinery required for protein synthesis

Can platelets be genetically modify by transfection with mRNA?

Platelet engineering to create cell therapies using mRNA-lipid nanoparticles

Hypothesis:

Platelets transfected with optimized mRNA elements will express exogenous protein.



J. Leung*, C. Strong*, KE Badior*, M Robertson, X Wu , MA Meledeo, E Kang, M Paul, Y Sato, H Harashima, Cap AP, D Devine, E Jan, PR Cullis, CJ Kastrup, ***Science Advances***, 2023

Direct transfection of donor platelets has advantages compared to approaches using stem cells

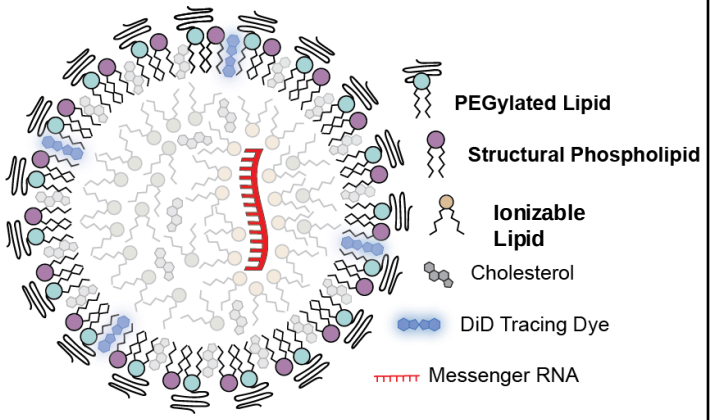
- Compatible with blood banking processes

- More available and similar to circulating platelets, than platelet-like particles produced *in vitro* from stem cells production

- Faster to administer than bone marrow transplant

Our approach to creating platelet cell therapies

Optimize mRNA-LNP Composition for Platelet Transfection



Maximal Protein Expression
Minimal Platelet Activation

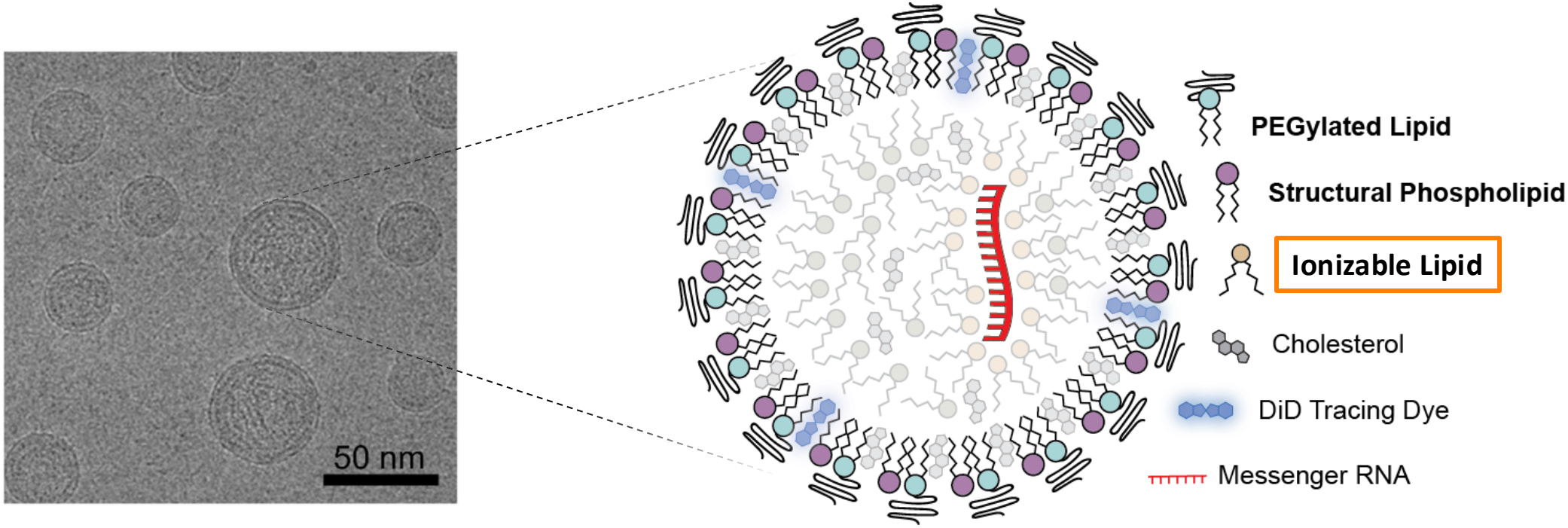
Characterize transfected platelets and optimize the platform for clinical compatibility

Retained responsiveness
Coagulability
Morphology

Evaluate mRNA-LNP products in vivo

Hemostasis
Circulation
Cargo Exchange/Transfer

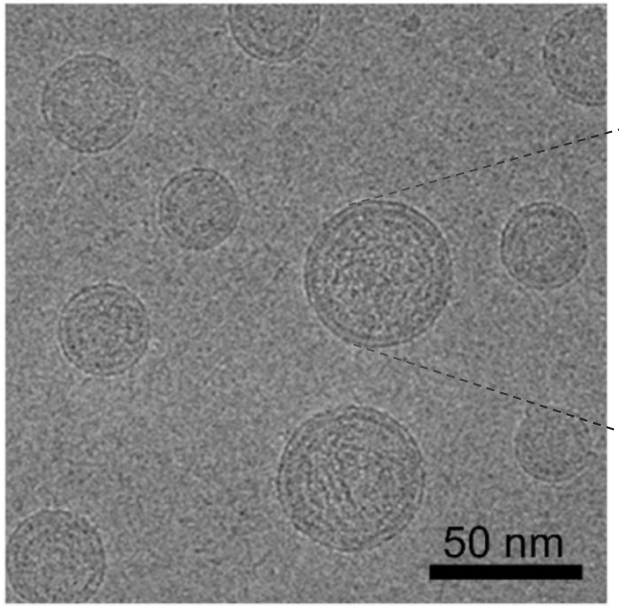
Lipid nanoparticles (LNPs) delivery mRNA into cells

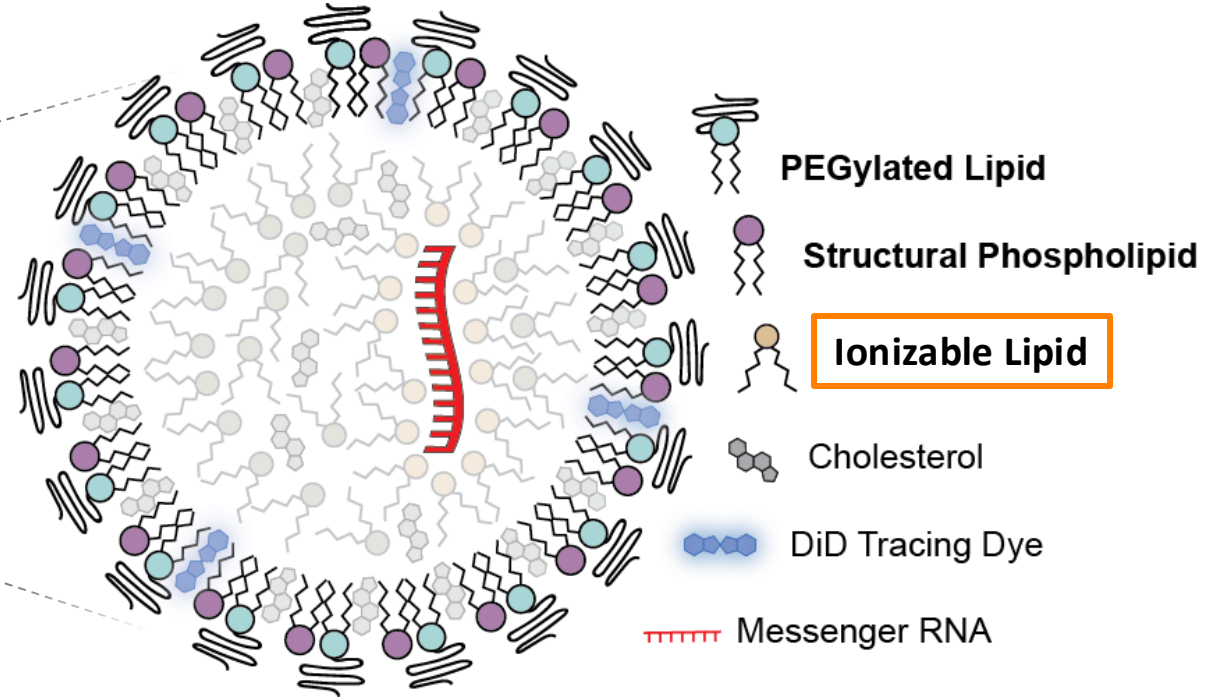


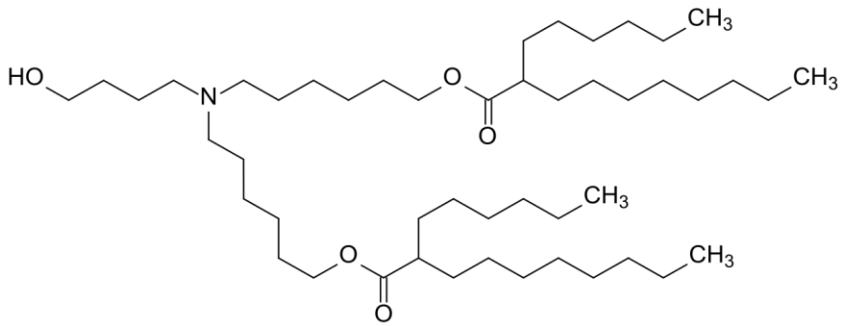
Use lipid formulations developed by
Pieter Cullis (UBC) and Marco Ciufolini (UBC)
Similar formulations approved by FDA in 2018 (ONPATTRO) and in two SARS-CoV-2 mRNA in vaccines (Pfizer/BioNTech/Acuitas and Moderna)

Ionizable lipids self-assemble into LNP under acidic condition with RNA.

Lipid nanoparticles (LNPs) delivery mRNA into cells



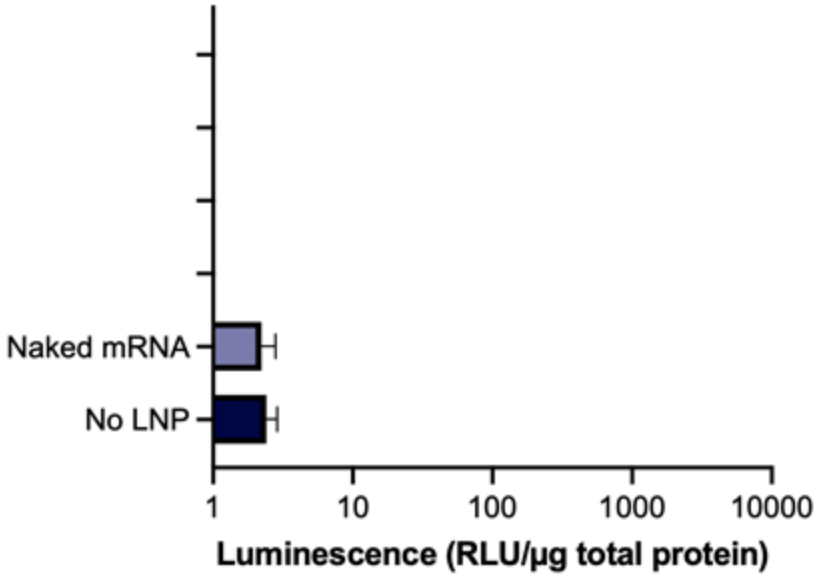




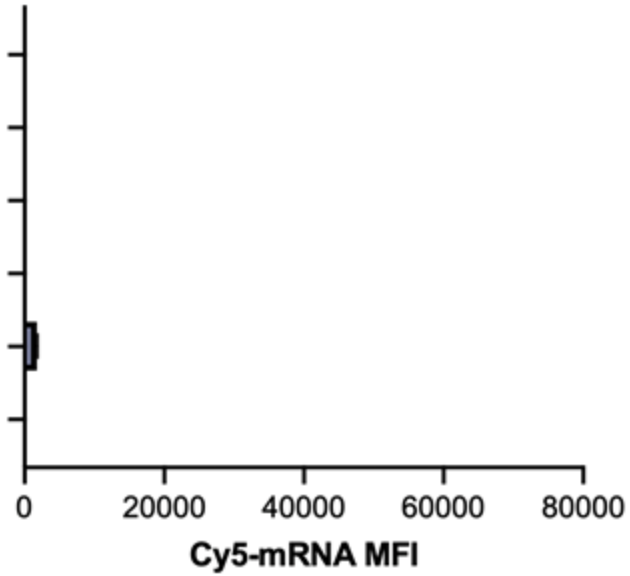
Ionizable lipids self-assemble into LNP under acidic condition with RNA.

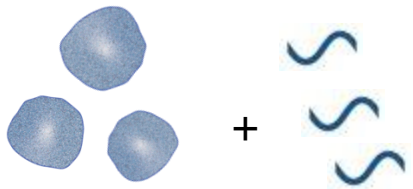
Platelets require specialized transfection agents

Protein Expression



mRNA uptake into platelets





Washed Platelets

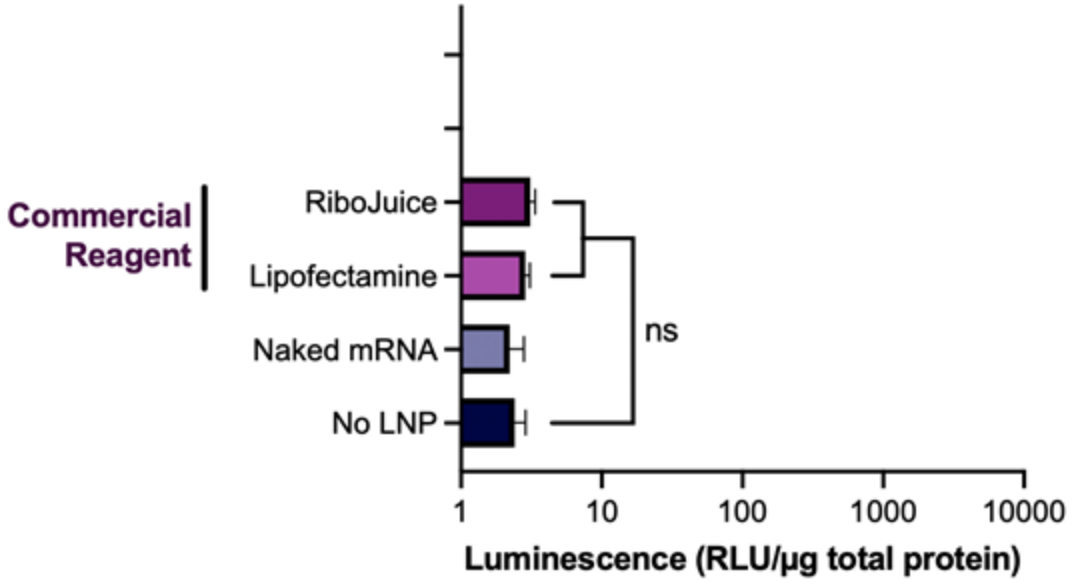
+

Free RNA

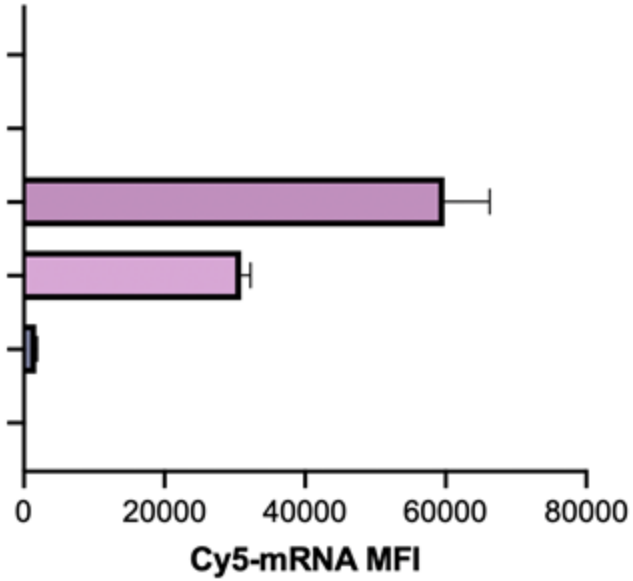
J. Leung*, C. Strong*, KE Badior*, et al. ***Science Advances***, 2023

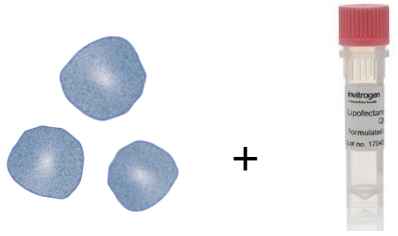
Platelets require specialized transfection agents

Protein Expression



mRNA uptake into platelets



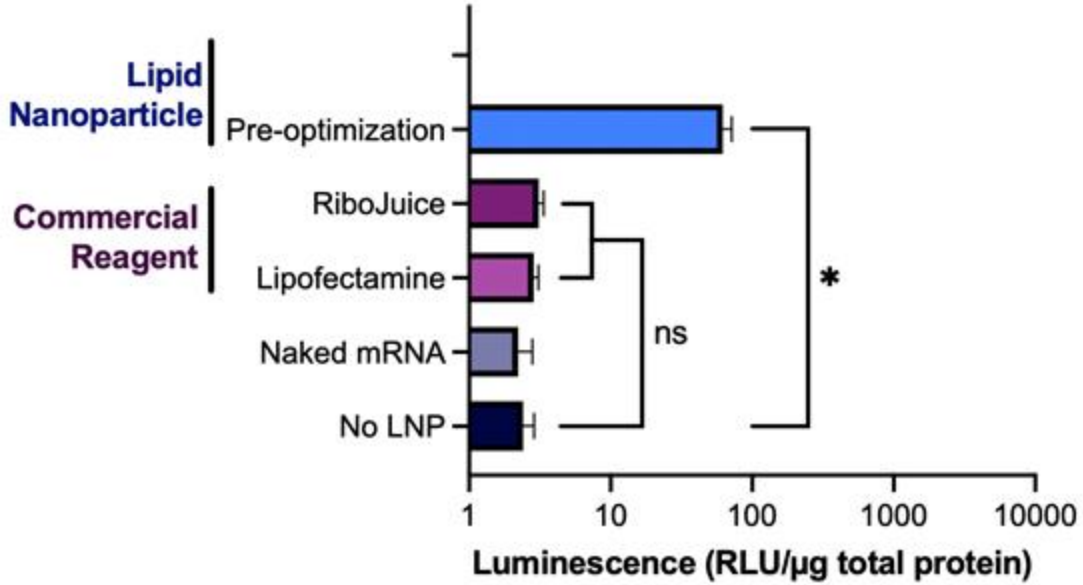


Washed Platelets + Commercial Reagents

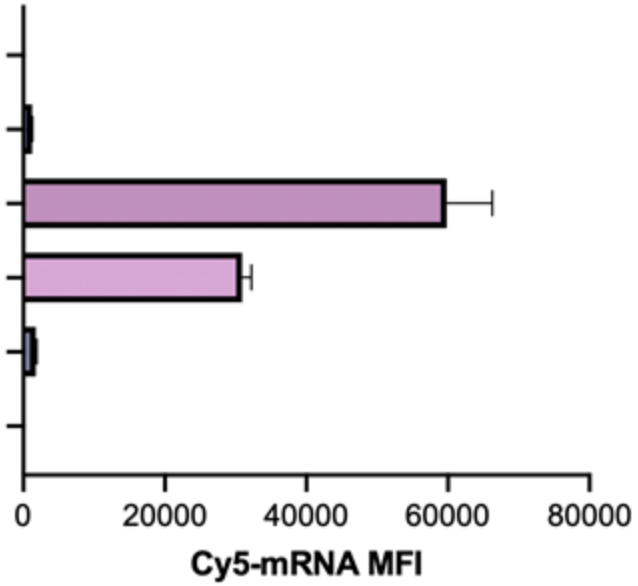
J. Leung*, C. Strong*, KE Badior*, et al. ***Science Advances***, 2023

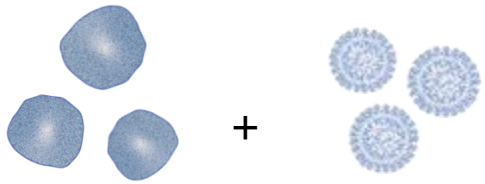
Platelets require specialized transfection agents

Protein Expression



mRNA uptake into platelets



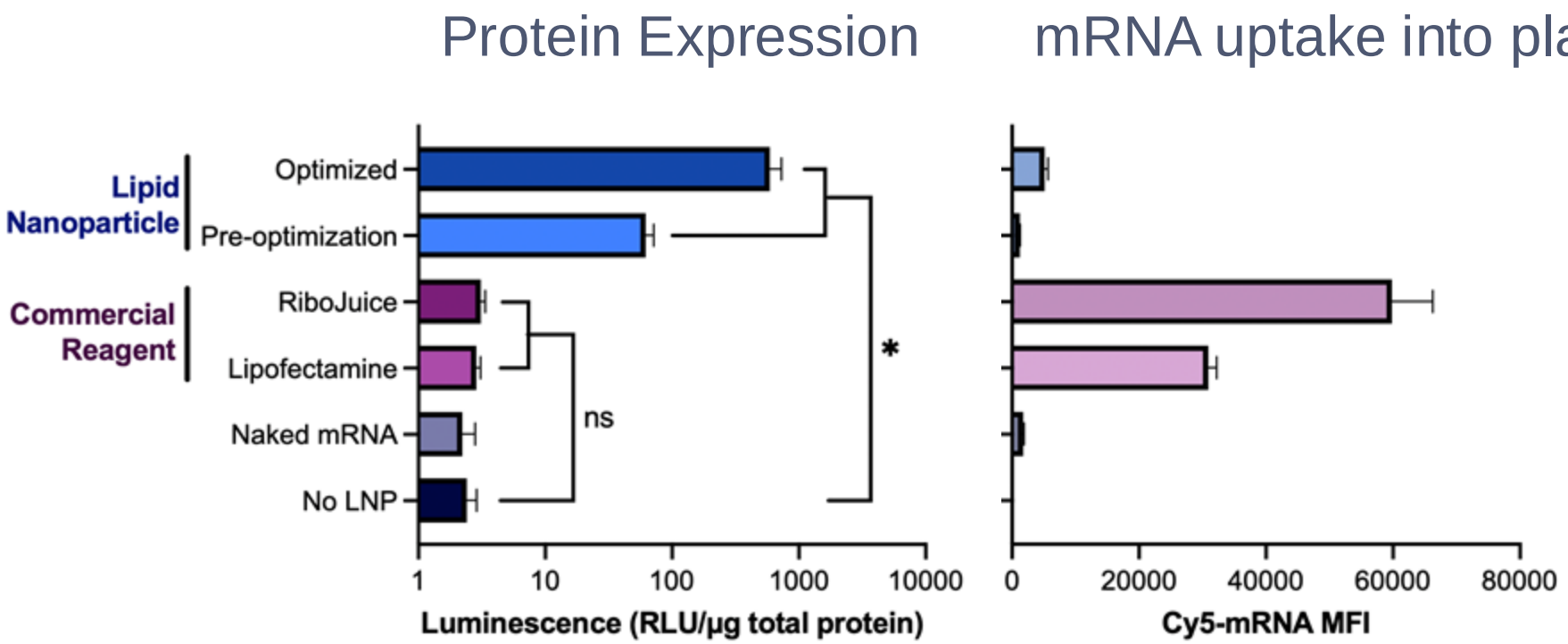


Washed Platelets

Lipid Nanoparticles

J. Leung*, C. Strong*, KE Badior*, et al. ***Science Advances***, 2023

Platelets require specialized transfection agents



10-100 fold increase in [illegible]ence depending on LNP formulation

Washed Platelets

Lipid Nanoparticles

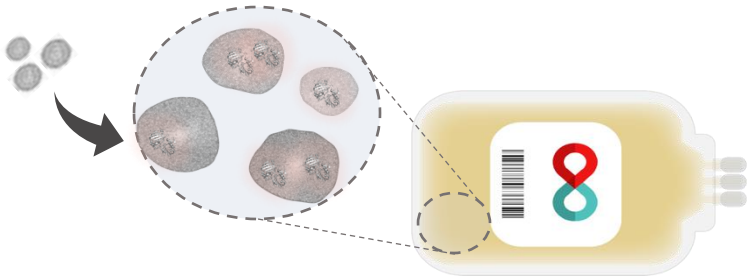
J. Leung*, C. Strong*, KE Badior*, et al. ***Science Advances***, 2023

Our approach to creating platelet cell therapies

Optimize mRNA-LNP Composition for Platelet Transfection

Maximal Protein Expression
Minimal Platelet Activaiton

Characterize transfected platelets and optimize the platform for clinical compatibility

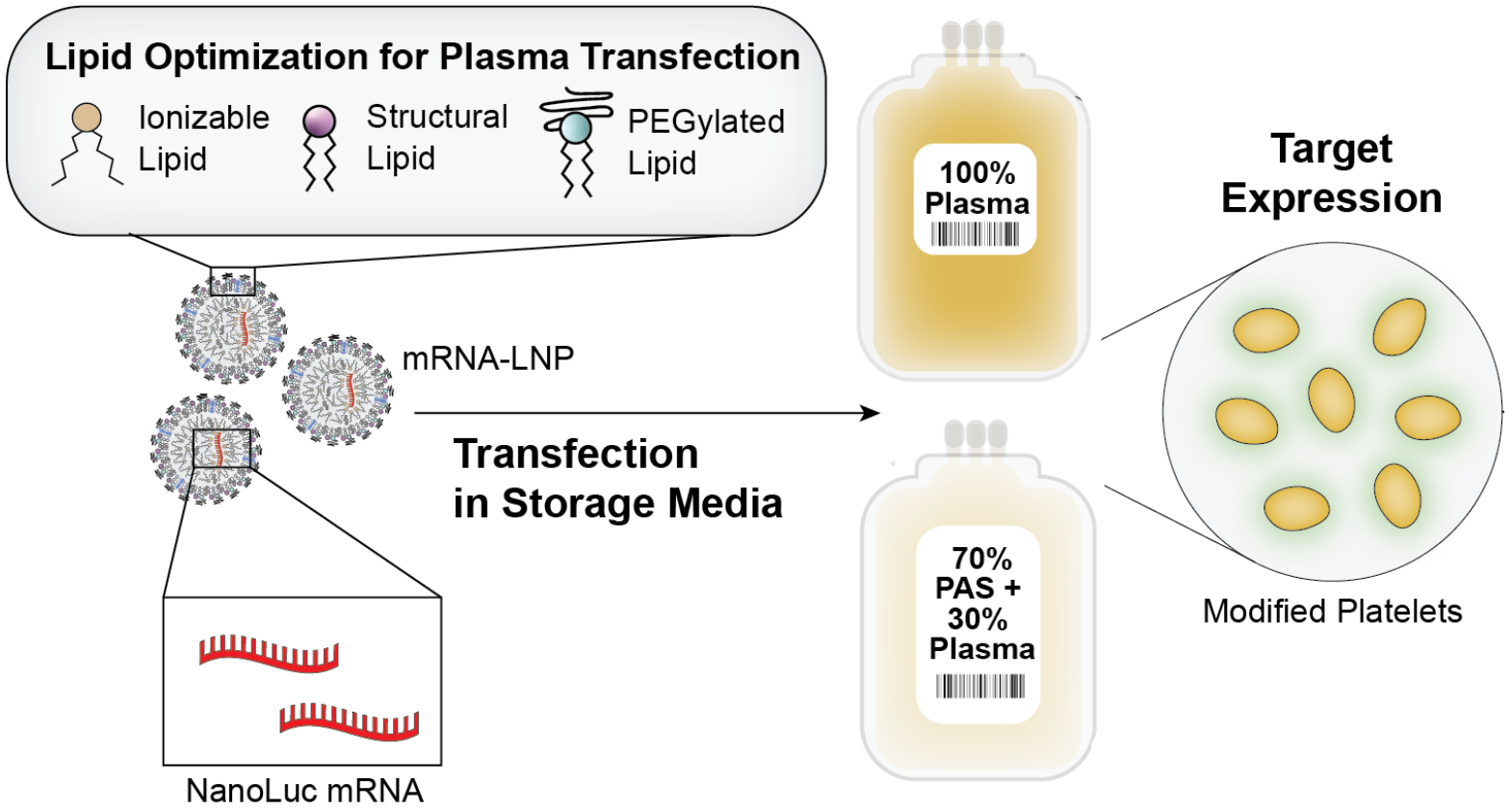


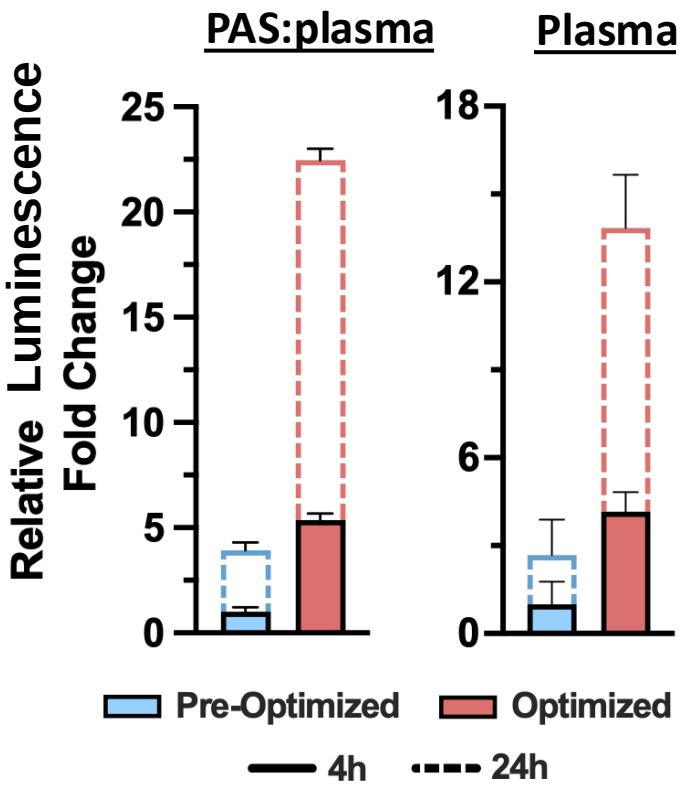
Retained responsiveness
Coagulability
Morphology

Evaluate mRNA-LNP products in vivo

Hemostasis
Circulation
Cargo Exchange/Transfer

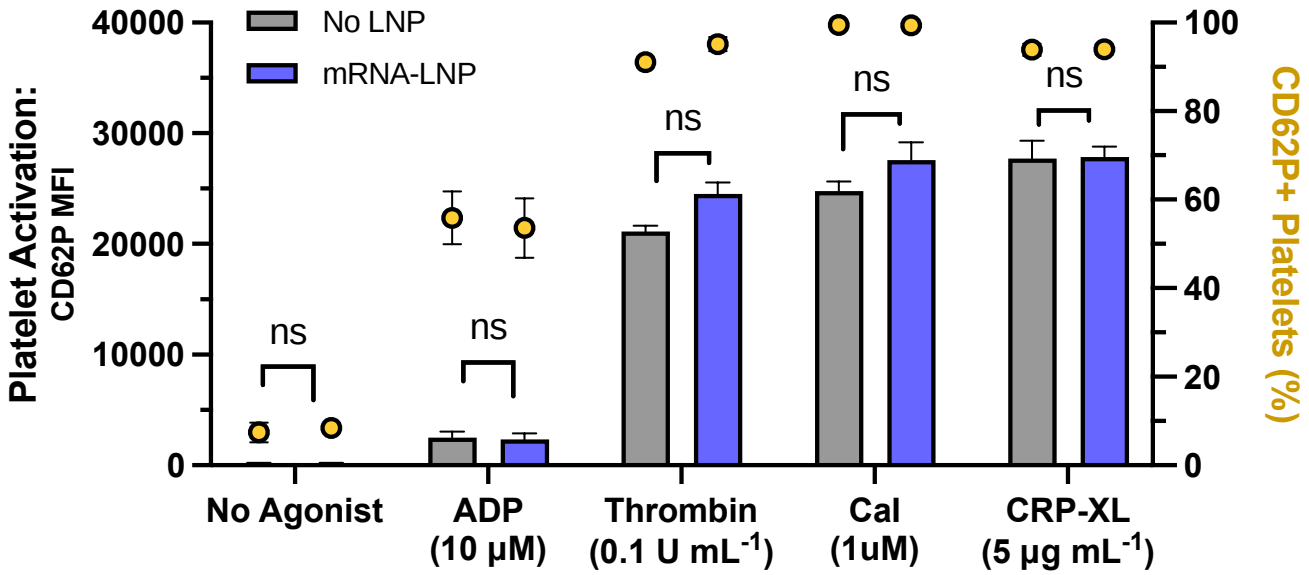
Platelets can be engineered with mRNA-LNP directly in their storage media

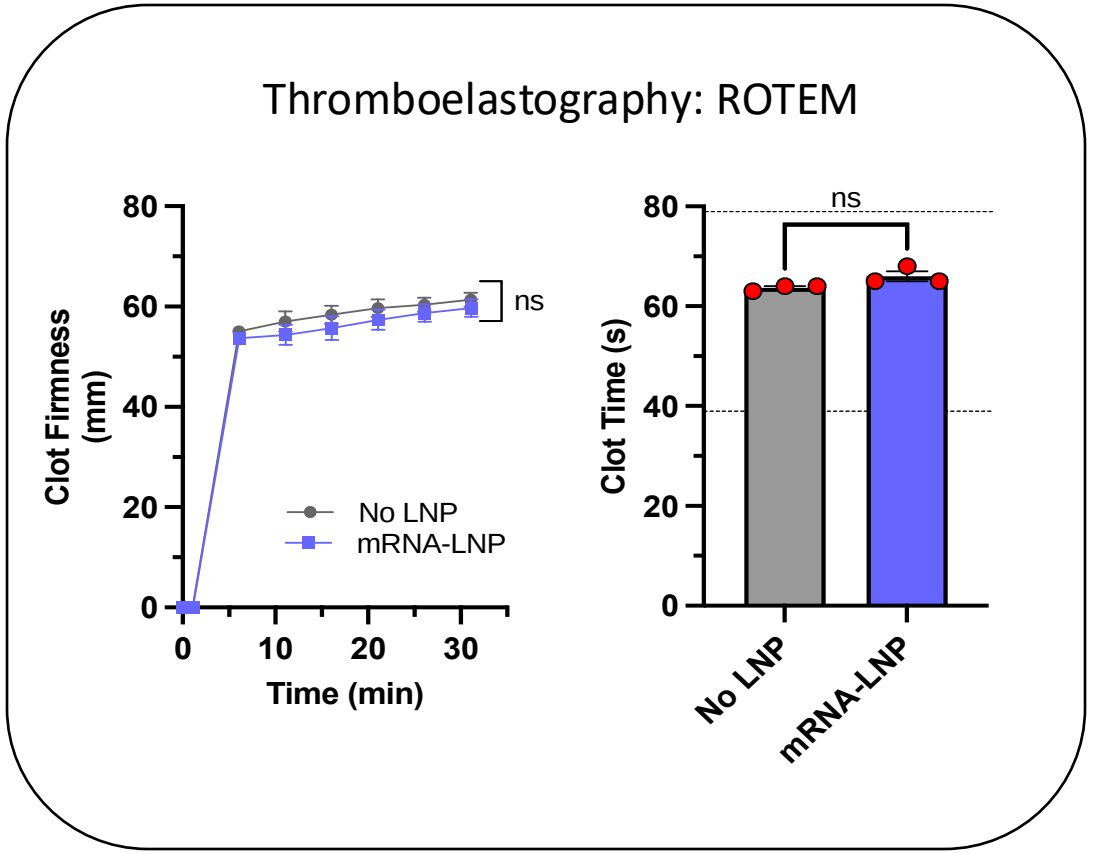




C. Strong*, J. Leung*, E. Kang*… D Devine, E Jan, PR Cullis, CJ Kastrup, ***Blood***, 2024

mRNA-LNP transfected platelets maintain their response to agonists and contribute to clot formation and stiffness

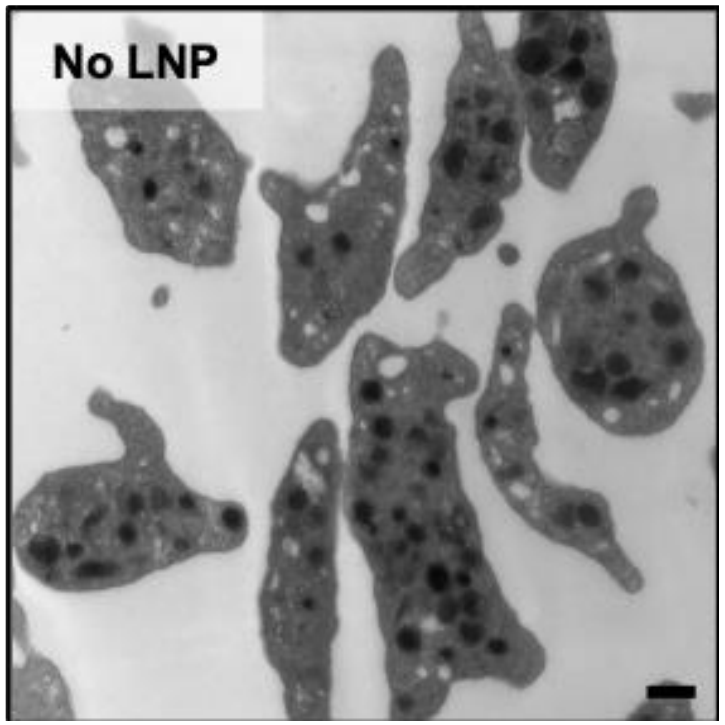




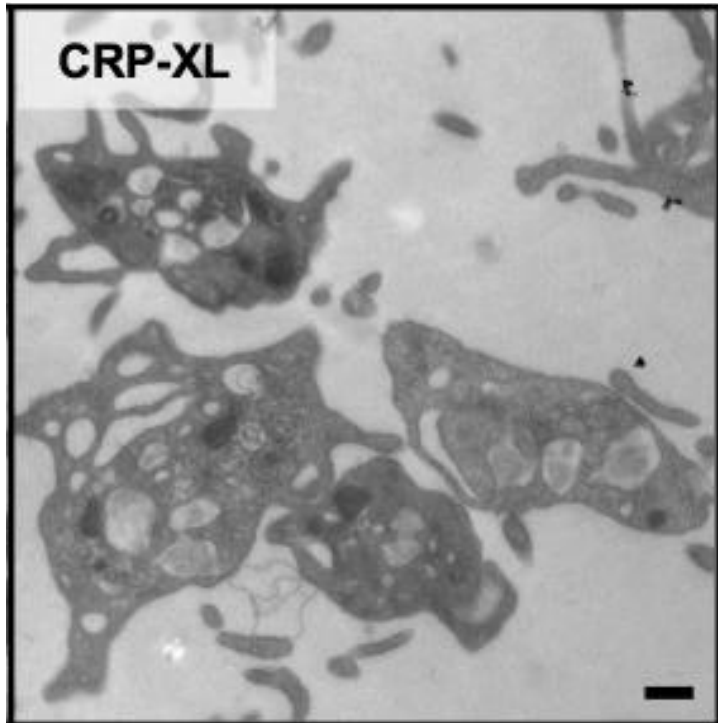
C. Strong*, J. Leung*, E. Kang*… D Devine, E Jan, PR Cullis, CJ Kastrup, ***Blood***, 2024

mRNA-LNP transfected platelets have normal morphology

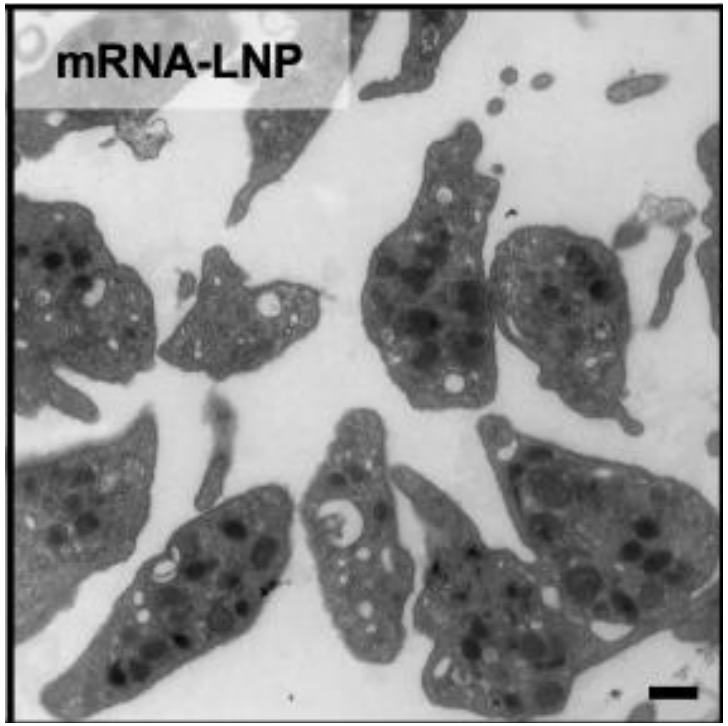
Resting Platelet



Activated Platelet



LNP Transfected Platelet



C. Strong*, J. Leung*, E. Kang*… D Devine, E Jan, PR Cullis, CJ Kastrup, ***Blood***, 2024

Our approach to creating platelet cell therapies

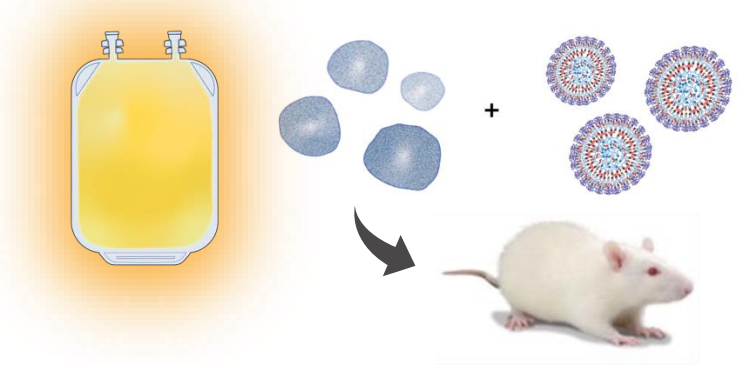
Optimize mRNA-LNP Composition for Platelet Transfection

Maximal Protein Expression
Minimal Platelet Activation

Characterize transfected platelets and optimize the platform for clinical compatibility

Retained responsiveness
Coagulability
Morphology

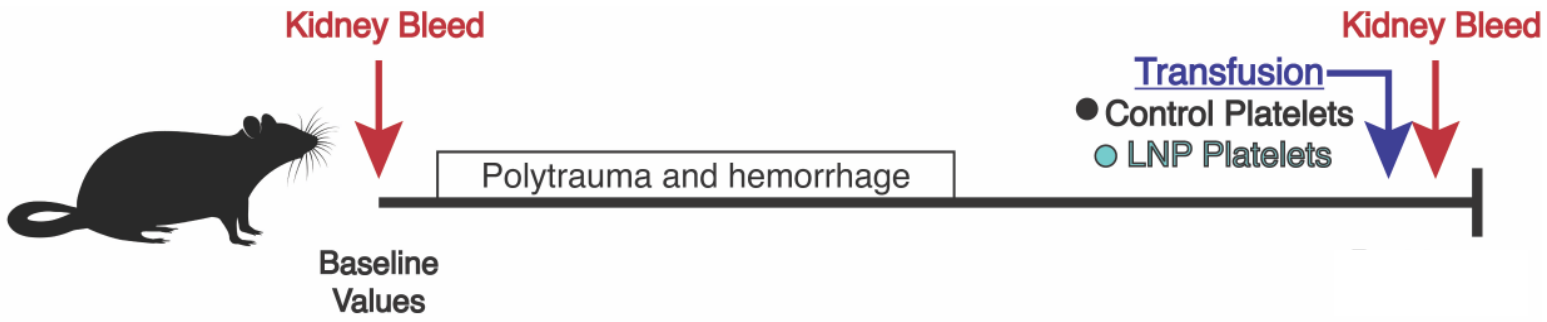
Evaluate mRNA-LNP products in vivo



Hemostasis
Circulation
Cargo Exchange/Transfer

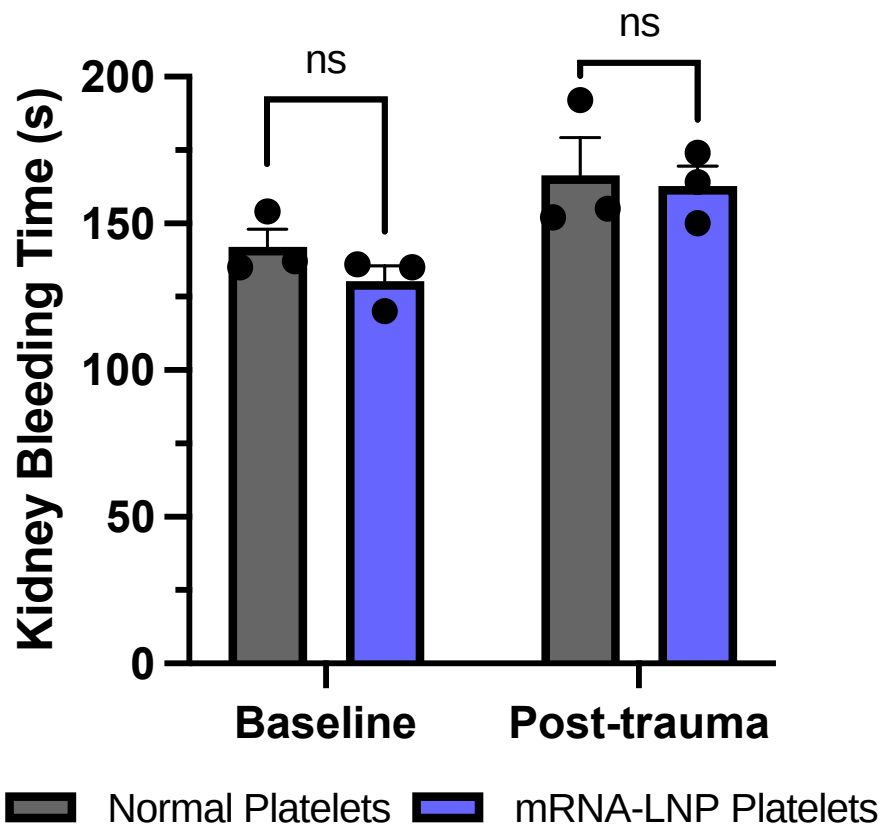
Edited platelets are tolerated *in vivo* and contribute to hemostasis

Rat model of polytrauma



Expect no difference in bleeding (mRNA expresses luciferase)

- With **Andre Cap**, **Adam Meledeo** & **Xiaowu Wu** (US Army Institute of Surgical Research)

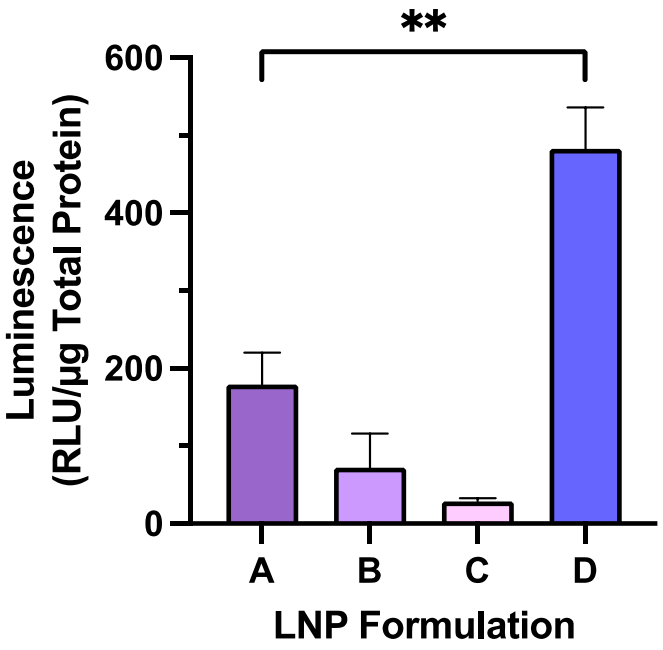


No statistical difference in bleed time or blood loss

J. Leung*, C. Strong*, KE Badior*, et al. ***Science Advances***, 2023

Can edited platelets be transfused and contribute to hemostasis?

Transfection of rat platelets

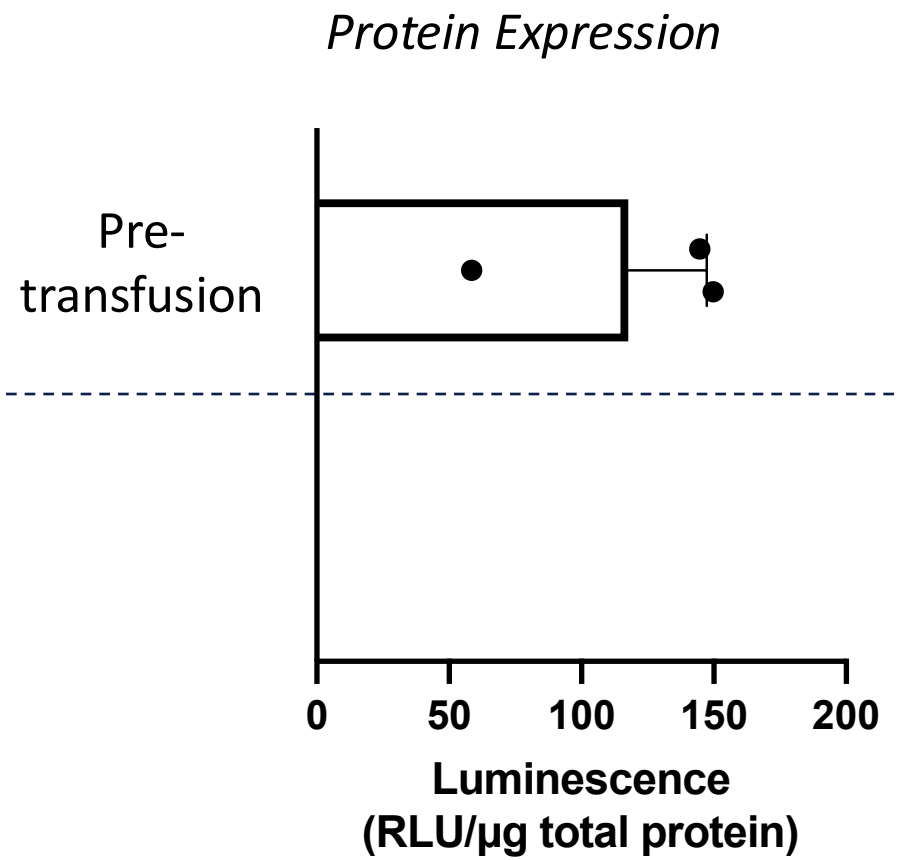


Rat model of polytrauma

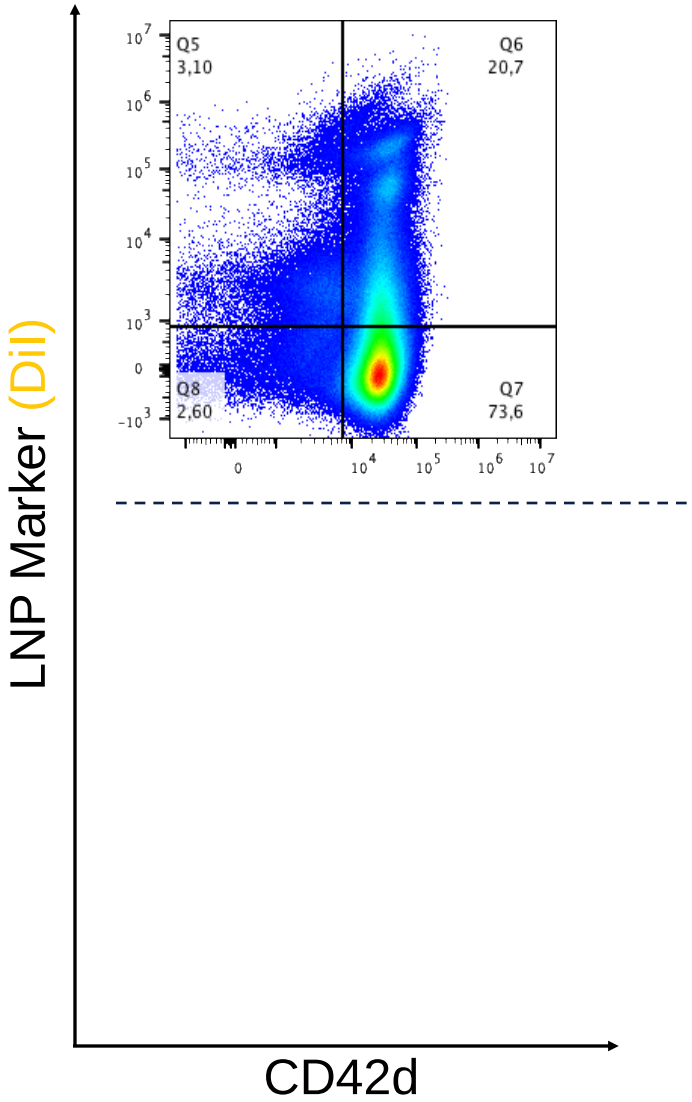
Expect no difference in bleeding
(mRNA expresses luciferase)

- With **Andre Cap**, **Adam Meledeo** & **Xiaowu Wu** (US Army Institute of Surgical Research)

Transfected platelets circulate after transfusion

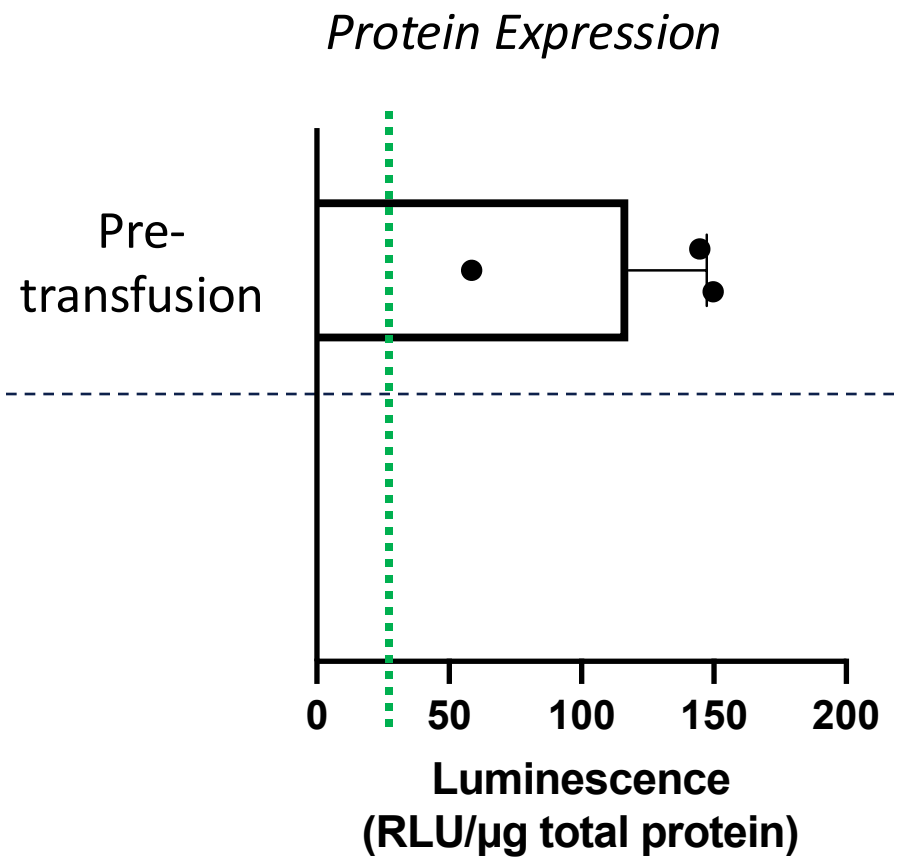


Platelets express nanoLuc
~20% labeled for LNP



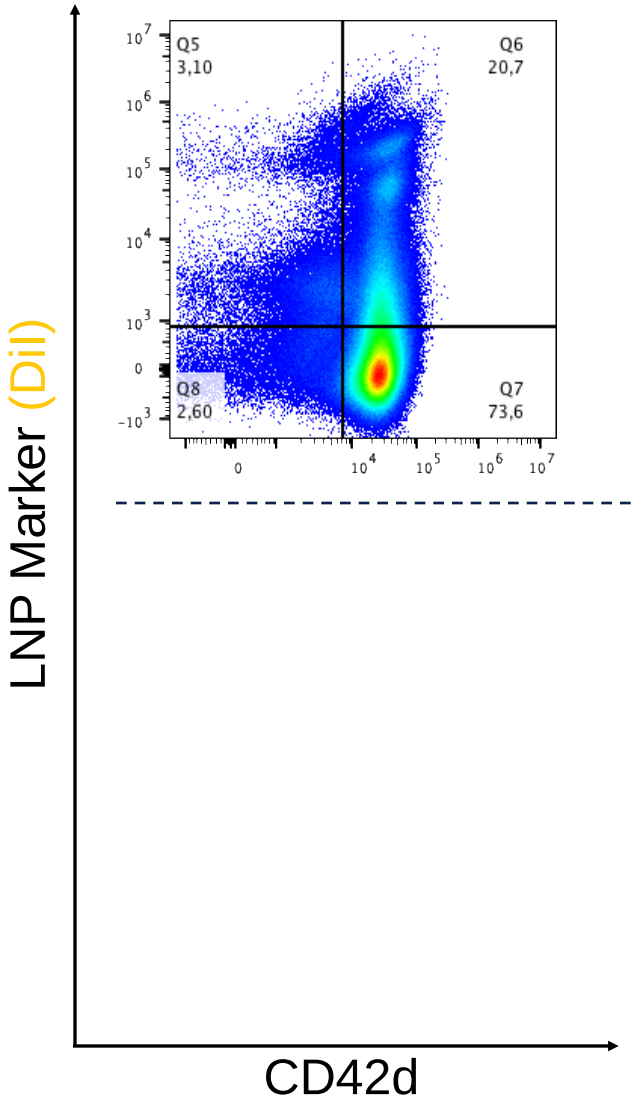
J. Leung*, C. Strong*, KE Badior*, et al. ***Science Advances***, 2023

Transfected platelets circulate after transfusion



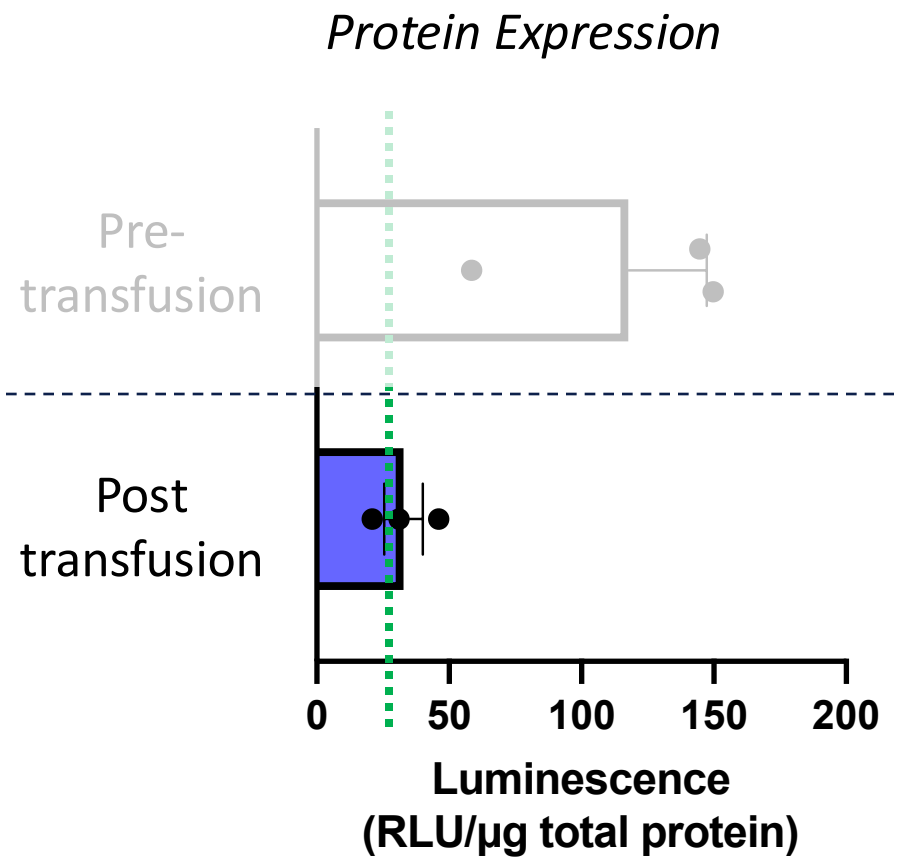
Platelets express nanoLuc
~20% labeled for LNP

LNP-platelets were diluted 20-fold upon transfusion.



J. Leung*, C. Strong*, KE Badior*, et al. ***Science Advances***, 2023

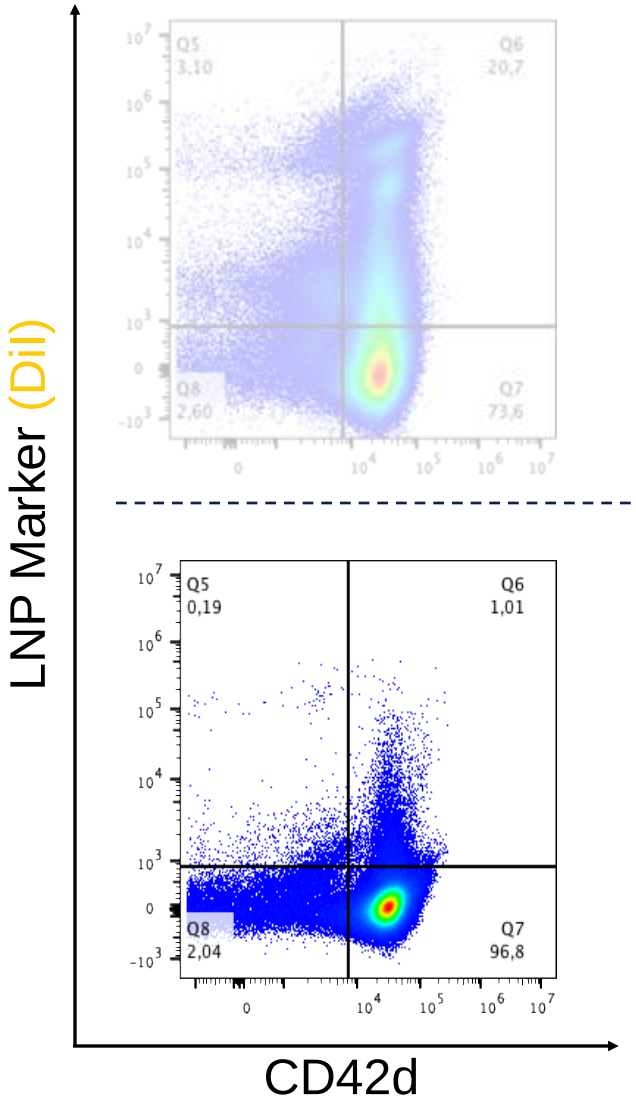
Transfected platelets circulate after transfusion



Platelets express nanoLuc
~20% labeled for LNP

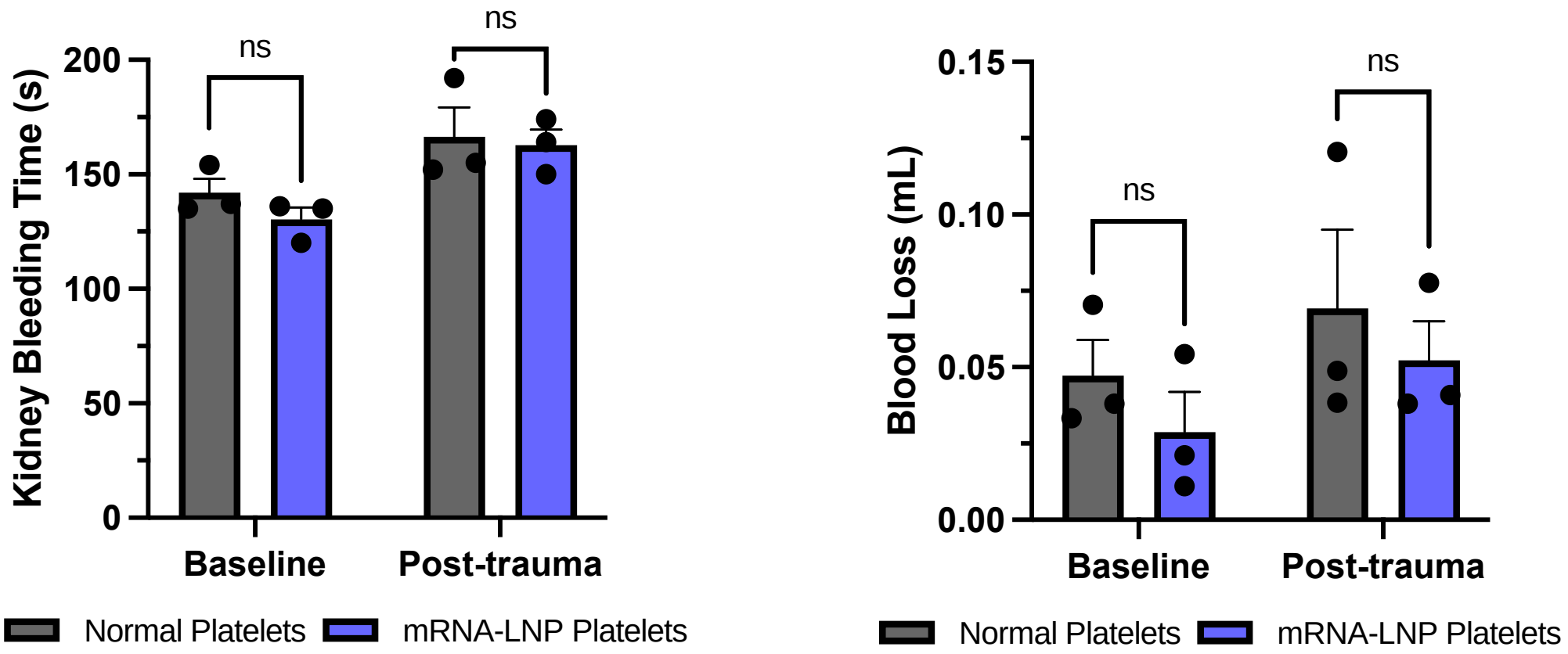
LNP-platelets were diluted 20-fold upon transfusion.

NanoLuc detected in platelets,
~1% labeled for LNP



J. Leung*, C. Strong*, KE Badior*, et al. ***Science Advances***, 2023

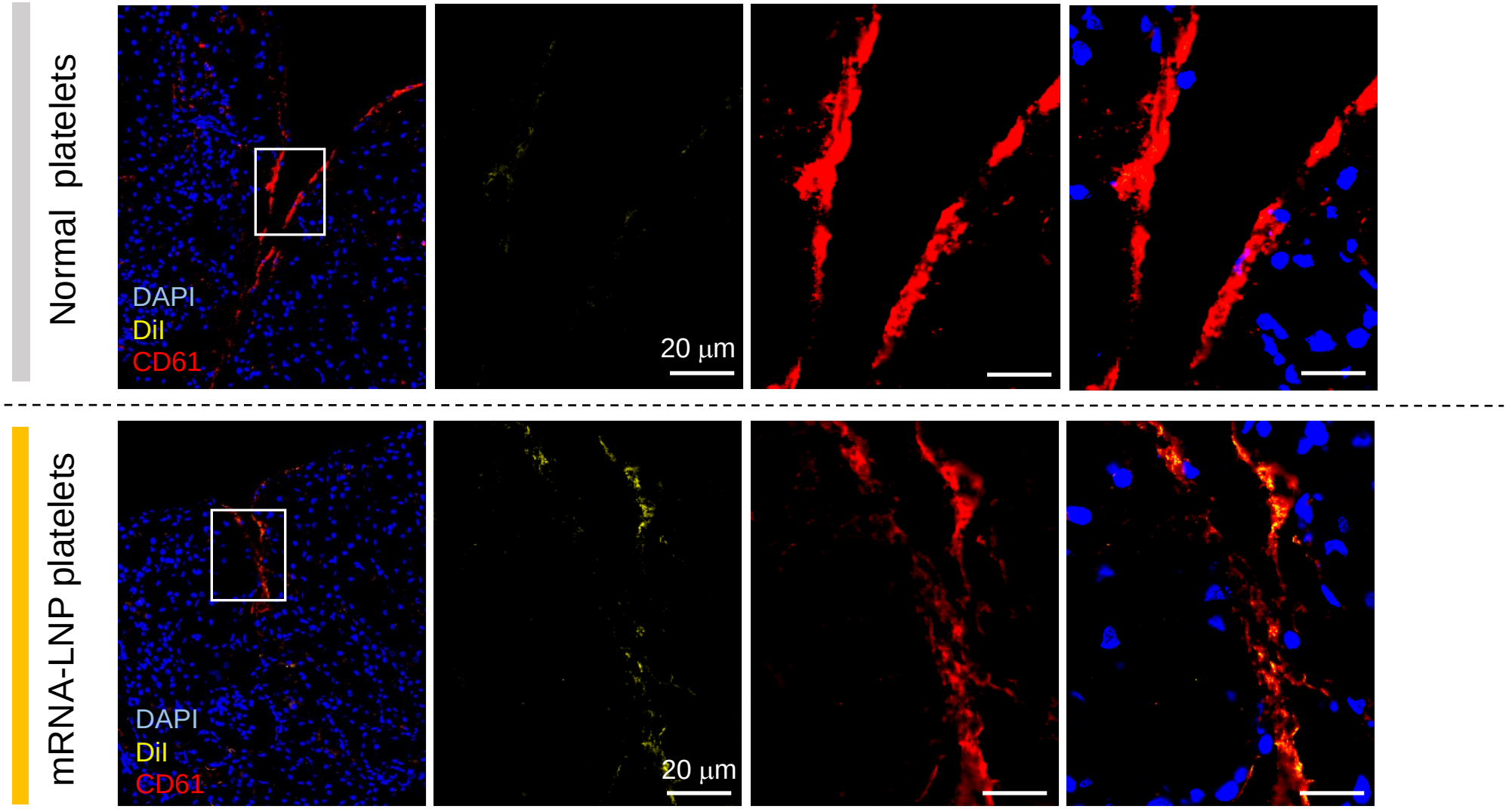
Edited platelets are tolerated *in vivo* and contribute to hemostasis



No statistical difference in bleed time or blood loss

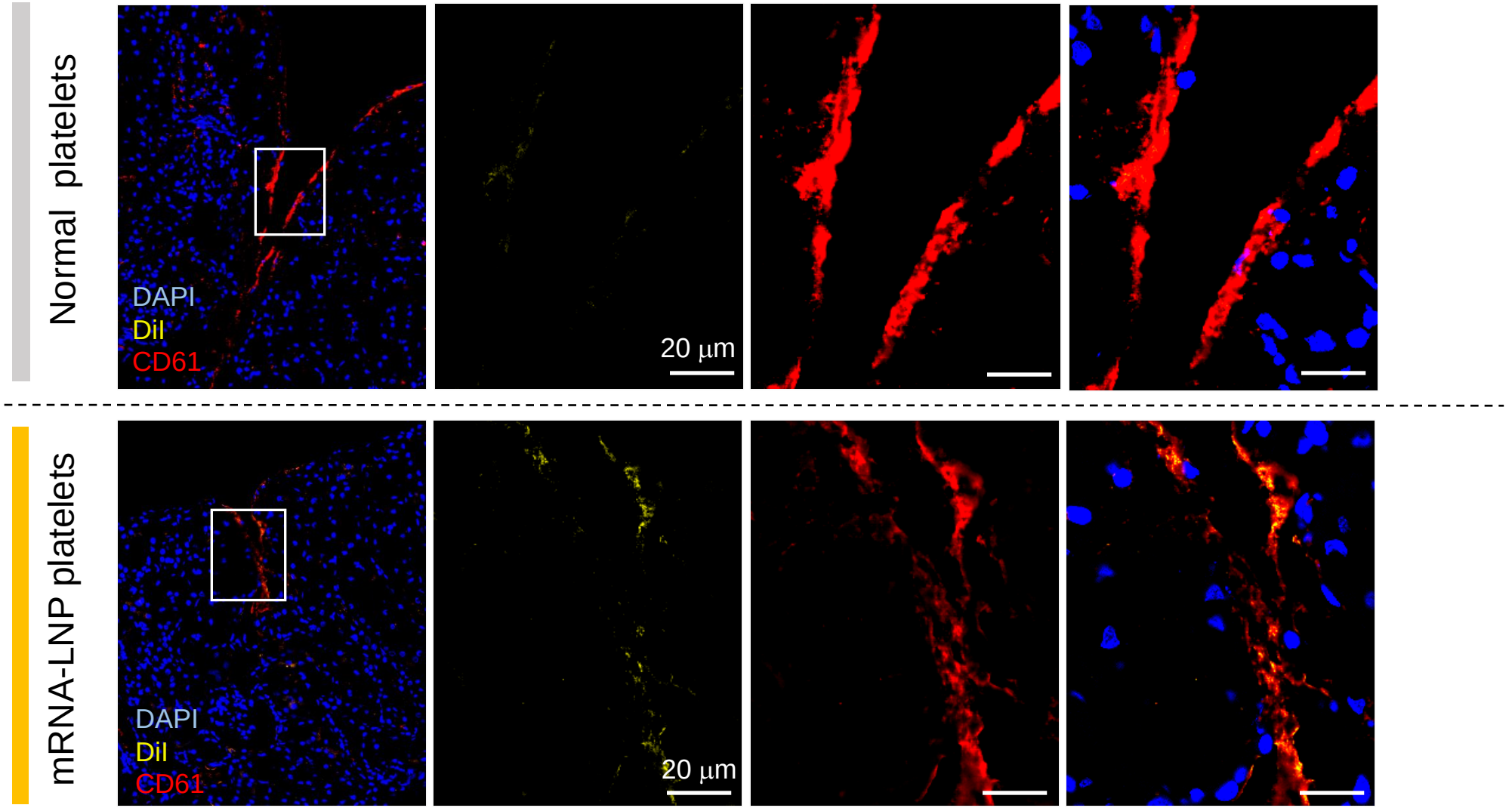
J. Leung*, C. Strong*, KE Badior*, et al. ***Science Advances***, 2023

Transfected platelets accumulate at vascular damage: Local delivery of mRNA and protein



Leung*, Strong*, Badior*, Robertson, Wu , Meledeo, Kang, Cap, Devine, Jan, Cullis, Kastrup, ***Science Advances***, 2023

Transfected platelets accumulate at vascular damage:
Local delivery of mRNA and protein

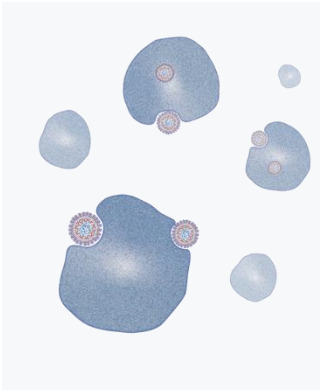


Leung*, Strong*, Badior*, Robertson, Wu , Meledeo, Kang, Cap, Devine, Jan, Cullis, Kastrup, ***Science Advances***, 2023

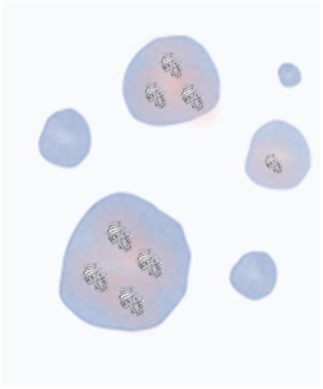
A Current Direction: Using transfusable platelets to locally deliver RNA and protein

Platelets naturally circulate to and accumulate at extra-hepatic disease sites, where they interact with a wide variety of other cell types.

Platelets + mRNA-LNP



Modified Platelets



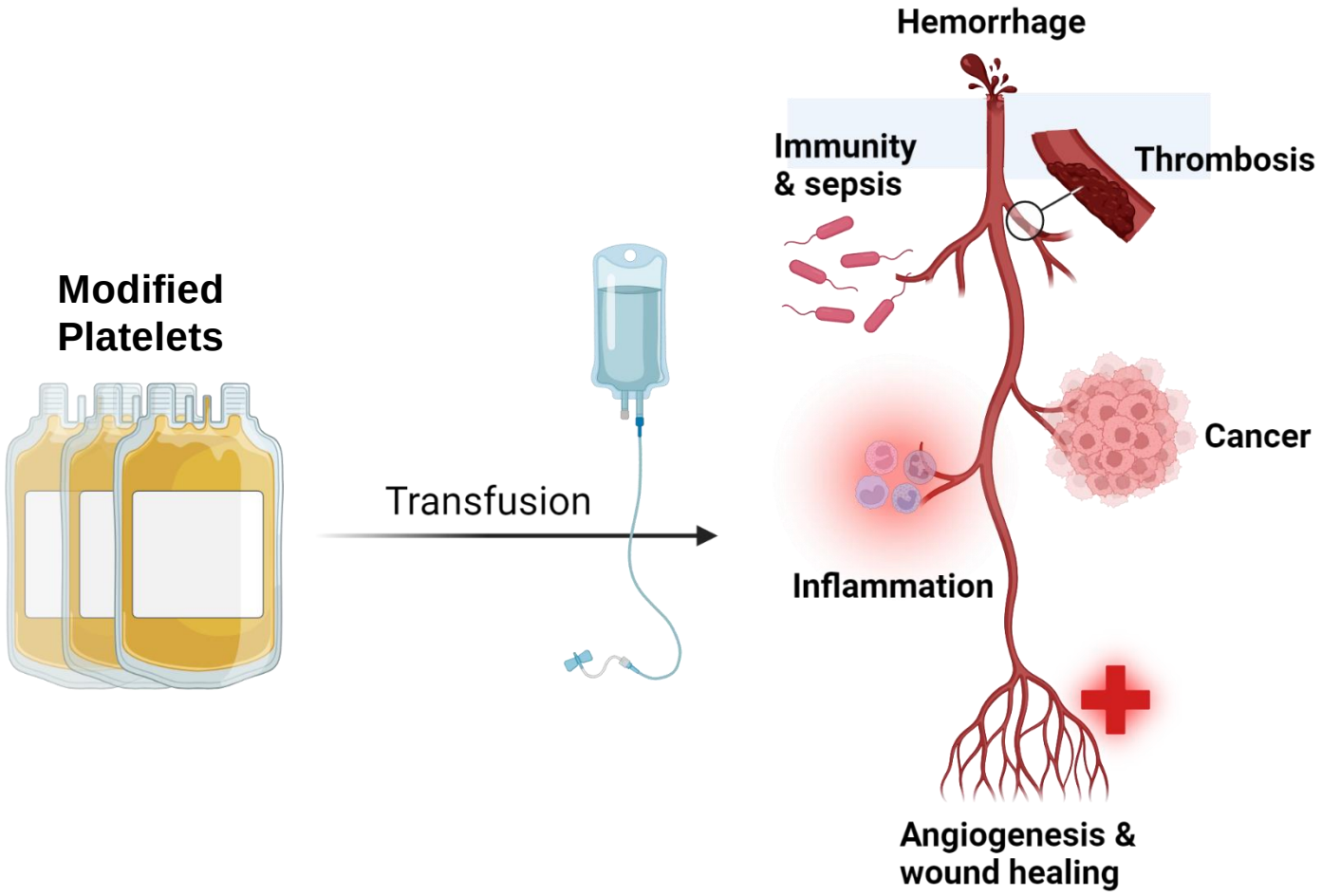
RNA-Modified Platelets

Platform Technology

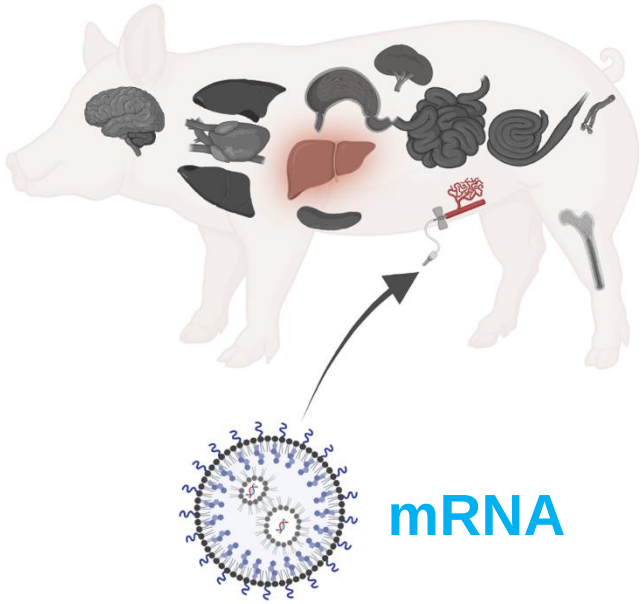
- Improved Transfusions
- Targeted Therapeutics
- Research Tool

A Current Direction: Using transfusable platelets to locally deliver RNA and protein

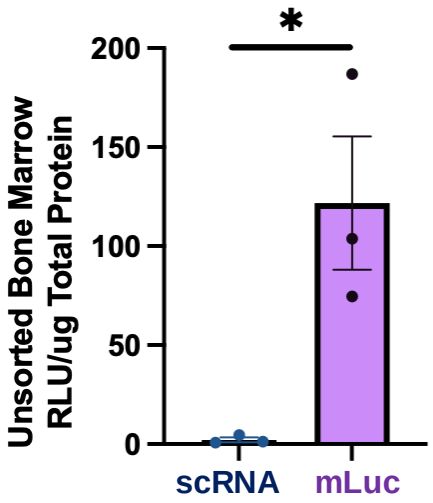
Platelets naturally circulate to and accumulate at extra-hepatic disease sites, where they interact with a wide variety of other cell types.

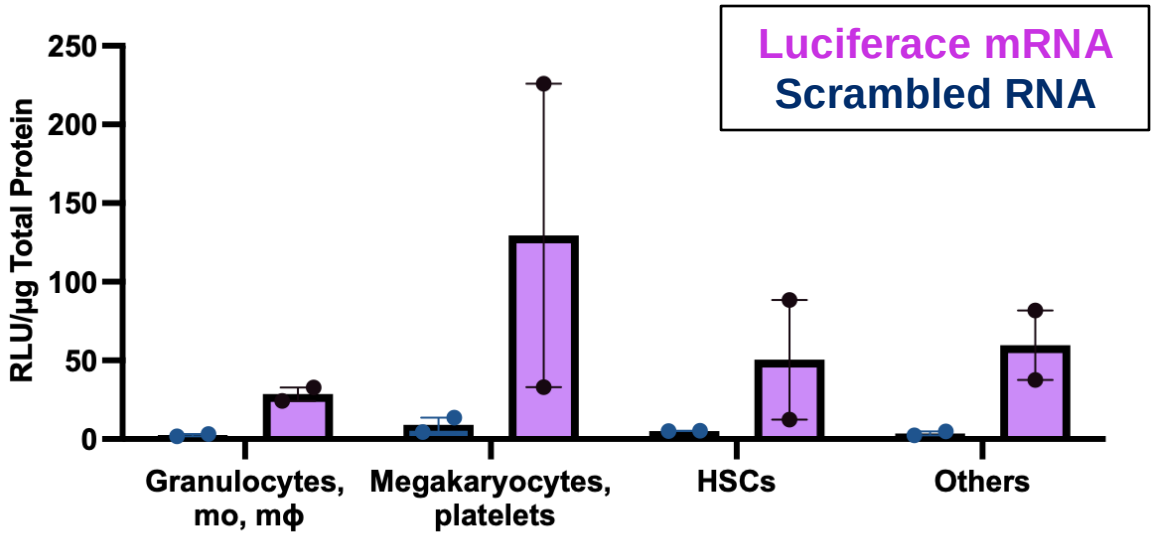


Future: Controlling protein production in all tissues



Expression of protein from mRNA-LNP in the bone marrow

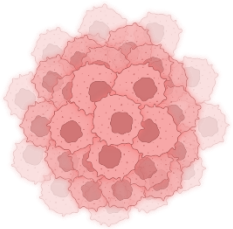
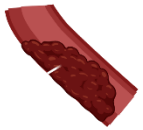
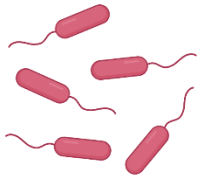
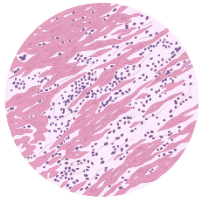
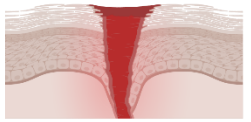
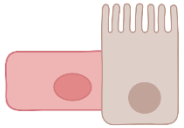
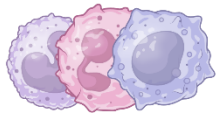
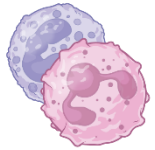
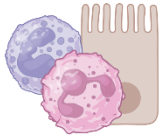
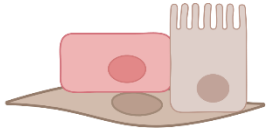




Ferraresso F, Badior K, Seadler M, Wietrzny A, Zhang Y, Cau MF, Haugen A, Dyer MR, Cullis PR, Jan E, Kastrup CJ
Molecular Therapy – Methods & Clinical Development 2024

Example applications: transfusable RNA-loaded platelets

Transfusable platelets as a versatile product to treat extra-hepatic diseases and conditions

	 Cancer	 Hemorrhage	 Thrombosis	 Sepsis	 Inflammation	 Tissue damage
Transfer target 	 tumor cells	 endothelial, epithelial cells	 neutrophils, monocytes, macrophages	 leukocytes, lymphocytes	 leukocytes, lymphocytes, epithelial cells	 endothelial, epithelial cells, fibroblasts
Loaded RNA 	pro-apoptotic (mRNA) oncogenes (siRNA)	clotting factors (mRNA) antifibrinolytic proteins (mRNA) platelet activators (mRNA) fibrinolytic proteins (siRNA)	fibrinolytic proteins (mRNA) platelet activators (mRNA) antifibrinolytic proteins (siRNA)	chemokines, cytokines (mRNA)	pro-inflammatory cytokines (mRNA) MAPK inhibitors (mRNA) anti-inflammatory cytokines (siRNA)	growth factors (mRNA) metalloproteases (mRNA)

Katherine Badior
Massimo Cau
Francesca Ferraresso
Emma Kang
Lih Jiin Juang
Jerry Leung
Manoj Paul
Noah Peng
Madelaine Robertson
Halen Turner
Monica Seadler
Amy Strilchuk
Colton Strong

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

NMIN
NANOMEDICINES INNOVATION NETWORK
RÉSEAU D'INNOVATION NANOMÉDECINES

Canada's Department of National Defence

DRDC
RDDC

versiti™
Blood Research Institute

CBR
THE CENTRE FOR BLOOD RESEARCH

Canadian Blood Services
BLOOD
PLASMA
STEM CELLS
ORGANS
& TISSUES

DEPARTMENT OF THE ARMY • UNITED STATES OF AMERICA •
THIS WE'LL DEFEND
1775