University of California San Francisco

ZUCKERBERG SAN FRANCISCO GENERAL Hospital and Trauma Center

Changes in Platelet RNA Architecture with Storage: *Uncovering Important RNA Metabolism Considerations for Precision Transfusion Medicine*

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Disclosures

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- Coagulant Therapeutics
- Haemonetics
- Cellphire

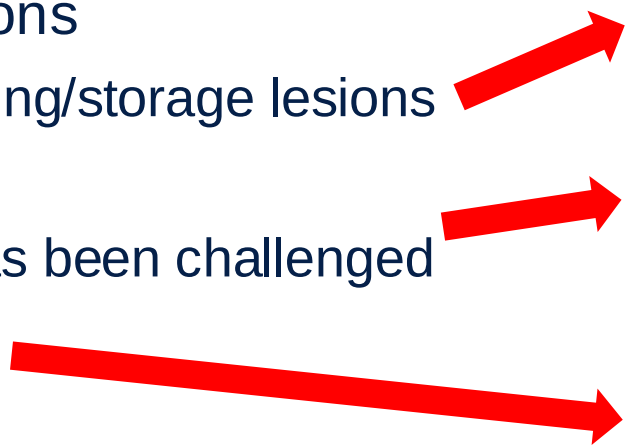
Family

- Husband, founder of CaptureDx


Platelets

The challenges… and solutions

- Trauma patients are still dying from failures in hemostasis
- Platelets are central in hemostasis
- Platelet transfusions
  - Platelet processing/storage lesions
  - Short shelf life
  - Effectiveness has been challenged
  - Unmet demand



- Increase platelet supply & shelf/life

- Decrease processing/storage lesions

- Alternative methods and products

COAGULATION

Comparison of platelet quality and function across apheresis collection platforms

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jth

ORIGINAL ARTICLE

Cold-stored platelet hemostatic capacity is maintained for three weeks of storage and associated with taurine metabolism

Susan M. Shea[1,2] | Julie A. Reisz[3] | Emily P. Mihalko[2] | Katelin C. Rahn[2] | Rassam M. G. Rassam[2] | Alisha Chitrakar[4] | Fabia Gamboni[3] | Angelo D'Alessandro[3] | Philip C. Spinella[1,2,5] | Kimberly A. Thomas[1,4,6]

TRANSFUSION SCIENCE ADVANCES | RESEARCH ARTICLE

Genetically engineered transfusable platelets using mRNA lipid nanoparticles

Jerry Leung[1,2,3,4†], Colton Strong[1,2,3†], Katherine E. Badior[5†], Madelaine Robertson[1,2,3,4], Xiaowu Wu[6], Michael A. Meledeo[6], Emma Kang[3,7], Manoj Paul[5], Yusuke Sato[8], Hideyoshi Harashima[8], Andrew P. Cap[6], Dana V. Devine[2,3,7,9], Eric Jan[2], Pieter R. Cullis[2,4], Christian J. Kastrup[1,2,3,5,10*]

Platelet transfusions are essential for managing bleeding and hemostatic dysfunction and could be expanded as a cell therapy due to the multifunctional role of platelets in various diseases. Creating these cell therapies will require modifying transfusable donor platelets to express therapeutic proteins. However, there are currently no appropriate methods for genetically modifying platelets collected from blood donors. Here, we describe an approach using platelet-optimized lipid nanoparticles containing mRNA (mRNA-LNP) to enable exogenous protein expression in human and rat platelets. Within the library of mRNA-LNP tested, exogenous protein expression did not require nor correlate with platelet activation. Transfected platelets retained hemostatic function and accumulated in regions of vascular damage after transfusion into rats with hemorrhagic shock. We expect this technology will expand the therapeutic potential of platelets.

› Blood. 2024 Aug 27:blood.2024024405. doi: 10.1182/blood.2024024405. Online ahead of print.

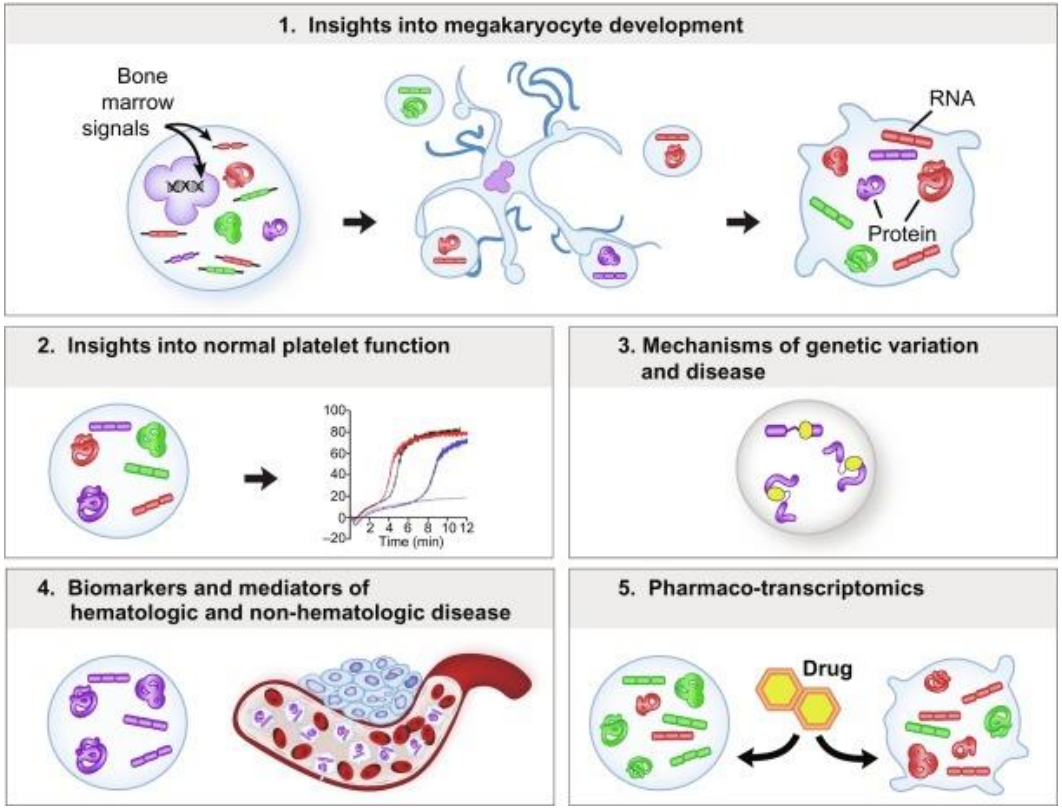
Genetic Engineering of Transfusable Platelets with mRNA–Lipid Nanoparticles is Compatible with Blood Banking Practices

Colton Strong [1], Jerry Leung [1], Emma Kang [1], Katherine E Badior [2], Madelaine K Robertson [1], Nicolas Pereyra [1], Elyn Marie Rowe [1], Amanda Wietrzny [2], Brenda Ma [1], Zechariah Noronha [1], Deaglan Arnold [3], Marco A Ciufolini [3], Dana V Devine [4], Eric Jan [1], Pieter R Cullis [5], Christian J Kastrup [4]

Affiliations + expand

PMID: 39190426 DOI: 10.1182/blood.2024024405

The platelet RNA window



Platelet processing/storage

2019 Reference: Jesse W. Rowley, Andrew S. Weyrich, Paul F. Bray. The Platelet Transcriptome in Health and Disease, Platelets (Fourth Edition), 2019

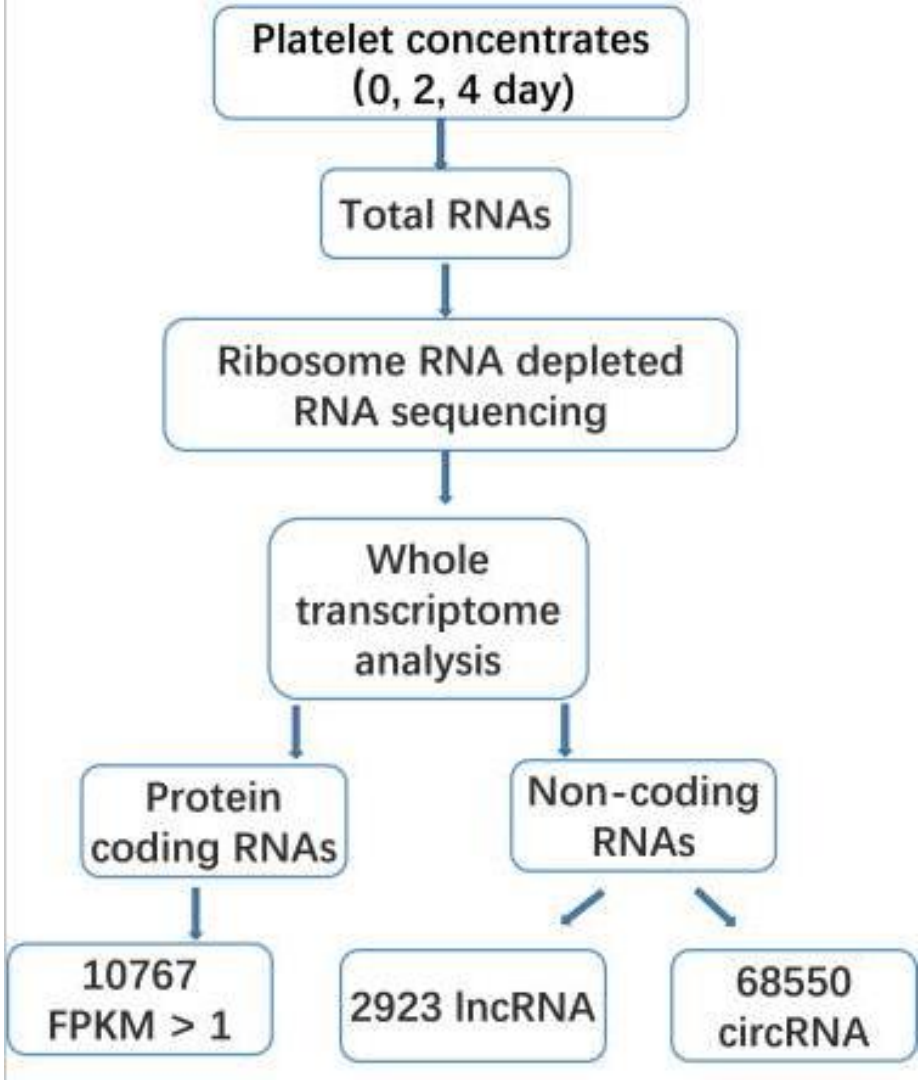
COLLECTION, PRODUCTION AND STORAGE OF BLOOD COMPONENTS

Original article

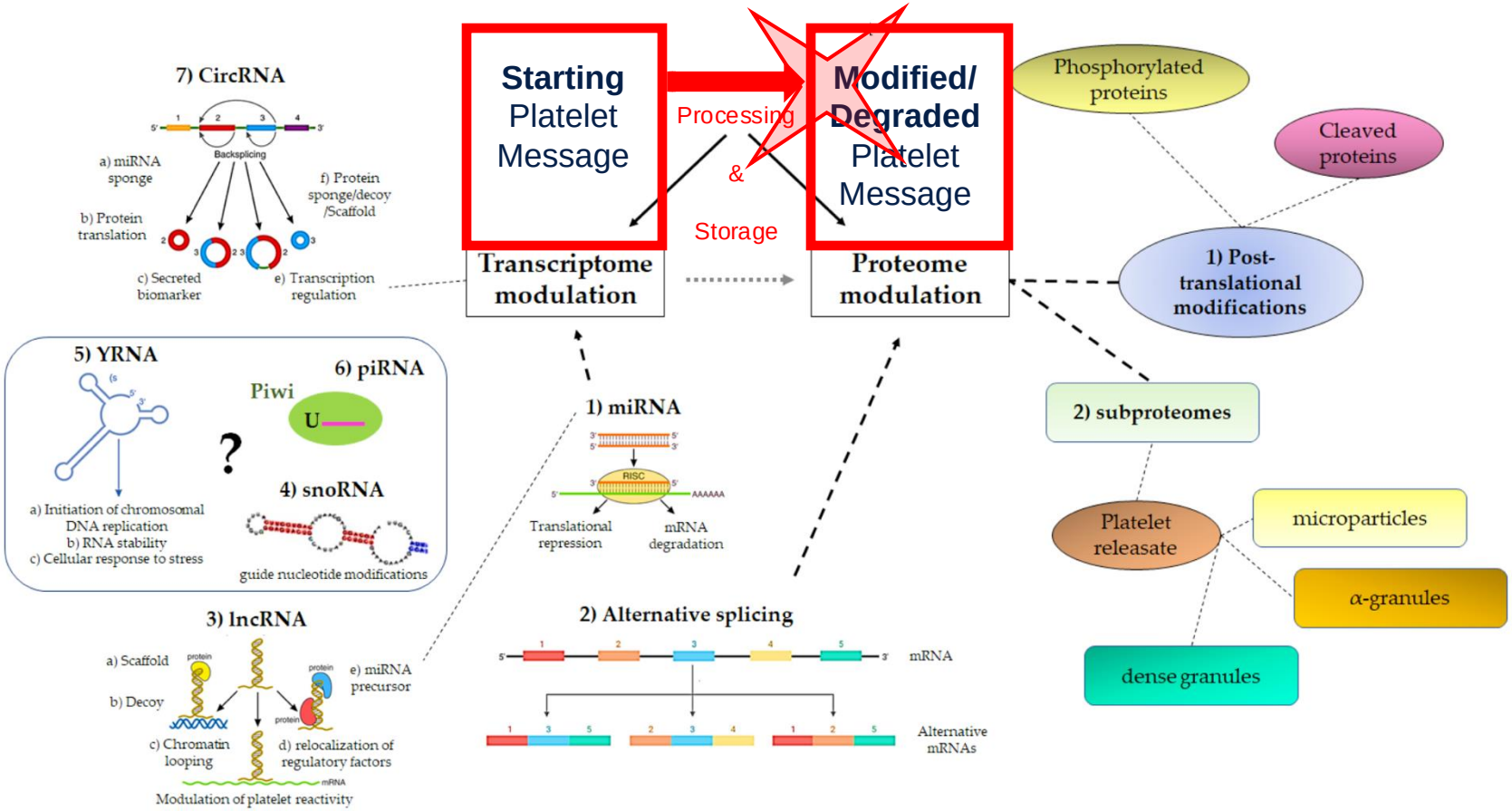
Whole transcriptome analysis of platelet concentrates during storage

Hasiyati Heililahong[1,2*], Peipei Jin[2*], Hang Lei[1,3], Haihui Gu[4], Baohua Qian[4], Xuefeng Wang[1,3], Jing Dai[1], Xiaohong Cai[1,3]

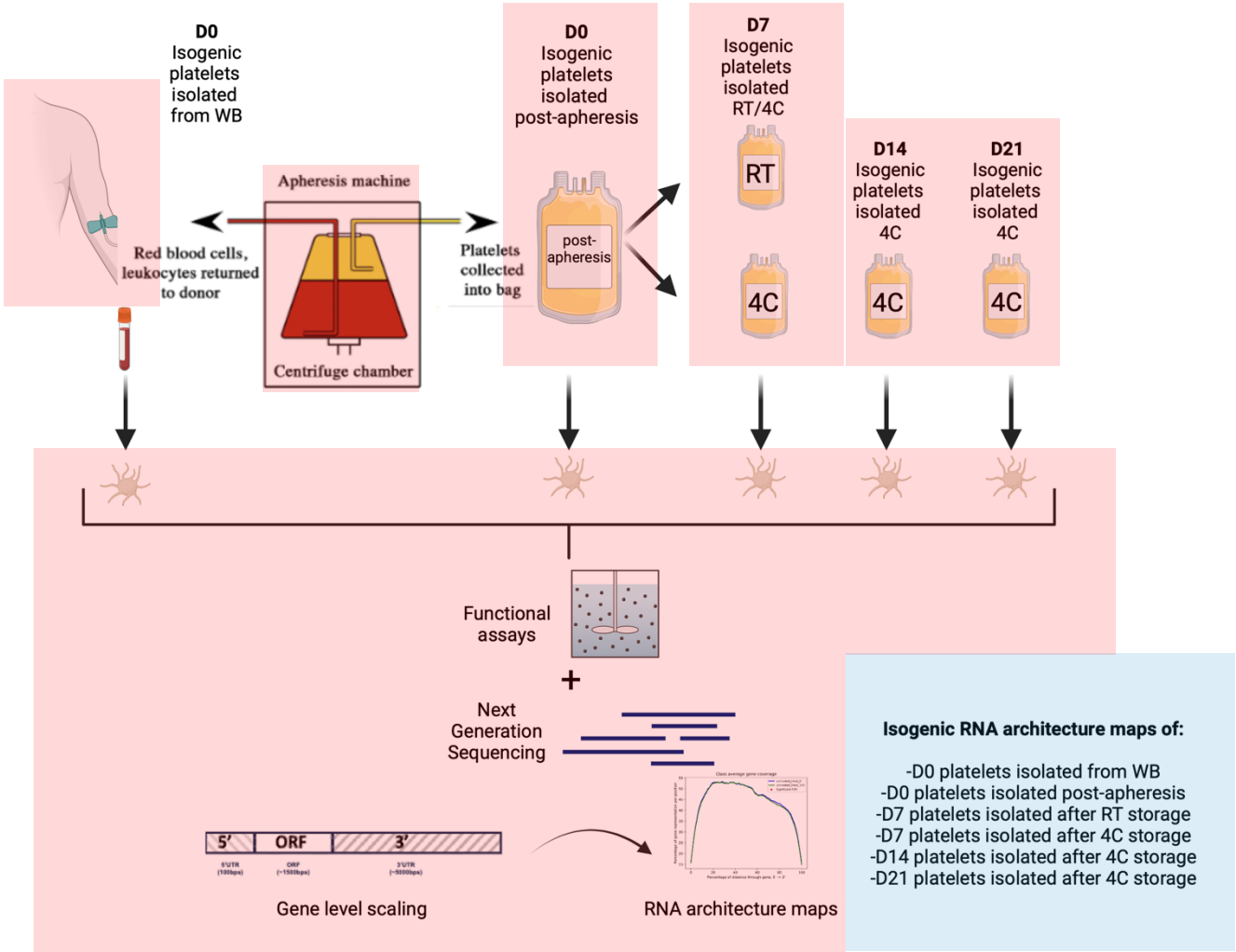
- Differentially expressed RNA in platelets out to 4 days at room temperature



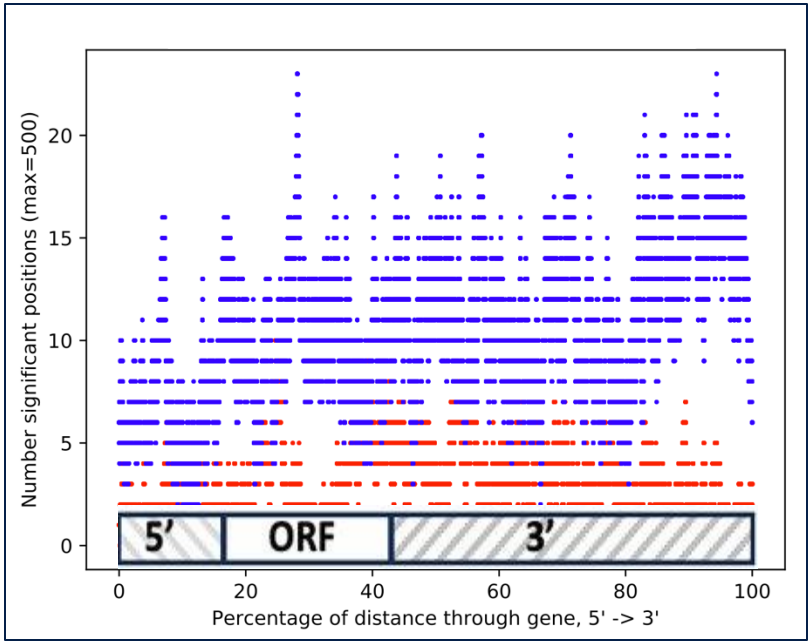
2023 Reference: Heililahong H, Jin P, Lei H, Gu H, Qian B, Wang X, Dai J, Cai X. Whole transcriptome analysis of platelet concentrates during storage. Blood Transfus. 2023

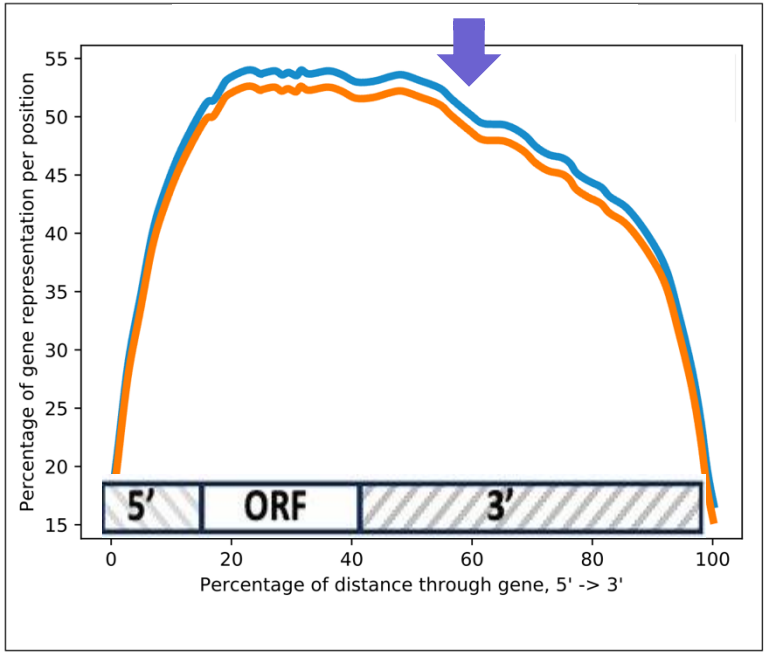


2023 Reference: Cimmino G, Conte S, Palumbo D, Sperlongano S, Torella M, Della Corte A, Golino P. The Novel Role of Noncoding RNAs in Modulating Platelet Function: Implications in Activation and Aggregation. Int J Mol Sci. 2023

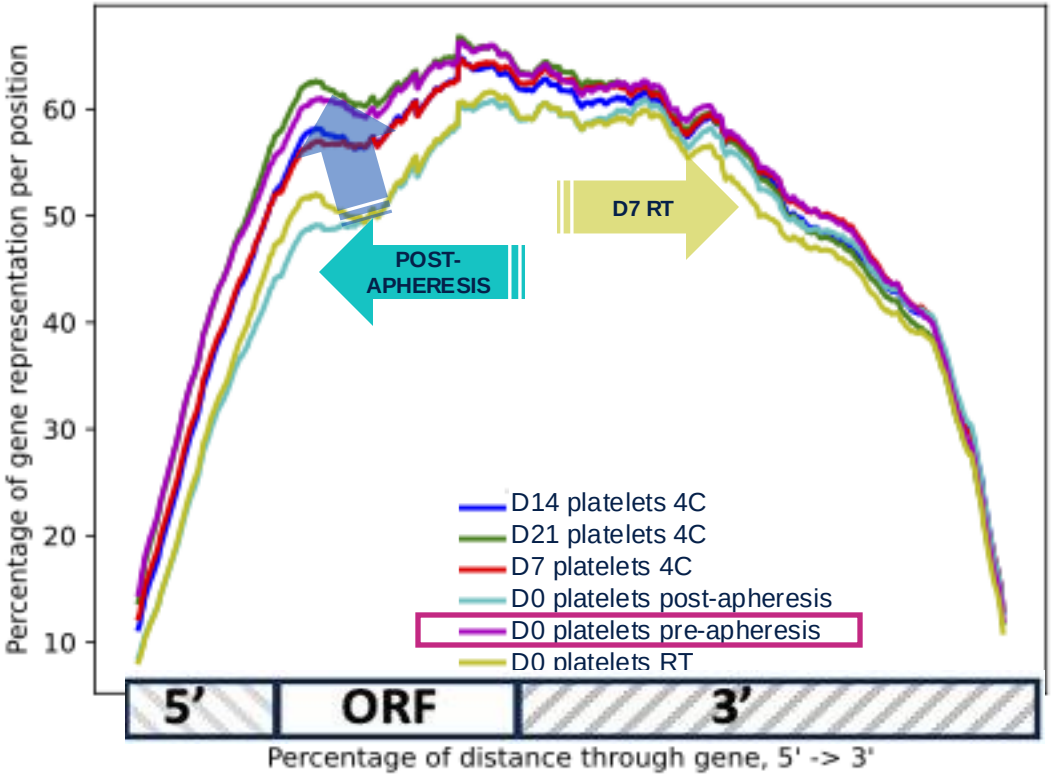

D0
Isogenic platelets isolated from WB
Apheresis machine
Red blood cells, leukocytes returned to donor
Platelets collected into bag
Centrifuge chamber
D0
Isogenic platelets isolated post-apheresis
post-apheresis
D7
Isogenic platelets isolated RT/4C
RT
4C
D14
Isogenic platelets isolated 4C
4C
D21
Isogenic platelets isolated 4C
4C
Functional assays
+
Next Generation Sequencing
5'
ORF
3'
Gene level scaling
RNA architecture maps
Isogenic RNA architecture maps of:
-D0 platelets isolated from WB
-D0 platelets isolated post-apheresis
-D7 platelets isolated after RT storage
-D7 platelets isolated after 4C storage
-D14 platelets isolated after 4C storage
-D21 platelets isolated after 4C storage

Platelet RNA architecture maps





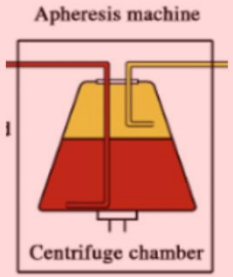
Platelet RNA architecture maps of processing/storage



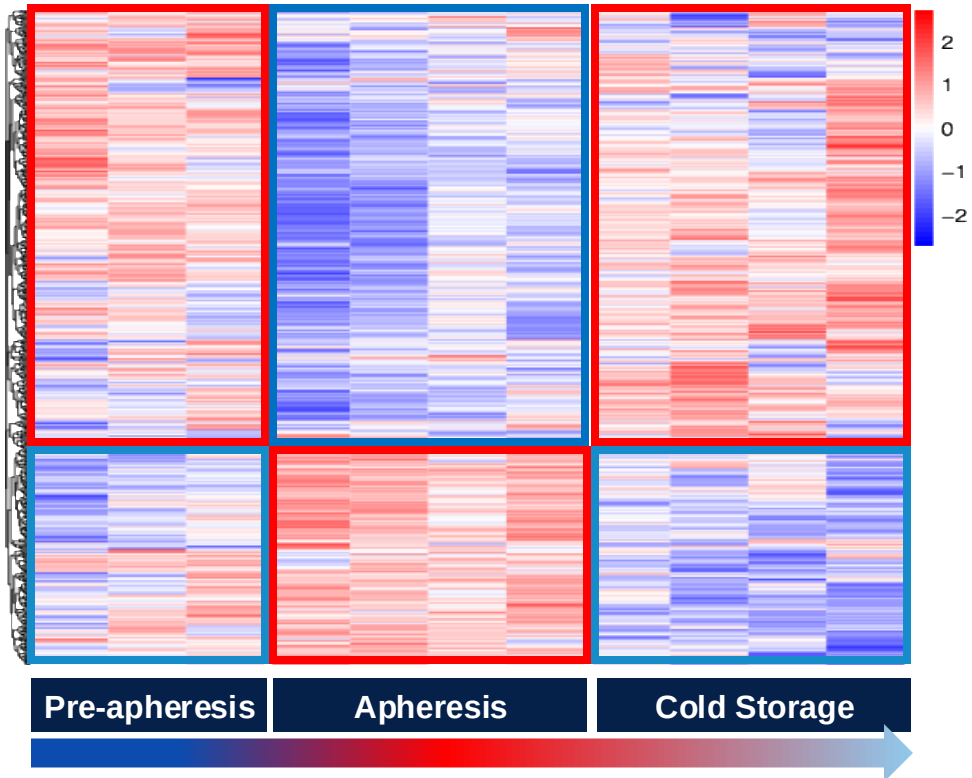
Summary of findings

- Platelet apheresis, storage temperature, storage time cause enormous RNA architecture changes

RNA architecture patterns of processing/storage

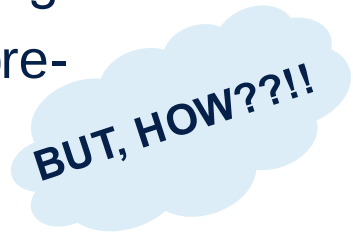
	Platelet RNA Architecture Effects of Apheresis	Platelet RNA Architecture Effects of RT Storage (7d)	Platelet RNA Architecture Effects of Cold Storage (7/14d)	Platelet RNA Architecture Effects of Cold Storage (3 wk)
				
	Apheresis	RT storage (7d)	Cold storage (7d, 14d)	Cold storage (3wk)
End processing	5' end degradation	3' end degradation	5' end recovery	5' end recovery

RNA architecture patterns of processing/storage by PCA features

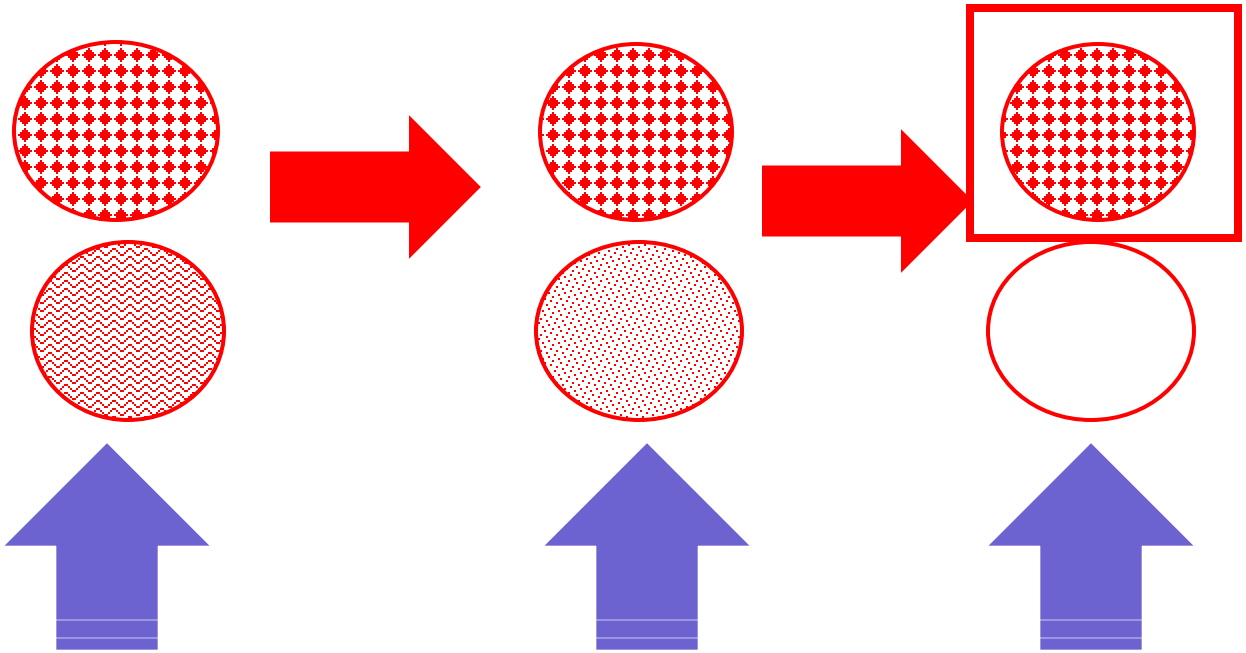


Summary of findings

- Platelet apheresis, storage temperature, storage time cause enormous RNA architecture changes
- There are 3 different RNA architecture effects with processing and storage of platelets:
  - Apheresis causes loss of the 5’ end of genes
  - Room temperature storage causes loss of the 3’ end of genes
  - Cold storage causes RNA architecture to appear like pre-apheresis platelets
    - Hypothesis:
      - Loss of a certain genotype(s) of platelet RNA→ Subpopulations of platelets?

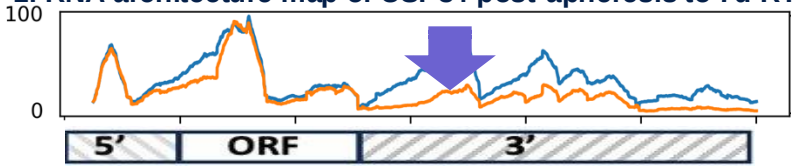


Concept of preferential loss of genotype (s)/ subpopulations

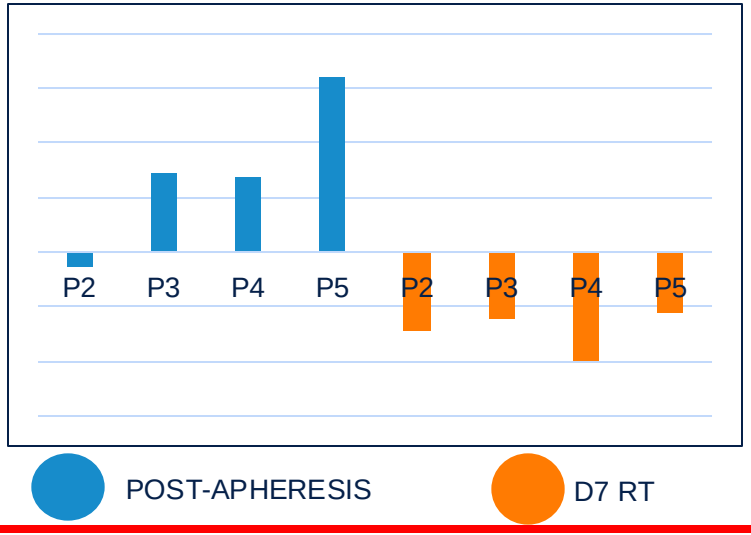


Gene specific RNA architecture changes and platelet function (USP34)

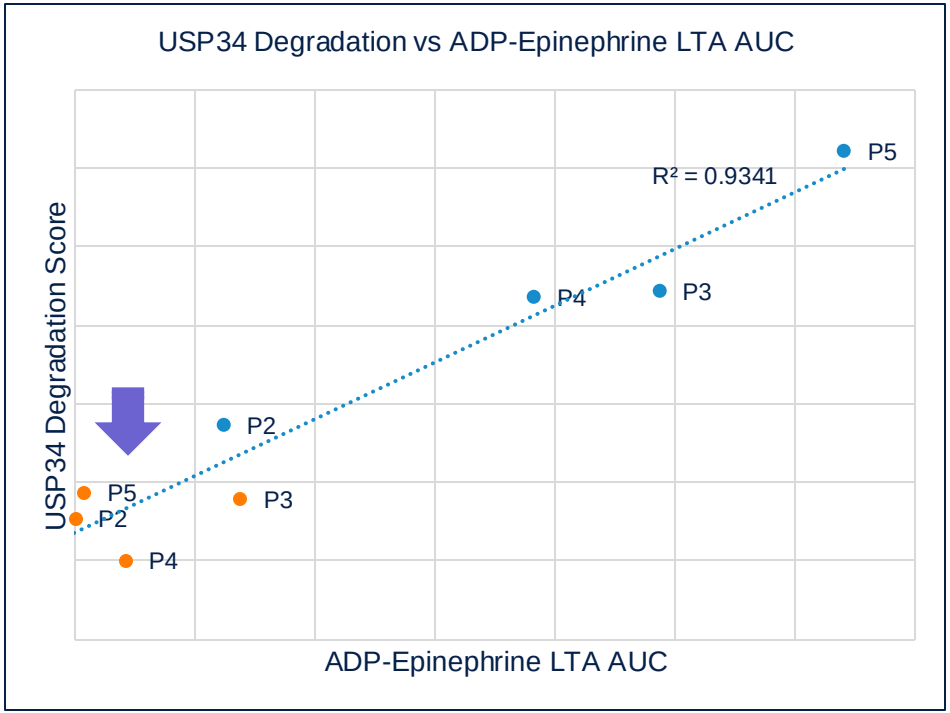
1. RNA architecture map of USP34 post-apheresis to 7d RT



2. USP-34 Specific RNA degradation PCA score



3. USP34 RNA Degradation score vs Platelet Functional Data



Summary of findings

- Platelet apheresis, storage temperature, storage time cause enormous RNA architecture changes
- There are 3 different RNA architecture effects with processing and storage of platelets:
  - Apheresis causes loss of the 5’ end of genes
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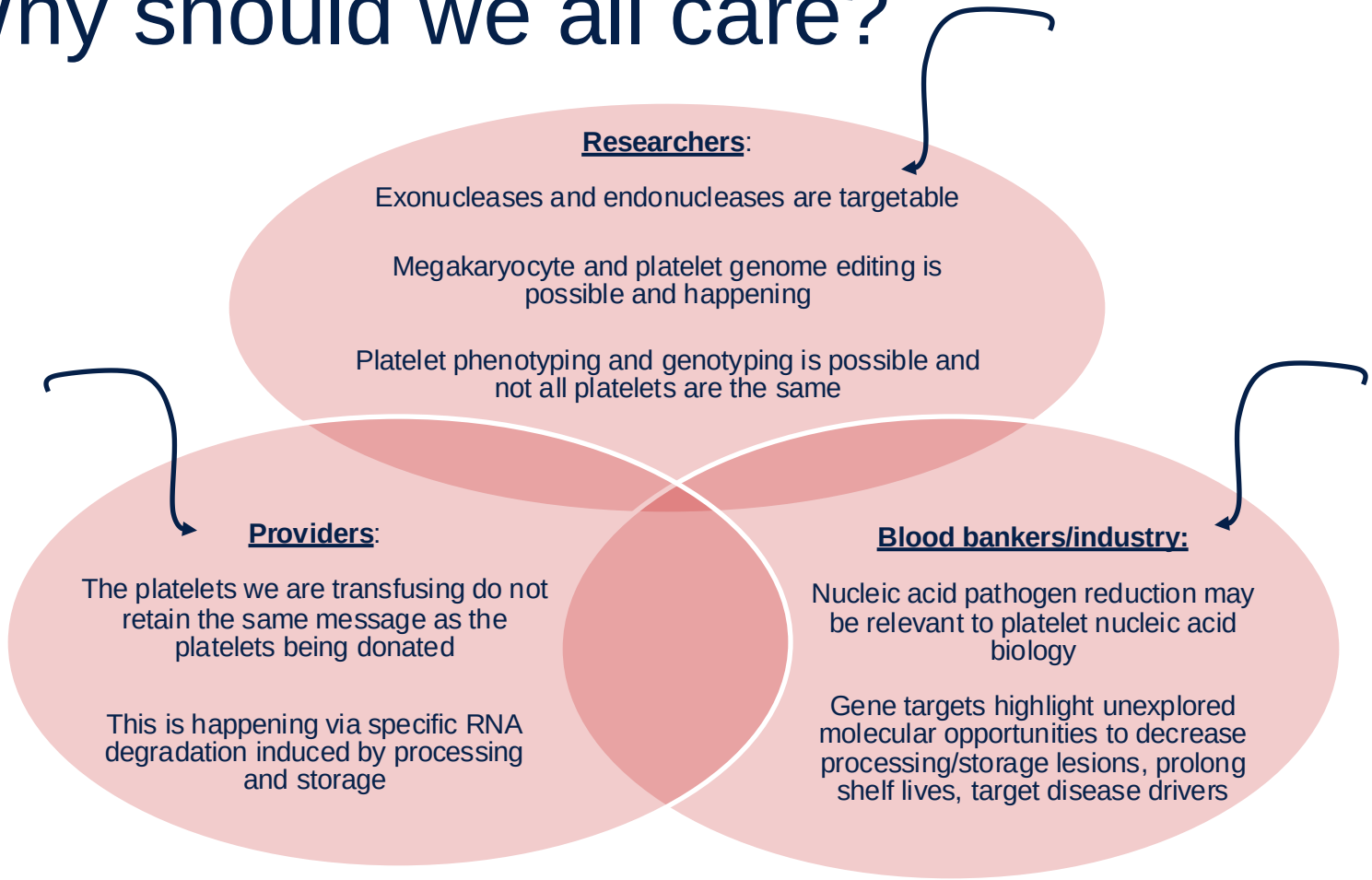
  - Cold storage causes RNA architecture to appear like pre-apheresis platelets
- Gene specific RNA architecture changes are associated with function of platelets
  - Hypothesis: These may be platelet “clock” genes

Why should we all care?



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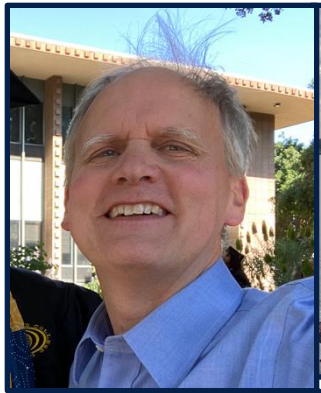

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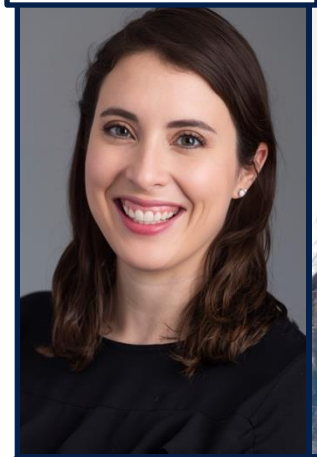

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