Laboratory of Cellular Hematology
Site Visit May 26, 2021

Overview of site visit presentations

Jaro Vostal, M.D., Ph.D, Laboratory Chief

Products reviewed in LCH

- **BIOLOGIC PRODUCTS**
- **Red cells** apheresis, 24 hour hold, pathogen reduced
- **Platelets** apheresis, 24 hour hold, cold stored, pathogen reduced, frozen platelets, platelet-like hemostasis agents
- **Plasma** apheresis, 24 hour hold, pathogen reduced, cryo precipitate, freeze dried plasma,
- **Whole Blood**

- **DEVICES**
- Blood collection and storage bags
- Apheresis instruments (platelet, red cell, plasma, portable and stationary)
- Leukocyte reduction filters
- Leukocyte counting devices
- Pathogen reduction processes
- Bacterial detection-culture based and rapid
- Blood bank cell counters
- Sterile connectors
- Automatic blood separators/expressors
- Blood warmers and pumps

Scope of regulatory review

- Pre-clinical evaluations of products and devices (safety and efficacy)
  - In vitro tests of processed transfusion products (platelets, red cells, plasma) biochemistry, cell physiology (activation)
- Medical device design, performance and impact on transfusion products
- Software for devices
- Post market product failures and adverse events investigations

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Mission related research

CBER Strategic Goals 2021-2025

Goal 1: Facilitate the development and availability of safe and effective medical products through the integration of advances in science and technology

Goal 2: Conduct research to address challenges in the development and regulatory evaluation of medical products

LCH research 2021-2025

- Improved and novel pathogen reduction methods
  - expanded efficacy vs pathogens
  - potential for less toxicity
- New transfusion products with safer profiles
  - cold stored platelets
  - temperature cycled platelets
- Improved product availability
  - frozen platelets
  - extended cold platelet storage
- Better understanding of storage/processing lesions
  - changes in platelet and RBC miRNAs
- Animal models for human transfusion product efficacy
  - oxygen delivery
  - hemostasis

Safety and Efficacy of Cellular Transfusion Products

Objective of this program is to address issues of safety in current transfusion products and develop methods to evaluate efficacy of novel transfusion products

PI: Jaro Vostal, MD, PhD

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Site Visit Report Review

BPAC Nov 4, 2021

Accomplishments (2015-2020)

pathogen reduction, platelet cold storage and animal model development

Pathogen reduction

- Developed 5 new photosensitizers for UVA light-based naturally occurring molecules with bactericidal pathogen reduction efficacy in plasma with up to 6 log reduction
  - Vitamins K3 and K5, Vitamins B1 and B6, Benzophenone
  - *Xu F et al. FEMS Microbiol Lett. 2018*
  - *Xu F. et al. Photodiagnosis Photodyn Ther. 2020.*

- Introduced a new concept of synergy by pairing photosensitizers that can increase antibacterial efficacy by up to 10,000x fold.
  - *Feyissa Q. et al. Transfusion. 2020*

- Demonstrated viral reduction in whole blood with Vitamin K5
  - *He, Y. et al. Journal of Medical Virology. 2021*

Platelet cold storage-improvements to platelet quality after storage

- Conducted a clinical trial in healthy humans to demonstrate improved *in vivo* recovery and survival of temperature cycled platelets vs cold stored platelets after 7 days storage-
  - Temperature cycling is 11.5 hrs in cold (4-6^{o} C) and 0.5 hrs at 37^{o} C
  - Temp cycling increased *in vivo* area under curve 2.5 fold over cold stored platelets
  - Collaboration with American Red Cross laboratories
  - *Vostal JG, et. al. Transfusion. 2018.*

- Used pharmacological inhibition of MAPK in platelets with VX-702 to reduce cold storage-induced platelet lesions
  - *Skripchenko A, et al. PLOS One, 2021.*

Animal models of transfusion

- Validated a mouse model of *in vivo* human platelet circulation against humans in our clinical trial
  - Immunodeficient mouse (SCID) that can accept human platelets
  - SCID mice can be used to predict platelet in vivo recovery in humans
  - *Gelderman MP, et.al. Transfusion. 2020*

- Chronically anemic and immunodeficient (Bthal/SCID) mouse for demonstration of oxygen delivery by human red cells
  - can accept human red cells
  - Reduction of exercise-induced lactate build up by transfusion of human red cells
  - *Shah SN. Et al. PLoS One. 2016.*

- Human platelet transfusions outcomes in septic rat model
  - Immunodeficient rat (Rag2 -/-) accepts human platelets
  - Demonstrates macrophage – mediated platelet aggregates in spleen
  - *Li Y, et al. Front Med. 2019*

Future plans- Vostal

- Pathogen reduction
- of red cells and whole blood with pairs of photosensitizers to improve efficacy
- evaluate quality of treated cells

- Platelet cold storage
- extend cold storage up to 21 days with temp cycling and pharmacological inhibitors of activation
- Plan a collaboration for clinical trial of cold stored platelets with Vx-702 to define kinetics in healthy humans

- Animal models
- humanized mice to evaluate human platelet hemostasis

Investigation of Potential Toxic Effects of Biologic and Engineered Nanoparticles and Microparticles in Blood and Blood Products and their Biomarker Applications

Program Objectives/ Research Projects

1. In vitro evaluation of effects of engineered nanomaterials on vascular endothelial cells (FDA Nanotechnology CORES Grant)

2. Investigation of cell membrane and protein microparticles in blood and blood products, their biomarker applications and potential role in vascular injury

PI: Jan Simak, Ph.D.

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BPAC Nov 4, 2021

Accomplishments (2015 -2020)

Project 1: In vitro evaluation of effects of engineered nanomaterials on vascular endothelial cells

- PAMAM dendrimer model of effects of spherical NPs on cultured endothelial cells *(Patel M. et al.: Nanotoxicology 2019)*
- Design and ongoing validation of the panel of in vitro assays *(Simak J., In: Handbook of Immunol. Prop. of Eng. Nanomaterials, 2016; Filipova M. et al.: PLoS One 2018)*
- Protein particle model using protein corona on engineered NP core

  for investigation of adverse effects of protein particles in biologics

  – work in progress in collaboration with D. Scott's Lab (OTAT)

Accomplishments (2015 -2020), cont.

Project 2: Investigation of cell membrane and protein microparticles in blood and blood products, their biomarker applications and potential role in vascular injury

- Comprehensive analysis of platelet extracellular vesiculome using a panel of high resolution analytical and imaging methods (*De Paoli S. et al.: CMLS 2018*)
- Distinguished four different pathways of PEV release from activated platelets (*De Paoli S. et al.: CMLS 2018*)
- Characterization of 6% DMSO cryopreserved platelets: platelet membrane disintegration, irreversible platelet damage and marked release of PEVs with thrombin generation procoagulant activity: PEVs contribute about 25% of procoagulant activity of cryopreserved platelet products (*Tegegn T. et al.: Journal of Extracellular Vesicles 2016*)

Future Plans-Simak

- Development of assays for characterization of membrane and protein submicron particles in biologics – focus on nanoparticle tracking analysis (NTA), atomic force microscopy (AFM), asymmetric flow field flow fractionation (AF4), high resolution flow cytometry (FC)

- Development of new methods of platelet cryopreservation to achieve preservation of fully functional platelets
  a) Investigation of different cryoprotectants and ice recrystallization inhibitors
  b) Investigate cryoprotective effects of various engineered nanomaterials for platelet cryopreservation
  c) Investigate the cold resistance of PLTs from cold/freeze tolerant species

Ex Vivo Stored Blood Component Safety and Quality:
Evaluation of Novel Methods for Pathogen Reduction and Functional Regulation in Blood Components

Objective of this program is to identify and evaluate promising methods and technologies effective in pathogen inactivation, while preserving functional properties of the treated blood components

PI: C. D. Atreya, PhD

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BPAC Nov 4, 2021

Accomplishments (2015-2020)

Evaluation of 405 nm light as a novel PRT for blood component safety

- **7** Publications in peer-reviewed journals
- **1** Patent application filed by FDA
- **1** Cooperative Research And Development Agreement (CRADA)

Evaluation of miRNA-based regulatory mechanisms in *ex vivo* stored blood cells with reference to platelet function

- **9** Publications in peer-reviewed journals

Whole blood miRNA analysis of hemophilia A patients: Discovery of miRNA regulation as a mechanism for hemophilia A

- **5** Publications in peer-reviewed journals

1 Patent pending, total publications 21

Rationale for 405 nm light as a tool for blood safety

- Microbicidal efficacy of 405 nm light has been well established as an alternative to ultraviolet light
- Can be used at levels that are lethal to microorganisms, without harming the exposed mammalian cell
- *In situ* PI treatment w/o external photosensitizers is possible, unlike with UV-A or -B light
- The light-excited endogenous porphyrins produce reactive oxygen species, including H_2O_2 and singlet oxygen, leading to oxidative damage and microbial cell death

Dai et al. 2009; Smith et al. 2009; McDonald et al. 2011; 2013; Maclean et al. 2011; 2013; 2016; Ramakrishnan et al. 2016 ; Leanse et al. 2021

405 nm light effect: Proof-of-concepts

Pathogens tested

Light dose range: 230-270 J/cm^2
Pathogen reduction: ≥ 5 Logs

Gram-positive bacteria

Staphylococcus aureus

S. epidermidis

Bacillus cereus

Gram-negative bacteria

Escherichia coli

Pseudomonas aeruginosa

Klebsiella pneumoniae

Acinetobacter baumannii

Yersinia enterocolitica

Virus

FCV (Nonenveloped RNA virus) –needs higher dose

Protozoan parasite

Trypanosoma cruzi

Effect on blood components

- Does not alter platelet *in vitro* metabolic parameters
- Retains platelet aggregation potential
- Preserves plasma protein integrity (studies ongoing)
- Preserves human platelet *in vivo* survival and recovery function in SCID mice
- Studies of the light effect on RBC is ongoing

Summary

- Studies with 405 nm light provided proof-of-concepts on microbicidal efficacy and preservation of plasma integrity and platelet functional parameters

- These concepts warrant further comprehensive evaluation towards an alternative to the existing PRTs

Evaluation of microRNA-based regulatory mechanisms in *ex vivo* stored platelets

Objective: Identify platelet functions relevant to *in vitro* quality that are regulated by miRNA

Background

- Platelets are terminally differentiated and enucleated; do not have gene transcriptional regulatory mechanisms
- But they have abundant microRNAs (miRNAs - small noncoding regulatory RNAs)
- Existence of miRNA-based post-transcriptional regulation of mRNAs is known in platelets
- Preservation of platelet shape, activation potential and ATP conservation in storage are important for platelet *in vivo* functions
- How these functions are regulated in stored platelets is not known

Ple et al. PLoS One, 2012; Lazar et al. Platelets, 2020

Summary

- Identified miRNA-223 regulation of platelet septine-2 and -6, which are important for preserving platelet shape
- Identified miRNA-320c and miRNA-181a regulation of platelet activation *via* regulation of RAP1b protein (Ras-related, which is important for platelet activation
- Identified miRNA-570 interaction with mitochondrial ATPase subunit g (ATP5L) in stored platelets

Dahiya and Atreya, MicroRNA 2019; 2020

Chattopadhyay, Dahiya and Atreya 2018

Dahiya et al, Platelets 2017

Future plans - Atreya

Evaluation of 405 nm light as a novel PRT for blood component safety

- Based on the proof of concepts developed so far, further comprehensive evaluation with following additional blood-borne pathogens will be evaluated:
  - Viruses: HIV, HTLV, HAV, HBV, HCV, HEV, WNV, ZIKAV, Dengue Virus etc.
  - Protozoan parasites: Plasmodium, Babesia
- Identify the optimal light dose that effectively inactivates pathogens
- Evaluate platelet and plasma coagulation function following treatment with the optimal light dose
- Examine pathogen reduction capability in stored red blood cells

Evaluation of miRNA-based regulatory mechanisms in *ex vivo* stored blood cells with reference to platelet function

- Continue examining the role of miRNAs and mRNAs identified in our microRNA/messenger profiling with reference to platelet function and quality
- Evaluate platelets after 405 nm light treatment to identify potential miRNA biomarkers of *in vitro* quality