Control of Oxidative Damage in RBC Transfusion

Andrew Dunham, PhD

Safer transfusions, better patient care. That’s our mission.
+ We are developing Hemanext™, a blood processing and storage system that provides a more physiologic red blood cell (RBC) with the goal of:
  - Reduced volume of RBC units transfused
  - Fewer morbidity and mortality complications than conventional RBC units

+ Focus on transfusion-dependent patient populations representing >50% of all RBC transfusions:
  - Chronically transfused (Sickle Cell Disease, Thalassemia, and Oncology)
  - Trauma
  - Other high volume procedures (e.g. GI, orthopedic, OBGYN, cardiovascular, transplant)

+ HQ: Bethesda, MD; Technology Center: Cambridge, MA; Manufacturing: Avon, MA

+ Have raised $36.8 million from private investors and ~$7 million from the National Institutes of Health (NIH) in earlier rounds
Organization

+ **Management Team (formerly of Bain & Company, Baxter Corp, Haemonetics Corp, Baxalta, Terumo BCT, Boston University and Los Alamos Labs):**
  - Martin Cannon, President and CEO
  - Tatsuro Yoshida, Technology Co-Inventor
  - Andrew Dunham, PhD, Chief Scientific Officer
  - Tony Pare, Chief Commercial Officer
  - Vic Dolecek, VP, Product Development
  - Lynn Mallett, CFO, Finance and Administration
  - Joseph Barrett, General Counsel

+ **Scientific Advisors:**
  - Paul M. Ness, MD, Johns Hopkins University School of Medicine
  - Larry Dumont, MBA, PhD, Blood Systems Research Institute
  - Jose Cancelas, MD, PhD, Hoxworth Blood Center at University of Cincinnati
  - Steven Spitalnik, MD, Columbia University
  - Christopher Silliman, MD, PhD, Bonfils Blood Center Research Department
  - Ralph Vassallo, MD, FACP, Blood Systems, Inc., Scottsdale, AZ.
  - Philip C. Spinella, MD, Washington University School of Medicine
  - Angelo D’Alessandro, PhD, University of Colorado
RBC Progressively Degrade Over Allowed Storage Period
New Findings – Interaction between oxygen and red blood cells

Current Beliefs
+ RBCs deliver O₂ so more O₂ is better
+ Blood collected from various donors has consistent %SO₂ values
+ Oxygen levels in RBCs do not change during storage
+ RBCs change as a function of time but this change is not clinically important

New Findings
+ Storing at <20% SO₂ improves RBC biochemical quality parameters
+ Day 0 RBC %SO₂ values vary from <10% to >90%
+ Oxygen leaches through bag increasing %SO₂ during storage
+ Clear superiority observed after resuscitation with Hemanext red cells
Causes and Consequences: Oxidative Damage

- **Root Causes**
  - High O2
    - Oxidative Damage
  - Nutrient Waste
    - ATP, 2,3-DPG
- **Specific Lesions**
  - Lipid Peroxidation
  - Protein Oxidation/Denaturation
  - RBC Senescence
- **Biologic Intermediates**
  - Hemolysis
  - Micro-Particles
  - Non-Viable RBC
  - PS
  - Lysolipids
  - Isocerostanes
  - Prostaglandins
  - Cytokines
  - Impaired NO Bioavailability
  - Advanced Glycation Endproducts
- **Physiologic Responses**
  - Cell Adhesion to EC Wall
  - Complement System Activation
  - Monocytes/Macrophage Stimulation/Overloading
- **Clinical Sequelae**
  - MOF
    - Lung
    - Heart
    - Kidney
    - Liver
    - Marrow
    - GI Tract
    - CNS
  - TRALI
  - TACO
  - TRIM
  - Malignancies
  - Infections
  - Post-Operative Pneumonia
  - Sepsis
  - Fever/Rigor
  - Myocardial Infarction
  - Cerebral Infarction
  - Dialium
  - Hemolytic Reactions
  - Venous Arterial Thromboembolism
  - Mortality
Current Standard of Care Balances Risks and Benefits of Blood Transfusions

Oxygen Carrying Capacity

Adverse Events
Blood Quality Matters to Transfusion Dependent Patients (More Than Any Others)

Adverse Events Originate from Degradation in Storage

+ The ideal transfusion:
  - Optimizes oxygen carrying capacity
  - Minimizes adverse outcomes

+ Degradation during storage reduces RBC efficacy and increases risk of the transfusion

+ Oxidative damage contributes profoundly to degradation during storage:
  - in any given unit of blood
  - driving heterogeneity in the blood supply

+ Risk of adverse events increases with transfusion volume

+ Risk is especially relevant to high volume recipients:
  - Thalassemia, Sickle, MDS, Trauma

+ Managing oxygen before storage will optimize the quality of any given unit and improve the consistency of the supply
Transfusion dependent hemoglobinopathies – opportunities for improvement

+ Increase interval between transfusions
+ Reduce annual blood exposure, reducing exposure to donors
+ Reduce iron load from transfusions
+ Reduce TRIM or iron related susceptibility to infections
+ Reduce susceptibility of developing allogeneic antibodies
+ Improve immediate oxygen delivery due to high 2,3-DPG / P50
+ Reduced interaction with endothelium from improved deformability
Managing %SO_2 Increases 24-hr Recovery Decreases Hemolysis after 42 Day Storage

24-hr In Vivo Recovery: Radiolabeled study testing viability after transfusion

Hemolysis: A process involving progressive RBC destruction

Dartmouth Hitchcock Medical Center: Dr. Larry Dumont
Dual-arm study: Control: LR-pRBC in AS3; Hemanext: Anaerobic LR-pRBC in OFAS3
Managing %SO₂ Reduces Volume Required for Resuscitation – Rat Model

Blood volume required to maintain 90% MAP (mL)

- **1 Week Old Blood**
  - Blood volume over time for Hemanext and Conventional methods.

- **3 Week Old Blood**
  - Blood volume over time for Hemanext and Conventional methods.

Hemanext
Conventional
Managing %SO₂ Improves Effectiveness of Oxygen Delivery – Rat Model

**Lactate concentration (mM)**

<table>
<thead>
<tr>
<th></th>
<th>1 Week Old Blood</th>
<th>3 Week Old Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Shock</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>10 min</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>60 min</strong></td>
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</tr>
</tbody>
</table>

**Hemanext**

- P < 0.05

**Conventional**

- P < 0.05

![Graph showing lactate concentration over time for 1 and 3 week old blood samples from baseline to 60 minutes after shock, with Hemanext and Conventional blood compared, indicating improved effectiveness of oxygen delivery with Hemanext.](image)
Managing %SO₂ Reduces Lung Injury and Inflammation – Rat Model

1 Week Old Blood

- CXCL1: P<0.02
- CD45+ neutrophils: P<0.01
- IL-6: P<0.02

3 Week Old Blood

- CXCL1: P<0.03
- CD45+ neutrophils: P<0.02
- IL-6: *
Managing %SO₂ Reduces Liver Damage – Rat Model

1 Week Old Blood

- Relative amounts (arb units)
- P < 0.03
- P < 0.04
- P < 0.02

3 Week Old Blood

- Relative amounts (arb units)
- P < 0.05
- P < 0.05
- P < 0.05

<table>
<thead>
<tr>
<th></th>
<th>AST</th>
<th>ALT</th>
<th>CXCL1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemanext</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Managing %SO₂ Reduces Kidney Damage – Rat Model

1 Week

- Serum Creatinine
- BUN
- u-NGAL

3 Weeks

- Serum Creatinine
- BUN
- u-NGAL

Relative amounts (arb units)

* P < 0.01

Hemanext
Conventional
Managing $%SO_2$ Reduces Spleen Damage – Rat Model

**CXCL1**

<table>
<thead>
<tr>
<th></th>
<th>1 Week Old Blood</th>
<th>3 Week Old Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Relative amounts (arb units)</strong></td>
<td><img src="image" alt="Graph showing relative amounts" /></td>
<td><img src="image" alt="Graph showing relative amounts" /></td>
</tr>
<tr>
<td><strong>CXCL1</strong></td>
<td><img src="image" alt="Graph showing CXCL1 levels" /></td>
<td><img src="image" alt="Graph showing CXCL1 levels" /></td>
</tr>
</tbody>
</table>

* $P < 0.03$
* $P < 0.02$
Hemanext Shifts Risk / Benefit Ratio of Blood Transfusions

Unrecovered RBC
Fe overload
Methemoglobin
Oxidized lipids
Cytokines
Transfusion events

Adverse Events

Oxygen Carrying Capacity

RBC survival
2,3-DPG
Deformability

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Dose response of %SO$_2$ on RBC quality was studied in a pool and split study. RBC samples were prepared at 6 O$_2$ levels and stored for 42 days: <3%, 5%, 10%, 20%, 55% and >90% SO$_2$. Resulting analysis showed optimum O$_2$ storage range. When %SO$_2$ <20:

- Predictors of efficacy: ATP, 2,3-DPG, and GSH/GSSG were improved
- Toxic by-products: Hemolysis, methemoglobin, and oxidized lipids were reduced

Currently %SO$_2$ levels in RBCs is uncontrolled: ranges from <10% to >90%.
- Mean %SO$_2$ of 45.9±17.5% on day zero and increased to >95% in 3 weeks

Uncontrolled starting %SO$_2$ levels, coupled with negative impacts of high oxygen saturation on red blood cell quality indicates that oxygen levels are an important source of variability in RBC quality.

Research and Blood Center %SO₂ distributions

Day 0

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research units</td>
<td>492</td>
<td>45.9%</td>
<td>17.5%</td>
</tr>
<tr>
<td>RIBC units</td>
<td>977</td>
<td>47.0%</td>
<td>21.0%</td>
</tr>
<tr>
<td>Sanguin units</td>
<td>1325</td>
<td>59.7%</td>
<td>18.2%</td>
</tr>
</tbody>
</table>
Simulated %SO₂ Increase During Storage

%SO₂ (day x) = %SO₂ (day 1) * t (x day)^(k); k = 0.3187 – 0.00356 * %SO₂ (day 1)

Yoshida et al., Blood Transfusion, 15 (2), 172-181. 3 2017
Percent $SO_2$ optimization minimizes oxidative damage and promote healthy metabolism.
RBC carries a large mass of highly reactive Fe\(^{2+}\) and O\(_2\) in hemoglobin

- Fraction of hemoglobin is oxidized by O\(_2\) to methemoglobin
- Robust enzyme-based anti-oxidant mechanism curtailed at 1-6° C

O\(_2\) depletion and control reduces hemoglobin oxidation

- Oxidizing hemoglobin makes methemoglobin, which does not transport oxygen
- Methemoglobin breaks down into materials that damage cell integrity and slow RBC metabolism
  - Iron (III) released from methemoglobin catalyzes lipid peroxidation cycle
  - Removes fuel for oxidative damage to proteins

O\(_2\) and CO\(_2\) depletion enhance RBC metabolic status

- Increases glycolytic flux to produce high ATP and 2,3-DPG levels
  - Increase in cytosolic pH by CO\(_2\) depletion
  - Sequestration of free 2,3-DPG and ATP by deoxy-hemoglobin, relieving feedback inhibition
  - Metabolic modulation stimulates glycolysis
Oxidation Exposure Index (OEI)

+ OEI is calculated to summarize the total exposure of a given RBC unit during storage
  - OEI is expressed in units of (%SO₂*Days)
  - %SO₂ increases at a rate published in Blood Transfusion¹
+ The OEI calculation is based on:
  - The starting %SO₂ of the unit
  - The empirically derived rate that %SO₂ increases during refrigerated storage in PVC¹
+ The OEI in Hemanext is calculated in a similar fashion
  - Hemanext starts at 5-15% SO₂ but does not increase during storage

¹ Yoshida et alae, Blood Transfusion, 15 (2), 172-181
Weekly Oxygen Exposure Index
10-90% SO₂ – Range, Mean +/- SD
Weekly Oxygen Exposure Index
10-90% SO₂ vs. Hemanext – Range, Mean +/- SD

Conventional
Hemanext

Oxygen Exposure Index (arb units)

Days of Storage
Weekly Oxygen Exposure Index
10-90% SO₂ vs. Hemanext – Range, Mean +/- SD

Conventional
Hemanext
Weekly Oxygen Exposure Index
10-90% SO₂ vs. Hemanext – Range, Mean +/- SD
The Hemanext System

+ Integrates easily into existing blood bank operations
Multi-Faceted Economic Value Proposition

+ **Chronically Transfused (Sickle Cell, Thalassemia, Oncology)**
  - Extension of interval between transfusion for chronically transfused
  - Reduced ancillary procedures (such as iron chelation) associated with lower quality RBC's

+ **Acute High Volume Transfusion Patients (Trauma, Complex Spine, Cardiovascular, Organ Transplant, etc.)**
  - Fewer infections, transfusion related complications, organ damage, and avg. length of stay in hospital
  - Reduced volume of units transfused
Key Upcoming Milestones

+ **4Q 2017**: FDA Pivotal Trial Initiated

+ **1Q 2018**: CE Mark Submission

+ **2Q 2018**: Commercial Launch in EU, Canada and Australia

+ **2Q 2018**: FDA Submission

+ **1Q 2019**: Commercial Launch in US
## Planned Preclinical Testing of Hemanext RBC

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
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</thead>
<tbody>
<tr>
<td><strong>Neutrophil Priming Test</strong></td>
<td>Neutrophil activation will be performed with RBC and supernatants from Hemanext products. <strong>Completed</strong> – no evidence of neutrophil activation</td>
</tr>
<tr>
<td><strong>Growth and proliferation of facultative and strict anaerobic bacteria</strong></td>
<td>Spike bacteria at different concentrations into leukocyte reduced red cell concentrates. Half of the unit will be processed with Hemanext RBC Processing System and then stored at 4°C for 42 days – <strong>On going</strong></td>
</tr>
<tr>
<td><strong>Rate of sickling of sickle red blood cells</strong></td>
<td>Incubate sickle and Hemanext RBC. The change in sickling rate will be measured after mixing with Hemanext RBC – <strong>June 2017</strong></td>
</tr>
<tr>
<td><strong>Hemolytic Potential</strong></td>
<td>Subject Hemanext RBC to varying shear stresses measuring hemolysis and red cell fragmentation – <strong>June 2017</strong></td>
</tr>
<tr>
<td><strong>Adherence to HUVEC</strong></td>
<td>Incubate Hemanext RBC with HUVEC at 37°C under static condition and varying shear stresses – <strong>Fall 2017, Paris</strong></td>
</tr>
<tr>
<td><strong>Neoantigenicity and T-Cell activation tests</strong></td>
<td>Measure expression of surface RBC antigens before and after processing Hemanext RBC Process – <strong>Fall 2017 Hoxworth Blood Center</strong></td>
</tr>
</tbody>
</table>
## Clinical Study Summary – Chronic Transfusion Initial Focus

<table>
<thead>
<tr>
<th>N</th>
<th>Type</th>
<th>Additive Solution</th>
<th>Start - End Date</th>
<th>Outcomes</th>
<th>SCD/Thal</th>
<th>MDS</th>
<th>HVT/Trauma</th>
</tr>
</thead>
<tbody>
<tr>
<td>~20</td>
<td>Pre-clinical – rats</td>
<td>AS3</td>
<td>Jan – May 2017</td>
<td>Oxygen delivery/ Organ damage</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>30</td>
<td>In vitro</td>
<td>PAGGSM</td>
<td>May – Jul 2017</td>
<td>Blood quality – CE mark</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>100</td>
<td>In vitro</td>
<td>AS3</td>
<td>Jul – Dec 2017</td>
<td>Blood quality – FDA</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>24</td>
<td>In vivo</td>
<td>AS3</td>
<td>Jul – Dec 2017</td>
<td>Blood quality/ 24 hour recovery – FDA</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>6</td>
<td>In vitro</td>
<td>PAGGSM</td>
<td>Jul – Nov 2017</td>
<td>Endothelial adhesion</td>
<td>x</td>
<td></td>
<td>x</td>
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<tr>
<td>2</td>
<td>In vitro</td>
<td>AS3</td>
<td>May – Jul 2017</td>
<td>Sickling kinetics after mixing with Hemanext</td>
<td>x</td>
<td></td>
<td></td>
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<tr>
<td>TBD</td>
<td>Whole units in SCD patients</td>
<td>PAGGSM</td>
<td>TBD</td>
<td>HbA retention</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>
Discussion and requests

+ Input from key opinion leaders in hemoglobinopathy treatment and clinical research
+ Potential collaboration opportunities?
  - Additional research activities?
  - Additional communication?
  - Other?
Thank You