Purpose

• To provide information about the US DoD Hemorrhage and Resuscitation Research and Development Program
  – Highlight RDCR and Blood Products efforts
  – Recent work on promising low volume approaches for next generation RDCR
Top cause of preventable DOW*:

- Hemorrhage 76%
- Burn 13%
- TBI 6%
- MOF 3%
- Airway 1%

*DOW: Died of Wounds at Role 3+

Kelly et al., 2008
### US Forces

**Blood Products Use OEF/OIF**

- **Transfused Patients**: 28,843
  - Percent U.S. Only: 23.1%
  - Percent Non-U.S.: 76.9%
- **Whole Blood**: 9,139*
  - Percent U.S. Only: 52.8%
  - Percent Non-U.S.: 47.2%
- **Red Blood Cells**: 155,035
  - Percent U.S. Only: 29.0%
  - Percent Non-U.S.: 71.0%
- **Platelets**: 9,173
  - Percent U.S. Only: 41.5%
  - Percent Non-U.S.: 58.5%
- **Fresh Frozen Plasma**: 92,297
  - Percent U.S. Only: 32.3%
  - Percent Non-U.S.: 67.7%
- **Cryoprecipitate**: 25,073
  - Percent U.S. Only: 40.4%
  - Percent Non-U.S.: 59.6%

*10.2% of total U.S. Wounded In Action (WIA) required whole blood transfusions

Source: Armed Services Blood Program Office as of November 2011
DoD Hemorrhage & Resuscitation Portfolio
Scope and Purpose

• **Scope**: The Hemorrhage and Resuscitation R&D program includes DoD efforts in the general areas of hemorrhage control, fluid resuscitation, blood products, transfusion, and pathophysiologic responses to traumatic hemorrhage, with a view ranging from basic and discovery research through clinical development.

• **Purpose**: Conduct research and development to provide improved methods, drugs, and devices to stop bleeding, restore lost blood volume, and mitigate the consequences of hemorrhage. Reduce mortality by up 16% overall.
Key Collaborations

US DoD Program
Key Partners
and Collaborations
DoD Hemorrhage & Resuscitation Portfolio
Strategic Approach

• The DoD Hemorrhage and Resuscitation Research Program is executed across six major efforts in three broad phases over time

• Objectives are aligned with major efforts and phases

• Developed with Joint Input
  – Reviewed and approved by Steering Committee and Joint Program Committee for Combat Casualty Care
### Strategic Approach: Major Efforts

<table>
<thead>
<tr>
<th>Major Effort</th>
<th>Goal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved Blood Products</td>
<td>Develop safer and more logistically supportable blood products for transfusion</td>
</tr>
<tr>
<td>Damage Control Resuscitation</td>
<td>Identify the best ways to use existing and newly developed blood products, drugs, and fluids (Including Transfusion Practices using existing products-CPGs)</td>
</tr>
<tr>
<td>Coagulopathy of Trauma</td>
<td>Elucidate mechanisms to identify diagnostic and therapeutic targets for the development of rapid diagnostics and drugs to prevent or treat coagulopathy of trauma</td>
</tr>
<tr>
<td>Immune/Inflammatory Modulation</td>
<td>Evaluate promising approaches and identify key mechanisms leading to the long-term ability to modulate inflammatory responses of the patient</td>
</tr>
<tr>
<td>Metabolic and Tissue Stabilization</td>
<td>Evaluate promising approaches and identify key mechanisms leading to long-term ability to modulate/stabilize metabolic responses (Including oxygen delivery)</td>
</tr>
<tr>
<td>Hemostatics</td>
<td>Evaluate/identify existing products and develop new products or procedures to control bleeding</td>
</tr>
</tbody>
</table>
Strategic Approach: Phases and Overarching Objectives

- Phases and Overarching Objectives
  - 1. Optimize the use of existing products for initial stabilization and resuscitative surgery (Near Term: 1-3 years)
  - 2. Optimize use of existing, improved, safer products and broaden availability to enable expanded use of life-saving products from role 1 through all levels of evacuation (Mid Term: 4-9 years)
  - 3. Modulate patient response to optimize outcomes at all levels from first responder through resuscitative surgery, ICU and transition to rehabilitative care using new drugs and technologies that eliminate the need for blood products (Long Term: 10+ years)
Improved Blood Products
Highlights

• Freeze-dried plasma development program
• Spray-dried plasma development
• Cryo-Preserved Platelets development program
• Red Blood Cell Extended Life development program
• Whole Blood Pathogen Reduction Technology
• Clinical Studies (Knowledge)
  – Effects of age of red cells
  – Frozen Blood Study
• Discovery Research
  – DARPA Red Cell Pharming Program
  – Improved whole blood and platelet storage
Damage Control Resuscitation

Highlights

• Navy Multifunctional Resuscitation Program

• Pre-hospital Use of Plasma for Traumatic Hemorrhage
  – 3 clinical studies (8 centers and ~950 patients) on use of plasma in prehospital setting as replacement of local standard (eg. NS). Goal is to gain information on the possible beneficial and negative effects of plasma in the prehospital setting

• Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR). Multicenter trial (~580 patients) to compare effects of 1:1:1 plasma to platelet to red cell transfusion versus 1:1:2 in trauma patients.

• On-going evaluation of various combinations blood products and fluids for resuscitation in animals
Hemostatics
Significant Activities

• DARPA Wound Stasis Program

• Platelet-derived Hemostatic Agent (PDHA) development

• NanoSys Advanced Trauma Dressing FDA approved

• Xstat Wound Dressing for Junctional Hemorrhage

• Interest in fibrinogen concentrate, PCCs, and other iv/systemic approaches

• Interest in endovascular approaches to hemostasis
Xstat Dressing

XStat works by applying a group of small, rapidly-expanding sponges into a wound cavity using a lightweight applicator.

In the wound, the XStat sponges expand and create a barrier to blood flow, present a large surface area for clotting, and provide gentle pressure.

No direct manual pressure is required.

Each XStat sponge contains a radiopaque marker for easy detection via X-ray.

The compact Xstat applicator includes a telescoping handle and a sealed valve tip.
Proof-of-Concept Studies

Transected femoral artery (4/9/2009)

Transected subclavian artery and vein (9/8/2009)
Summary of Animal Data

### Hemostasis at 4 minutes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hemostasis</th>
<th>No Hemostasis</th>
<th>Percent Successful</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Xstat Versions</td>
<td>45</td>
<td>11</td>
<td>80.4 %</td>
</tr>
<tr>
<td>Combat Gauze</td>
<td>2</td>
<td>6</td>
<td>25.0 %</td>
</tr>
</tbody>
</table>

### Hemostasis at 60 minutes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hemostasis</th>
<th>No Hemostasis</th>
<th>Proportion Successful</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Xstat Versions</td>
<td>52</td>
<td>4</td>
<td>92.9 %</td>
</tr>
<tr>
<td>Combat Gauze</td>
<td>2</td>
<td>6</td>
<td>25.0 %</td>
</tr>
</tbody>
</table>

### Survival at 60 minutes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival</th>
<th>No Survival</th>
<th>Proportion Successful</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Xstat Versions</td>
<td>54</td>
<td>2</td>
<td>96.4 %</td>
</tr>
<tr>
<td>Combat Gauze</td>
<td>3</td>
<td>5</td>
<td>37.5 %</td>
</tr>
</tbody>
</table>

Xstat preclinical animal testing collective outcomes for primary endpoints (feasibility study + development studies, Xstat n=56, Combat Gauze n=8).

USSOCOM and Army sponsored effort
Next Generation Resuscitation: Very Low Volume Approaches

- \( \leq 4 \text{ml/kg} \) treatment to stabilize casualty/increase survival after hemorrhagic shock
- Maintain casualty longer until full resuscitation and surgery
- Reduce pre-hospital fluid requirements
- In place of, or as an adjunct to fluids and blood products
- Approach – explore existing drugs (other indications) for potential use in shock
  - Immune Modulation
  - Metabolic and Tissue Stabilization
Lymphocyte Sequestration
Immune Modulation Strategy – T-cell Modulation

- **Lymphocyte depletion**
  - Thymoglobulin
  - Genzyme
  - FDA approved for organ transplantation
  - Available as IV infusion
  - Experimentally – Porcine Anti-Thymocyte Globulin (PATG)

- **Lymphocyte sequestration**
  - FTY720
  - Novartis
  - FDA approved for multiple sclerosis
  - Available as oral tablet or IV infusion
  - Sequesters lymphocytes in secondary lymphoid organs and reduces circulatory lymphocytes by acting as a sphingosine 1-phosphate (S1P) receptor antagonist
**Immunomodulation Strategies for the Treatment of Hemorrhagic Shock**

### Hypothesis:
Lymphocyte depletion or sequestration reduces the inflammatory response and improves survival following grade III liver injury and hemorrhagic shock in swine.

### Experimental Approach
- **Pre-PATG Infusions**
  - Pre-PATG (10mg/kg) Doses x4

- **Uncontrolled Hemorrhage**
  - Liver Crush

- **Pre-Hospital Phase**
  - Abdominal Packing/Temporary Closure
  - Liver Repair/Blood Transfusion

- **Hospital Care**
  - Post-op Care
  - Euthanasia/Necropsy

### Pre-injury Treatment – Proof of Concept
- Thymoglobulin pre-injury vs Placebo (n=8-9/trt)

### Post-treatment Study:
- FTY720 Post Injury vs Placebo (n=8-9/trt)

Navy study at NMRC (Elster, Tadaki et al.)
Results: Survival

Pilot (pre-treatment with PATG):

Mean survival time was improved in the PATG (54.3 ±11.6h) compared to the control (19.6 ±9.9h), p<0.05.

The overall survival was 78% (7/9) in PATG and 25% (2/8) in the control

FTY720 – Post Treatment (Hawksworth et al., 2012):

FTY720 Improves survival (p<.05) in the reperfusion phase
Results: FTY720 on T-Lymphocytes

FTY720 results in:
- Reduced peripheral T-lymphocytes
- Increased T-cells in Lymph nodes and spleen
Results: FTY720 Other

FTY720 reduced peripheral Neutrophils

FTY720 decreased neutrophil infiltration of the lung

FTY720 decreased inflammatory gene transcript expression in liver tissue

Figure 7. Lung tissue neutrophil infiltration at time of necropsy. Quantification of anti-MPO reactivity. Mean and SEM.
Effect of immunomodulatory agents on resuscitation of Non-Human Primates (Macaca fascicularis) after severe uncontrolled hemorrhage

**Status:** 40 animals complete

**Hypothesis:** Leukocyte depletion or sequestration drugs given 15 min after grade III liver injury will reduce organ injury and enhance survival by dampening key secondary injury immune responses

**Treatment Groups (n=6/trt)**

| Controls (Normal saline NS)                    |
| Controls (Normal saline NS) plus soft tissue injury |
| Thymoglobulin 5 mg/kg                          |
| Thymoglobulin 10 mg/kg                         |
| Thymoglobulin 20 mg/kg                         |
| FTY720 0.3 mg/kg                               |
| Best agent plus soft tissue injury             |
| Best agent plus soft tissue injury with agent at 30 min |

**Experimental Approach:**

**Time 0:** Closed-abdomen laparoscopic liver injury/hemorrhage.

**Time 15 minutes post injury:** Infuse test material

**Time 15 – 120 min:** \(\leq 20\text{cc/kg of resuscitation fluid}\)

**Time 120 min:** Begin hospital care-repair of liver laceration

**Time 120 – 240 min:** Hospital care with continuous monitoring and resuscitation and blood transfusion

**Time 240 min:** Recover from anesthesia

**Time 24 hr-2 weeks:** Daily blood samples; day 14 post injury the animals will be euthanized, necropsy and tissue samples collected for histologic and RNA analysis.

**Pay-off:** Preclinical proof of concept to support clinical development of an immune modulatory drug to reduce deaths/morbidity from hemorrhage shock
Complement Inhibition
Complement Inhibition

- Previous studies have shown that inhibition of C5a and Decay Accelerating Factor reduce intestinal and lung injury in hemorrhagic shock in mice, rats, and swine.

- C1-inhibitor licensed in US and Europe for hereditary angioedema

- C1 inhibitor is a member of the serpin family of protease inhibitors and inactivates a variety of proteases including
  - complement system proteases (C1r, C1s, mannose-binding protein-associated serine protease 2 (MASP-2)) – inhibits both the classical pathway and the lectin pathway
  - contact system proteases (factor XII, plasma kallikrein),
  - an intrinsic coagulation protease (factor XI),
Effects C1 Inhibitor on tissue damage in a porcine model of controlled hemorrhage

(Dalle Lucca et al., 2012)

- Swine (30-38 kg) were hemorrhaged using a controlled, isobaric Wiggers model of controlled hemorrhagic shock.
- The animals were randomly assigned to: one of four experimental groups:
  - H, hemorrhage + vehicle (n = 6);
  - H + C1-INH (100 IU/kg, n = 5);
  - H + C1-INH (250 IU/kg body weight, n = 5);
  - Control, sham operated (cannulated but not hemorrhaged, n = 6).
- Each dose was infused in a total volume of 120 mL. Shed blood was not returned to the animal.

Army Study at USAISR
C1-INH Effects on Gut

C1-INH at 250 IU/kg BW reduces gut injury and intestinal complement deposition.
C1-INH Effects on Lung

C1-INH at 250 IU/kg BW reduces lung injury and lung complement deposition
C1-INH Other

- C1-inh-250 (not C1-INH-100) improved Base excess and HCO3-
- Kidney Function: C1-INH-250 reduced BUN and creatinine
- Cytokine release: C1-inh-250 reduced TNF-alpha
- Conclusion, rhC1-INH attenuated tissue damage, lowered tissue complement activation and deposition, corrected metabolic acidosis, and reduced circulating TNF-alpha release.
- Next steps – swine poly-trauma, mechanistic studies, primates
Valproic Acid
Valproic Acid

- Histone deacetylases (HDACs) play a key role in homeostasis of protein acetylation in histone and nonhistone proteins. Regulate DNA expression via histone acetylation and deacetylation.

- Valproic acid is an 8 carbon HDAC Inhibitor that is currently approved as an anticonvulsant (anti-seizure) drug in the US (Depakene).

- Recent animal data suggest a protective effect of Valproate in hemorrhage is shock.

- Potential role in hemorrhagic shock with poly trauma was investigated

- Office of Naval Research sponsored work
Surviving blood loss without blood transfusion in a swine polytrauma model (Alam et al., 2009)

• 3 treatment groups (n = 6-8/group):
  – no treatment (control)
  – fresh whole blood (FWB) given after liver injury
  – intravenous VPA (400 mg/kg, given in prehospital phase).

• Animals were monitored for 4 h, with survival being the primary endpoint. Liver tissue was subjected to Western blot analysis.
VPA improves survival similar to FWB

VPA improved survival

VPA did not improve acidosis
VPA up-regulates cell-survival pathway
Next Steps

• Treatment with VPA without blood transfusion improves early survival in a highly lethal poly-trauma and hemorrhagic shock model.

• The survival advantage is due not to improvement in resuscitation but to better tolerance of shock by the cells, in part due to the preservation of the Akt survival pathway.

• Subsequent pig study failed to show benefit – investigators believe the effect was lower dose and loss during uncontrolled hemorrhage (Alam et al., 2011)

• US DoD (Navy) is sponsoring phase I/II clinical studies (dose escalation).
Estrogen
Program Overview

Current Performers: University of Alabama-Birmingham, Texas A&M Institute for Preclinical Sciences

Program Goals:

<table>
<thead>
<tr>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Develop treatments to allow 75% survival in a rodent model of hemorrhagic shock (60% blood loss)</td>
<td>Develop treatment to improve survival in a large animal model (60% total blood loss)</td>
</tr>
<tr>
<td>1. Maintainable for 3 hours without crystalloid (fluid) resuscitation</td>
<td>1. Demonstrate equivalent effects in second, larger animal experimental model</td>
</tr>
<tr>
<td>2. Small mass/volume treatment (≤1 ml/kg)</td>
<td></td>
</tr>
<tr>
<td>3. Recovery of normal function</td>
<td></td>
</tr>
</tbody>
</table>

SBL Candidates:

- Estrogen-derivatives
- Hydrogen Sulfide

SBL Pig Model: 60% Bleed without Resuscitation

- Sinclair Mini-pig: 35-50kg sexually mature male
- Well-characterized & reproducible surrogate model that sufficiently predicts the response in humans
  1. Used in 200+ publications on hemorrhage
  2. Similar to humans anatomically, physiologically and in the pathophysiological response
Conjugated Estrogens (E2, Premarin)

5mg/kg

Requires 20x25 mg vials/dose for a std human

Showing U-Shaped effect of Premarin
Conjugated Estrogens (E2, Premarin)

17 beta-estradiol (E2) Single Dose, 40 male rats

![Kaplan-Meyer Survival Curves - 6hrs (1mg/kg)](image)

- **E2 (n=20)**: 40.0%*
- **Control (n=20)**: 5.0%

*p<0.05

- Requires 20x25 mg vials/dose for a std human

Showing U-Shaped effect of Premarin

5mg/kg

onsdag 5. september 2012
Conjugated Estrogens (E2, Premarin)

17 beta-estradiol (E2) Single Dose, 40 male rats

- Kaplan Meyer Survival Curves - 6hrs (1mg/kg)
- E2 (n=20) vs. Control (n=20)
  - *p<0.05
  - E2: 40.0%*  vs. Control: 5.0%

Premarin Single Dose, 32 male rats

- 5mg/kg
- Premarin (n=16) vs. Control (n=16)
  - Premarin: 83.3%  vs. Control: 27.8%
  - # p <0.05 vs. Control group

Requires 20x25 mg vials/dose for a std human  

Showing U-Shaped effect of Premarin

Anthony E. Pusateri, PhD (301-619-9822/anthony.pusateri@amedd.army.mil)  
Unclassified  
Slide of (36)  
12 June 2012

onsdag 5. september 2012
Conjugated Estrogens (E2, Premarin)

17 beta-estradiol (E2) Single Dose, 40 male rats

Premarin Single Dose, 32 male rats

Premarin Single Dose, 27 male Sinclair mini-swine

Kaplan-Meier survival curves - 6hrs (1mg/kg)

E2 (n=20) 40.0%*

Control (n=20) 5.0%

5mg/kg

Premarin (n=16) 83.3%

Control (n=16) 27.8%

# p <0.05 vs. Control group

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Showing U-Shaped effect of Premarin

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onsdag 5. september 2012
Conjugated Estrogens (E2, Premarin)

17 beta-estradiol (E2) Single Dose, 40 male rats

Kaplan-Meier Survival Curves - 6hrs (1mg/kg)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>E2 (n=20)</th>
<th>Control (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>60</td>
<td>*p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>180</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>240</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>300</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>360</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Premarin Single Dose, 32 male rats

5mg/kg

<table>
<thead>
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<th>Time (min)</th>
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<th>Control (n=16)</th>
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<tr>
<td>0</td>
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<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>240</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>300</td>
<td>0.0</td>
<td>0.0</td>
</tr>
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</table>

Premarin Single Dose, 27 male Sinclair mini-swine

Kaplan-Meier survival estimates

Requires 20x25 mg vials/dose for a std human

Showing U-Shaped effect of Premarin

Anthony E. Pusateri, PhD (301-619-9822/anthony.pusateri@amedd.army.mil)
Ethinyl Estradiol Sulfates (EE-3-sulfate)

- Water soluble molecule that is excreted via the bile duct instead of the kidneys.
  - Associated with marked hemodynamic, hypertension and bradycardia response.
- 7x More Potent Than 17b Estradiol
- 35x more potent than Premarin
- Stable at Varied Ambient Temperatures
- Can be Formulated In Low Volume, High Dose
- Amenable To For Field Use for Self Aid (Auto-injector)
- API, Custom Synthesized For SBL

**Latest Results:**

**EE-3-Sulfate Single Dose—Male Rat Data**

- Vehicle control (n=8)
- EE3-SO4 (1 mg/kg), n=12

**EE-3-Sulfate Single Dose—Male Pig Data**

- Vehicle control
- EE3-SO4 (3 mg/kg), n=6

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Related Work

- Resuscitative Endocrinology: Single-dose Clinical Uses for Estrogen - Traumatic Hemorrhagic Shock (RESCUE - Shock): A Phase II Trial to Evaluate the Effects of A Single Dose of Intravenous Premarin for the Treatment of Patients With Hemorrhagic Shock (n=50)
- Similar study for TBI
- PI: Jane Wigginton, MD
- Dallas, TX
- Conducted under the NHLBI (NIH) Resuscitation Outcomes Consortium
Next steps

• DARPA/DoD Considering whether to move the new compound toward clinical development

• Results of the Premarin studies in patients may provide insight
Conclusions

• The DoD Hemorrhage and Resuscitation R&D Program is comprehensively organized and executed with Joint coordination, direction, and cooperation according to a strategic plan with near, mid, and long-term objectives

• Great near-term advances will likely be possible with improved blood products and hemostatic products

• Very low volume resuscitative approaches, such as Estrogen, Valproate, C1-Inhibitor, or FTY720, that modulate the inflammatory response or make tissues resistant to ischemia may represent the next generation in remote damage control resuscitation
Comments and Questions