



# Systemic Inflammatory Response: Translational Research

**Eric Elster MD FACS**

**CDR MC USN**

**Naval Medical Research Center**

**National Naval Medical Center**

**Walter Reed Army Medical Center**

# Multiple Injuries in Combat Wounded



# Translational Research Program



# Inter-service collaboration

- Clinical Centers
  - **NNMC - Biomarkers pilot underway (n = 48)**
  - **NNMC/WRAMC - Orthopaedic predictors (n = 52)**
  - **WRAMC - ESWT trial underway (n = 40)**
  - **NNMC – Prospective biomarker (start October)**
  - **BAMC – future ESWT site**
  - **University of Maryland – future biomarker site**
  - **Johns Hopkins Wound Center – future biomarker and EWST site**
- Research Labs
  - **NMRC – Central Player**
  - **AFIP/WRAIR – Microbiology support**

# Role of Inflammatory Response

- Wound healing
- Heterotopic Ossifications
- Blood Transfusion Requirements
- Wound Bioburden
- Resource Utilization
  - Hospital Stay
  - ICU
- Attenuation of Response
  - Global – Lymphocyte Depletion or Sequestration (Porcine studies)
  - Local – ESWT (Rodent studies)

“Following massive injury, physiological responses that were appropriate when applied locally become inappropriate and beyond regulation when systemically activated.”

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# When are wounds 'ready' to heal?

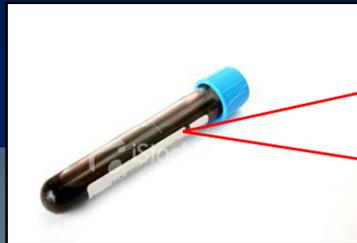
This wound healed



This wound dehisced

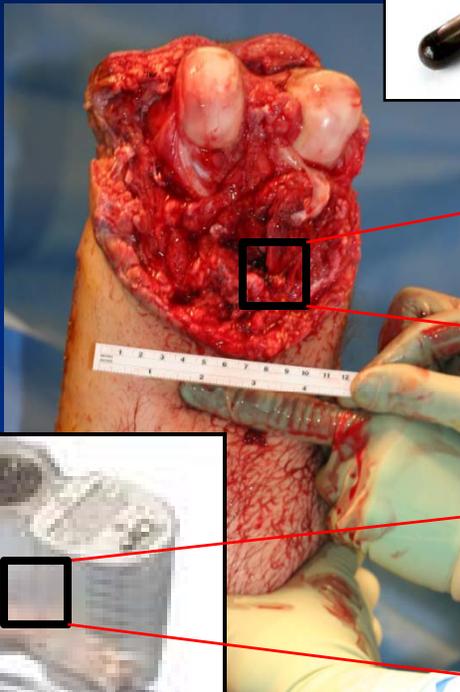


# Wound Healing Biomarkers



## Serum:

- Cytokines
- Chemokines



## Tissue biopsy:

- Wound healing associated genes
- Quantitative bacteriology



## Wound effluent:

- Cytokines
- Chemokines
- Quantitative bacteriology

# Inflammatory Biomarkers in Combat Wound Healing

Figure 2

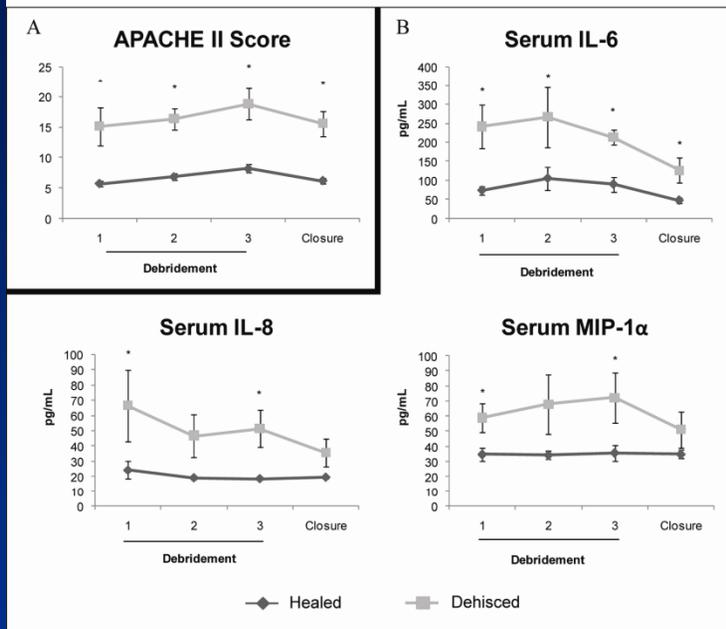
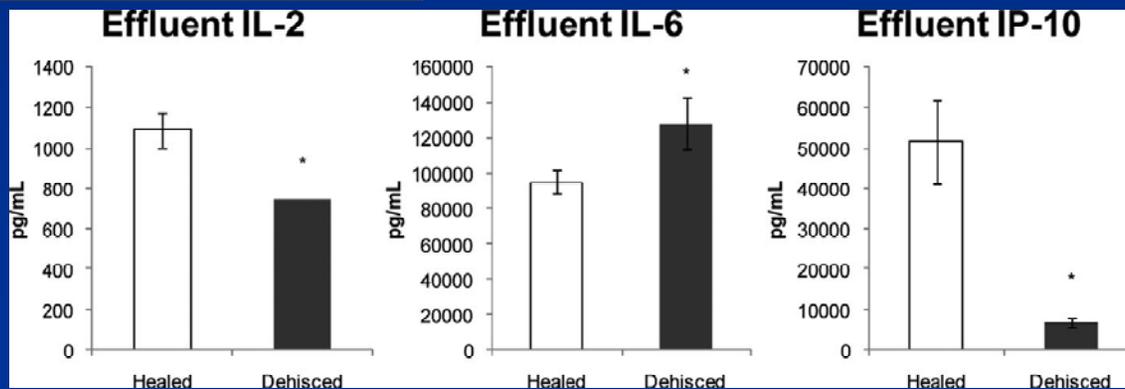
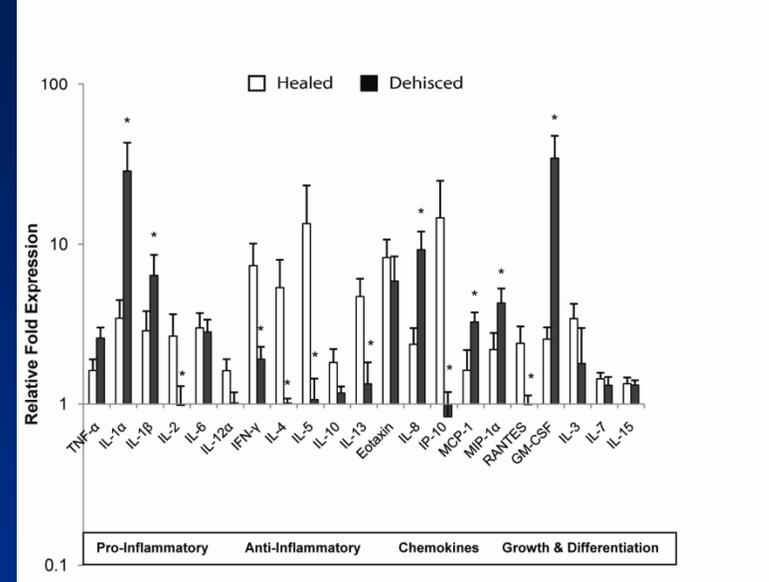
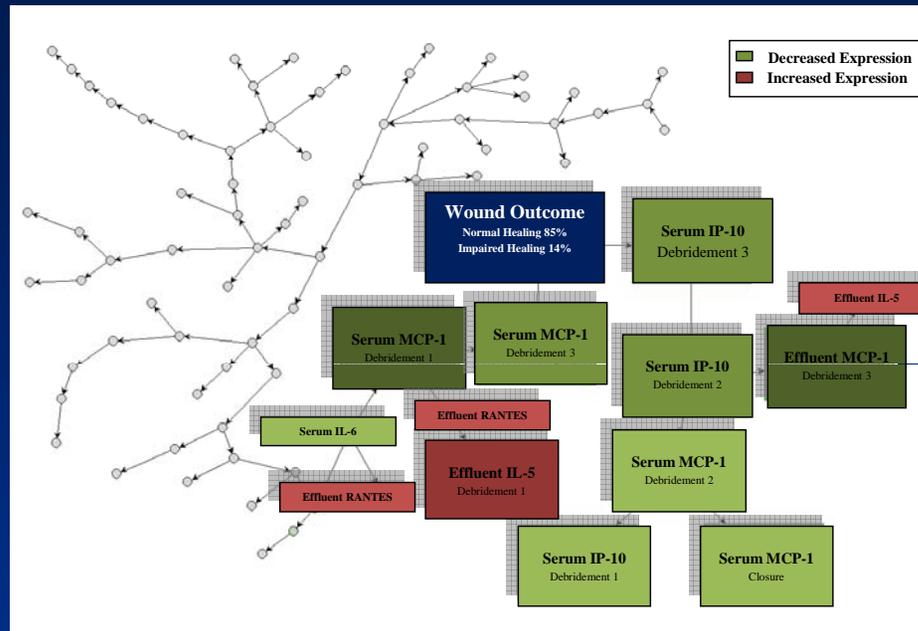


Figure 4



# Probabilistic (Bayesian) Model

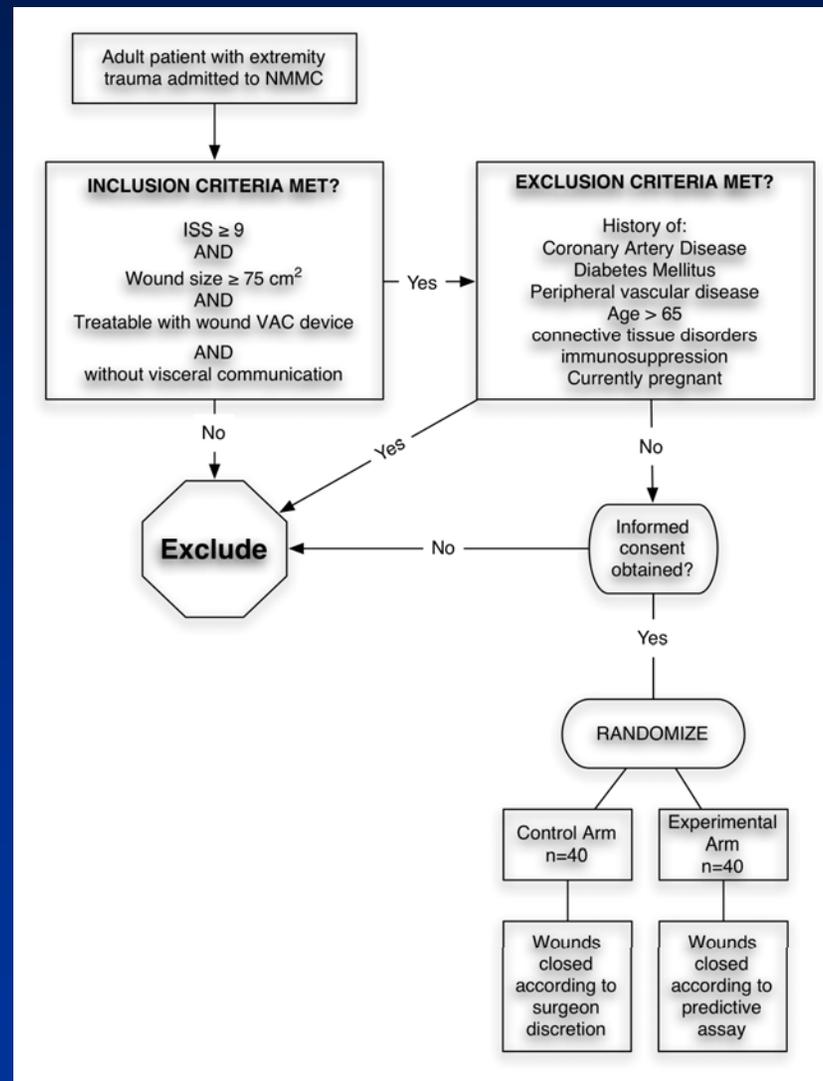


## Validation Analysis<sup>a</sup>

	Sensitivity	Specificity	Impaired Predictive Value	Normal Predictive Value
<b>Bayesian Biomarker Network</b>	88.9%	90%	80%	94.7%

<sup>a</sup> Leave-one-out method

# Prospective Biomarker Study



# Potential Cost Savings Wound Biomarkers

- The average cost per wound (I&D) = \$1,643
- 2007- 500 visits to the operating room for I&D
- Approximately 3.5 I&Ds per patient
- Annual cost for I&Ds alone was at least \$821,500
- Reduce the average number of I&Ds to 2.5 = total savings for I&D costs per year of \$234,949
- Reduce the mean LOS from 22 days to 18 days = additional \$1,944,800
- Total yearly cost savings = \$2,179,749.

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# Which wounds develop HO?

This wound developed HO

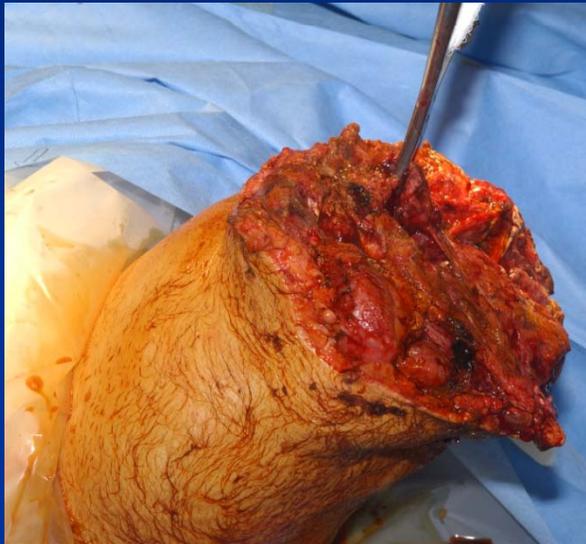


Same patient – No HO



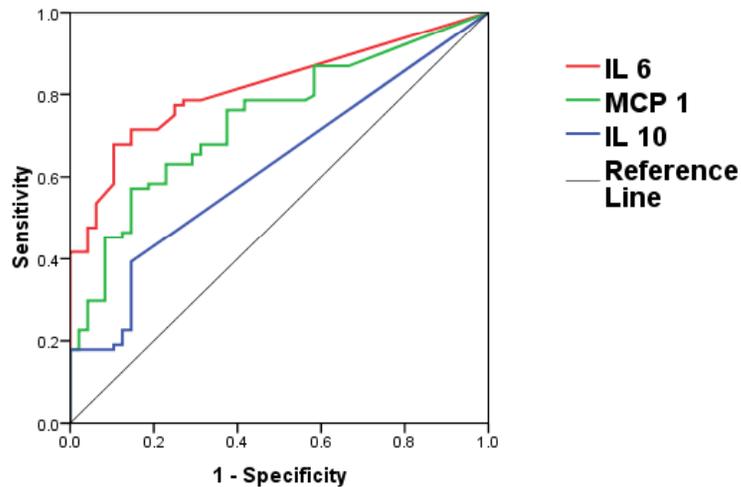
# HO Clinical Characteristics

Characteristic	P Value	Chi Square	Odds Ratio
Patient age (continuous variable)	0.01*	6.4	N/A
Age: < 30 vs. ≥ 30 years (OR for < 30 years)	0.007*	7.4	3.0
Injury Severity Score (continuous variable)	<0.001*	13.9	N/A
ISS: < 16 vs. ≥ 16 (OR for ISS ≥ 16)	0.02*	5.4	2.2
Multiple affected limbs (OR for multiple affected)	0.002*	10.1	3.9
Location (OR for residual limb)	0.048*	6.1	2.9
Mechanism of injury	0.25		
Blast and ISS ≥ 16	0.26		
TBI and ISS ≥ 16	0.69		
Blast and TBI	0.37		

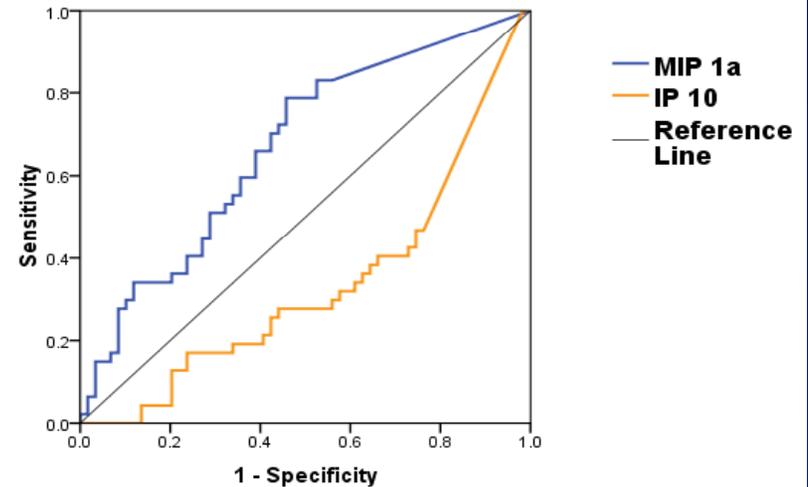


# ROC Curves

## Serum Biomarkers



## Effluent Biomarkers

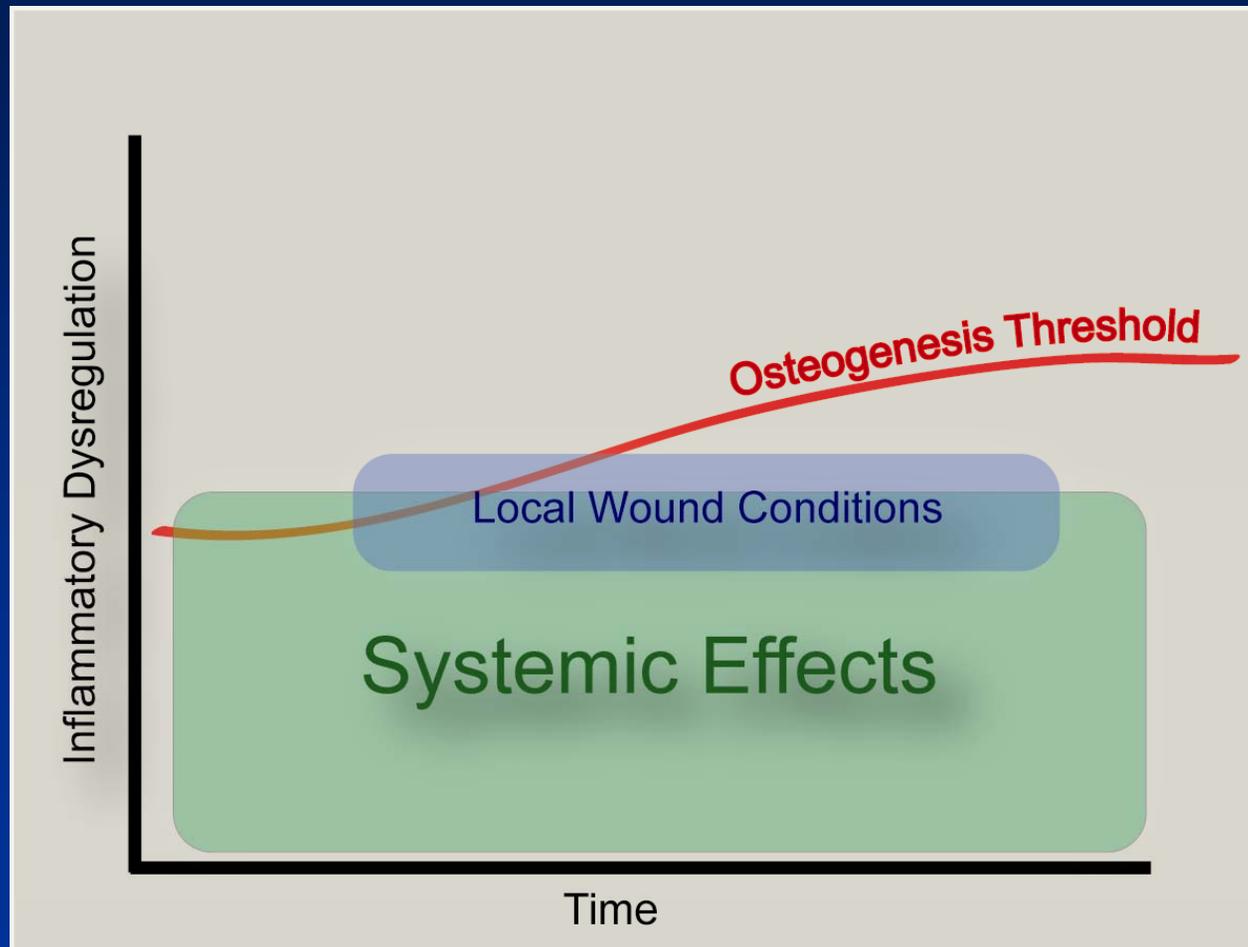


Biomarker	Area Under Curve	95% CI	P Value
Serum IL-6	0.801	0.728 Š 0.873	<0.001
Serum MCP-1	0.722	0.638 Š 0.806	<0.001
Effluent MIP-1 $\alpha$	0.681	0.581 Š 0.782	0.001
Effluent IP-10*	0.671	0.567 Š 0.774	0.002
Serum IL-10	0.625	0.531 Š 0.719	0.016

\*IP-10 predictive of not developing HO

Evans, Brown et al, JBJS In press

# HO and Inflammation



# Role of Inflammatory Response

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# Blood Transfusion Requirement is Associated with Systemic Response

- Study cohort
  - Mean age of  $22 \pm 1$ ,
  - Mean ISS of  $15.8 \pm 2.8$ ,
  - Mean Glasgow Coma Score
  - GCS of  $13.8 \pm 0.6$
  - Penetrating injuries (90% IED and 10% GSW).
- Group of serum cytokines whose elevation was associated with increased blood product utilization

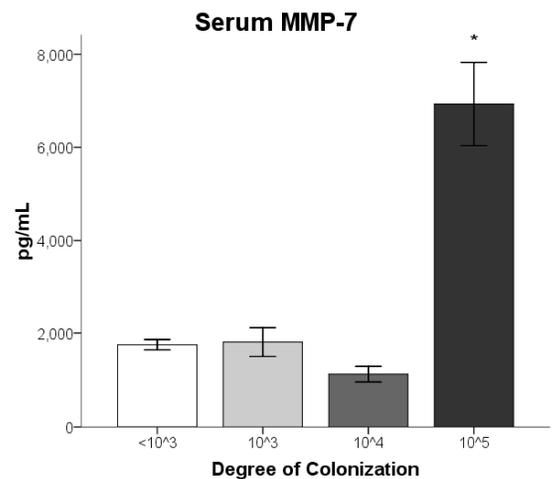
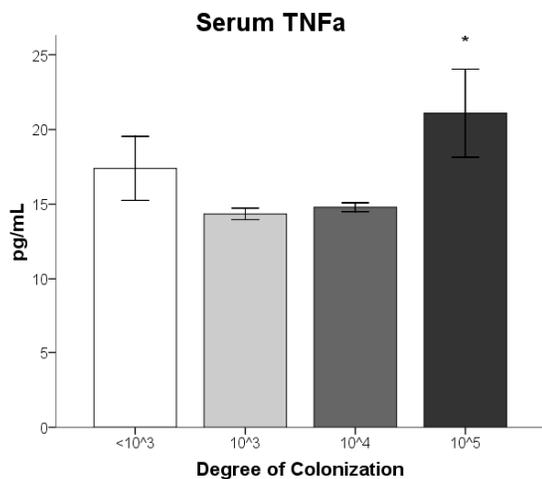
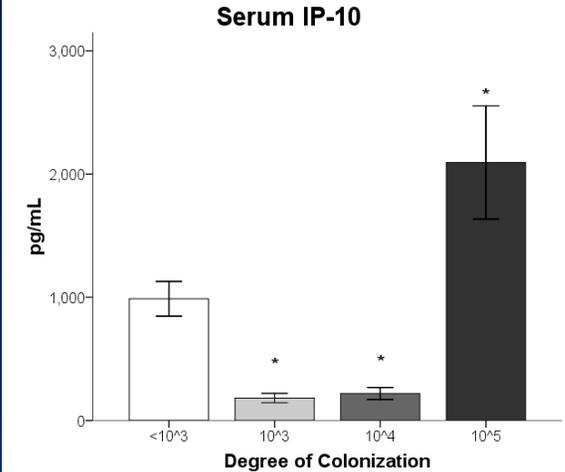
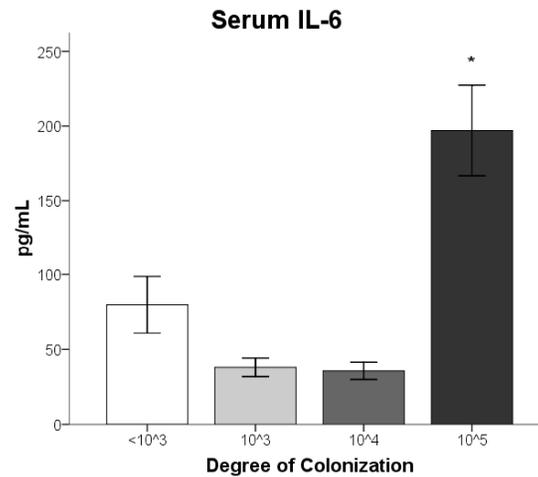
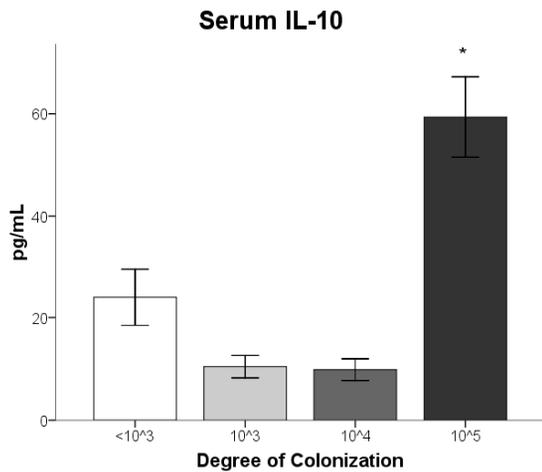
# Cytokines and Blood Transfusion

<i>Cytokine</i>	<i>Pairwise Correlation</i>	<i>p</i>
IL-10	0.94	< 0.001
IL-8	0.90	< 0.001
IP-10	0.67	0.0013
IL-6	0.61	0.004
IL-12p40	0.47	0.038

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# Systemic Response to Wound Colonization



$<3^{10}$  CFU/gm - undetectable  
 $3^{10}$  CFU/gm - colonized  
 $4^{10}$  CFU/gm - critical colonization  
 $\geq 5^{10}$  CFU/gm - infection  
\* $p < 0.05$  compared to  $<3^{10}$  CFU/gm

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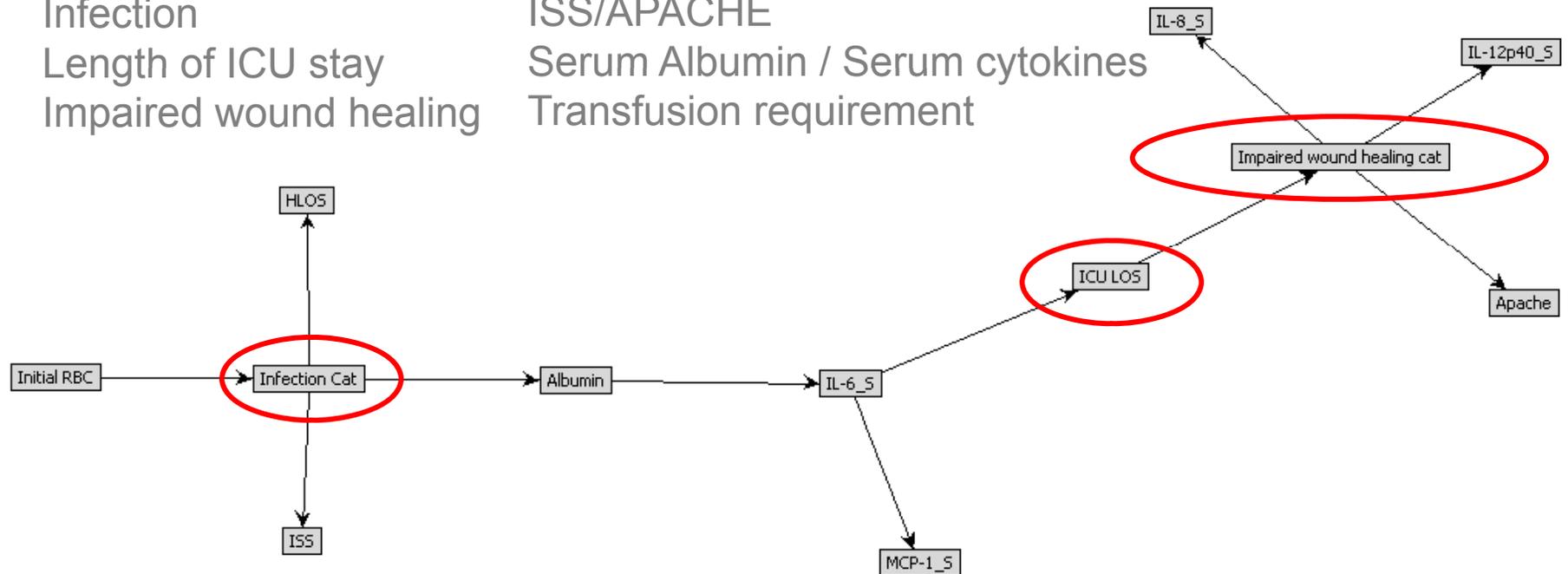
# Bayesian Belief Network Model Clinical Outcomes in War Wounded

## Outcomes:

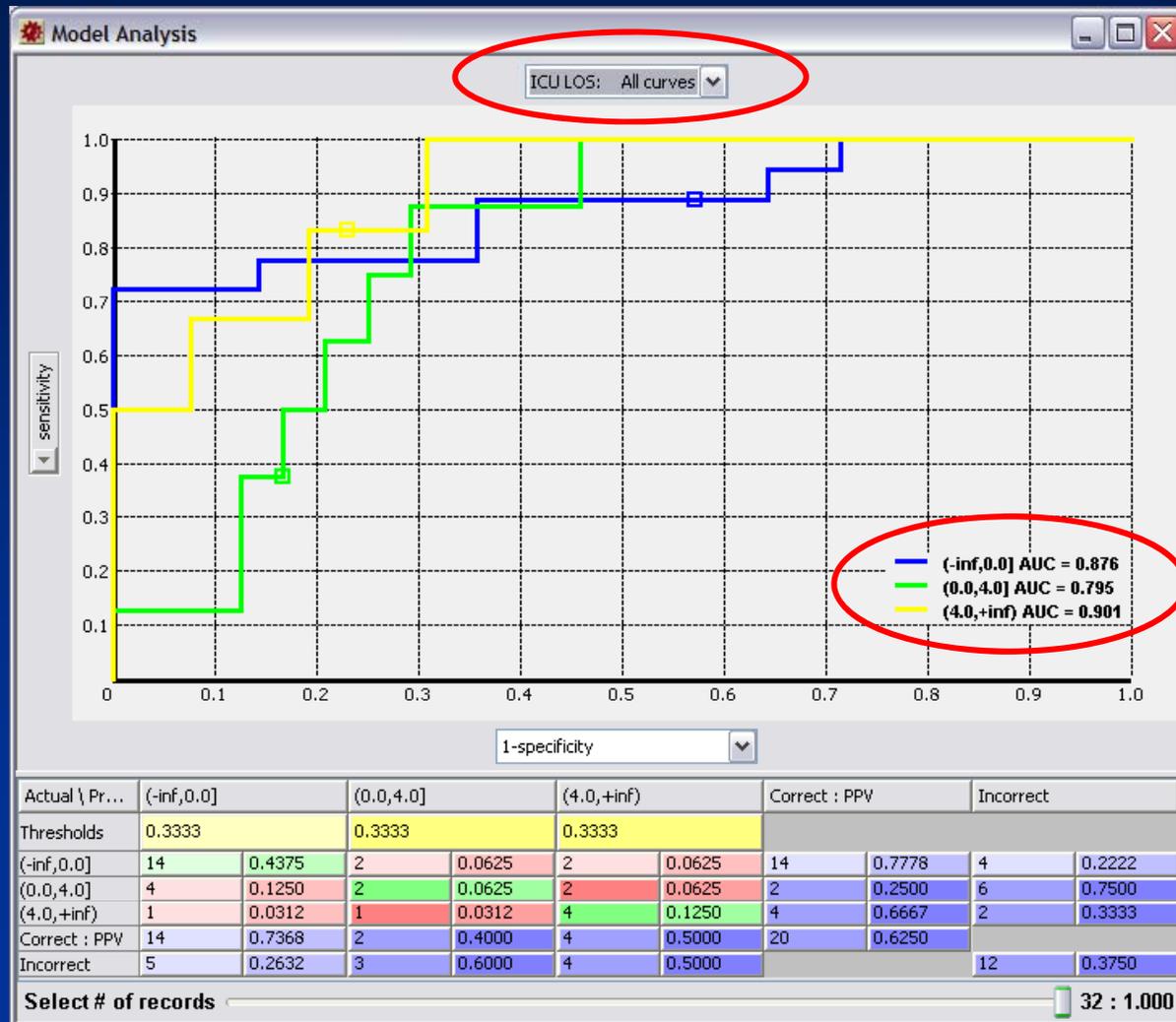
Infection  
Length of ICU stay  
Impaired wound healing

## Variables:

ISS/APACHE  
Serum Albumin / Serum cytokines  
Transfusion requirement



# Probabilistic Model ICU LOS

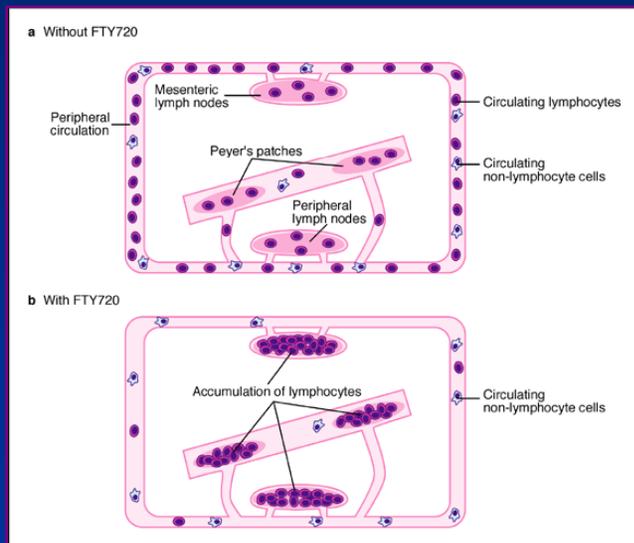


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# Systemic Immunomodulation

Lymphocyte depletion or sequestration agents given at the time of severe hemorrhage will attenuate innate immune molecular and cellular activation following ischemia and reperfusion

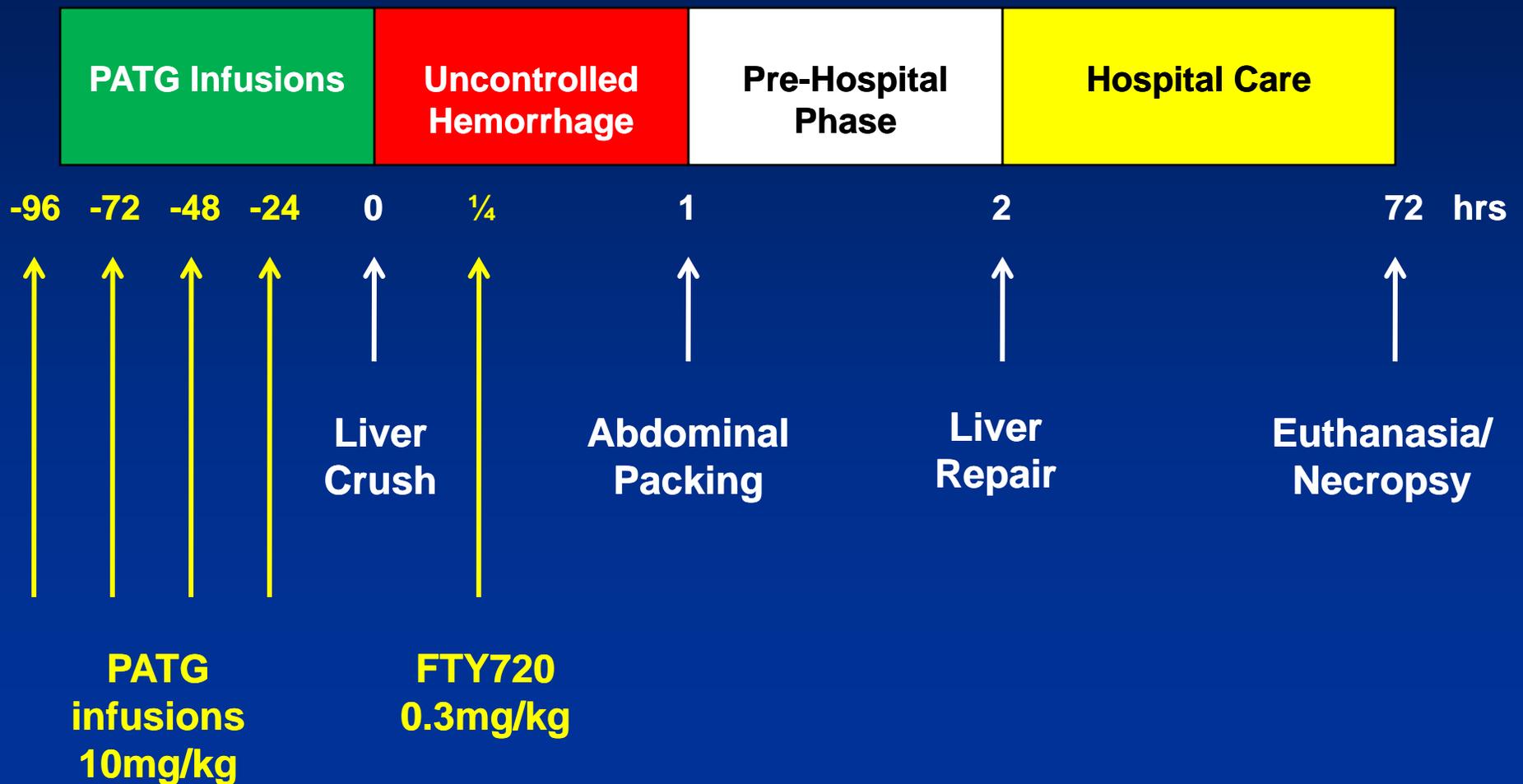


FTY720 - Novartis



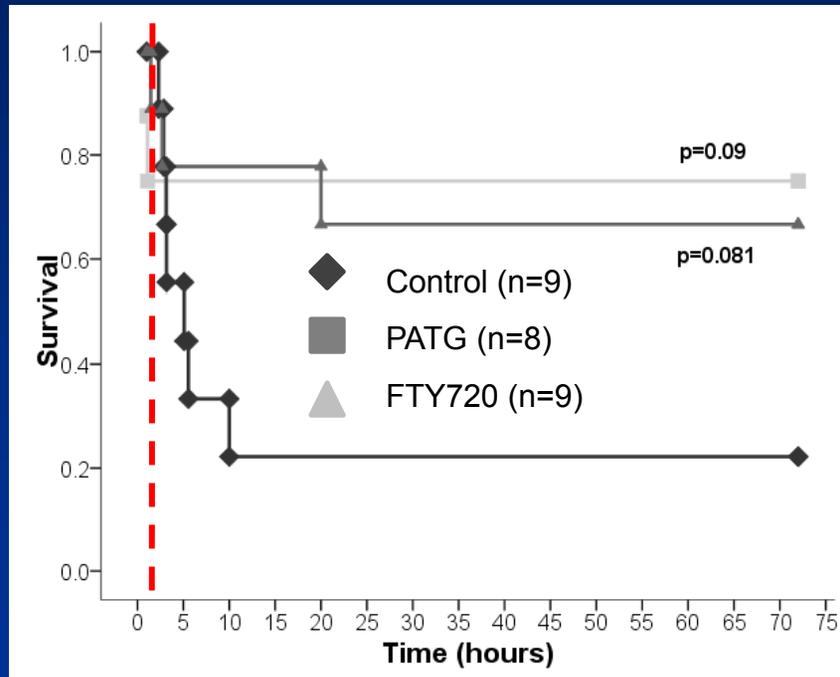
Thymoglobulin - Genzyme

# Porcine Shock Model

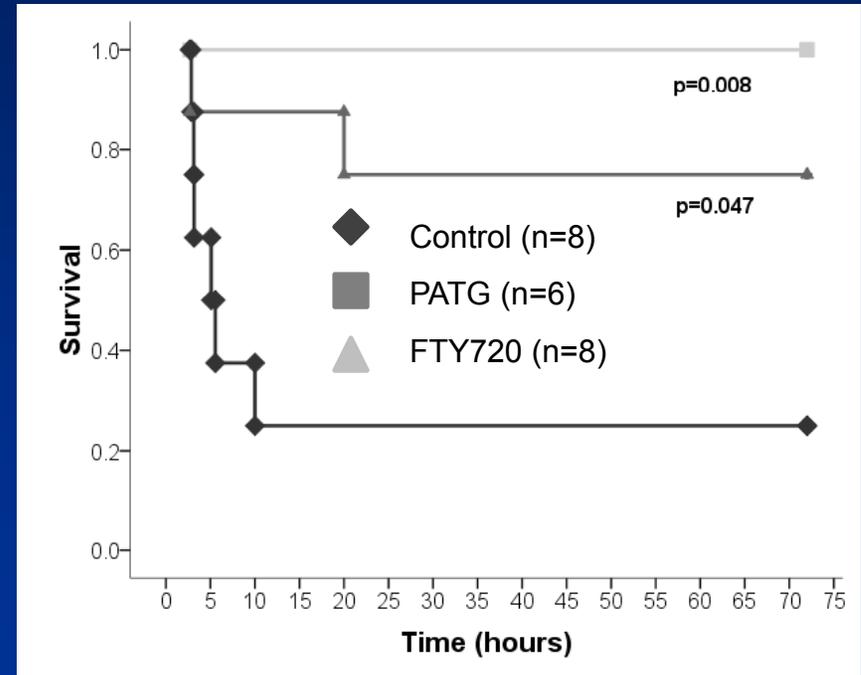


# Immune Modulation Improves Survival

## Overall Survival

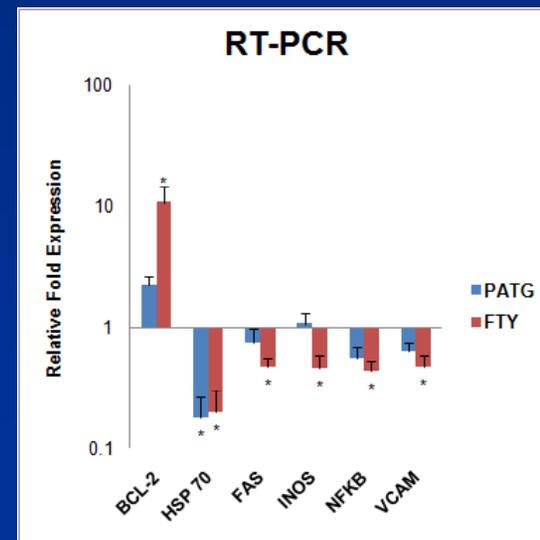
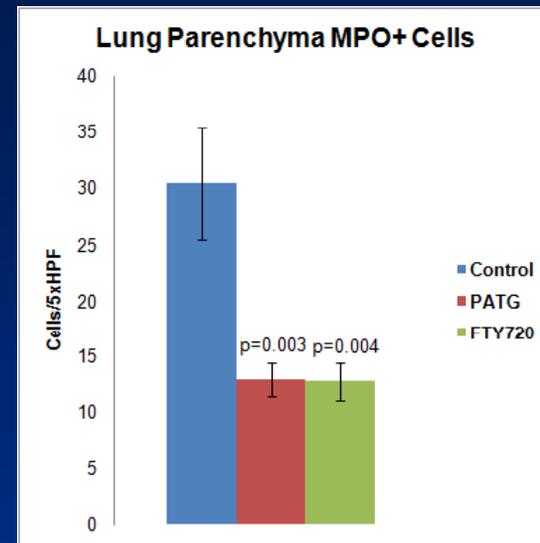
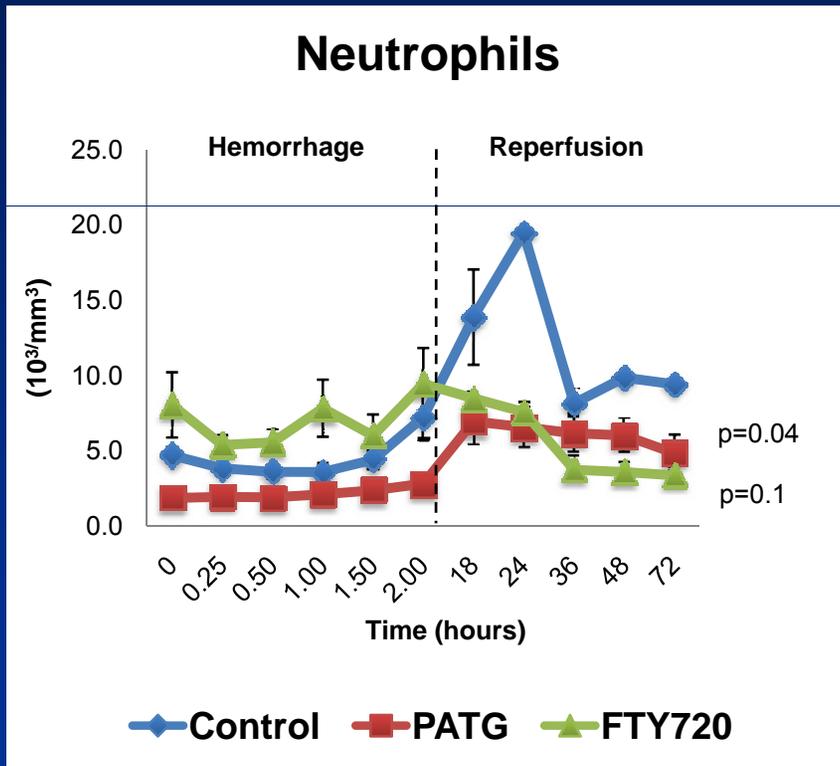


## Reperfusion Survival



*Hawksworth, Graybill et al.*

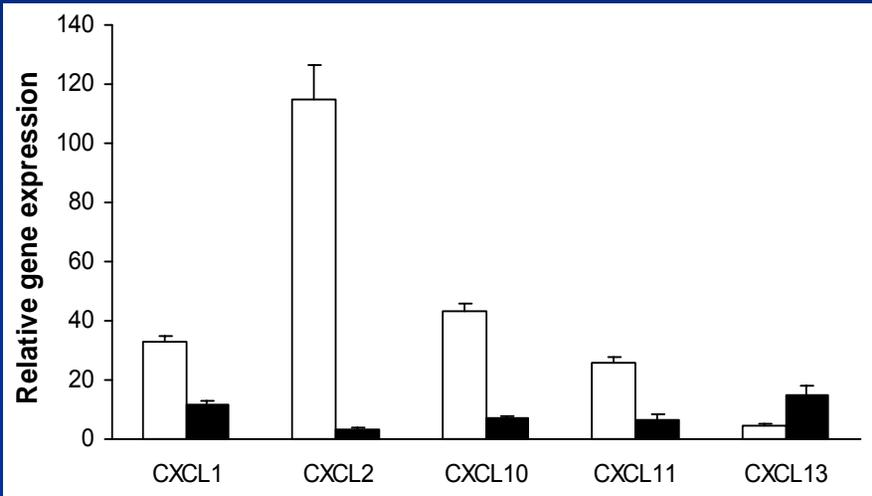
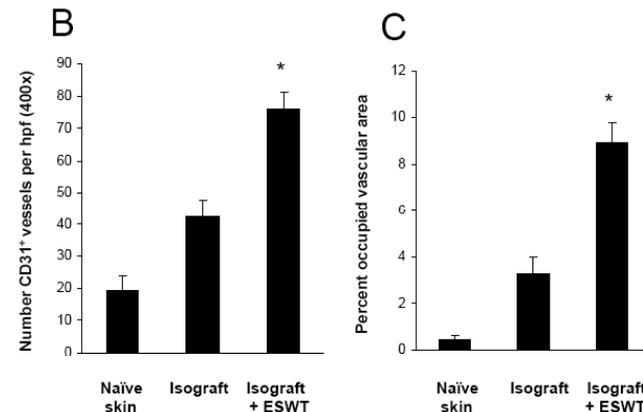
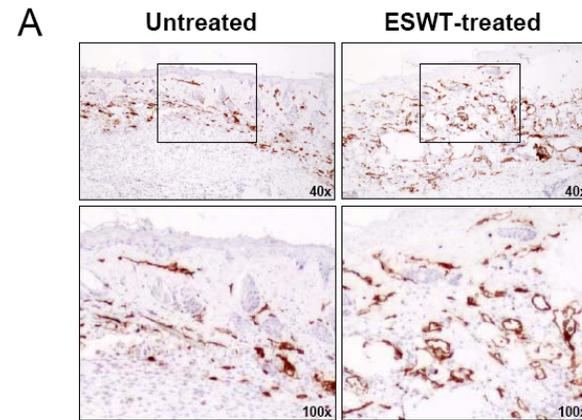
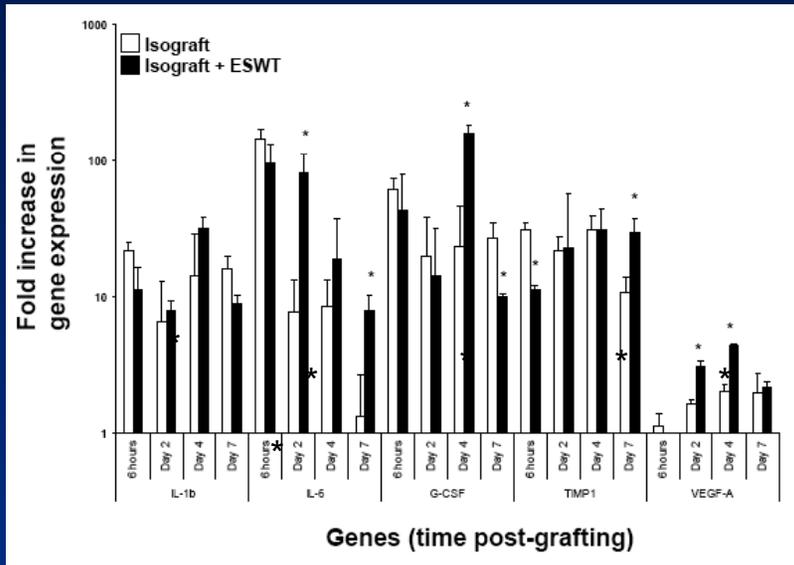
# Lymphocyte Immunomodulation Attenuates Innate And Cellular Response



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# ESWT Immunomodulates and Increases Angiogenesis In Small Animals



*Stojadinovic, Elster, Angiogenesis 2008*  
*Davis et al Int Wound J 2008*

# Conclusions

- War wound failures, HO, transfusion requirements and wound infection are all related to systemic inflammatory dysregulation
- These all result in increase resource utilization
- Inflammatory biomarkers can be measured in serum and in wounds and are potentially predictive in acute war wound healing, wound infection and HO
- Modulation of the inflammatory response to injury may provide novel future therapies
- The combination of diagnostics and therapeutics provide the potential for cost savings, improved care and faster return to duty

# Acknowledgements

- The multidisciplinary care of these patients would not have been possible without the dedicated efforts of everyone at WRAMC and NNMC. Both civilian and military personnel have rendered skilled and compassionate care for these casualties. All of our efforts are dedicated to those who have been placed in harm's way for the good of our nation.
- The views expressed are those of the authors and do not reflect the official policy of the Department of the Navy, Army, the Department of Defense, or the US Government.
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