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Medicine Research Center

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**33 YEARS**  
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# Immunothrombosis: what trauma induced coagulopathy taught us about COVID-19 therapies

Matthew D. Neal, MD FACS

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Co-Director of the Pittsburgh Trauma and Transfusion Medicine Research Center  
Co-PI of Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV4) DCC/CCC  
University of Pittsburgh



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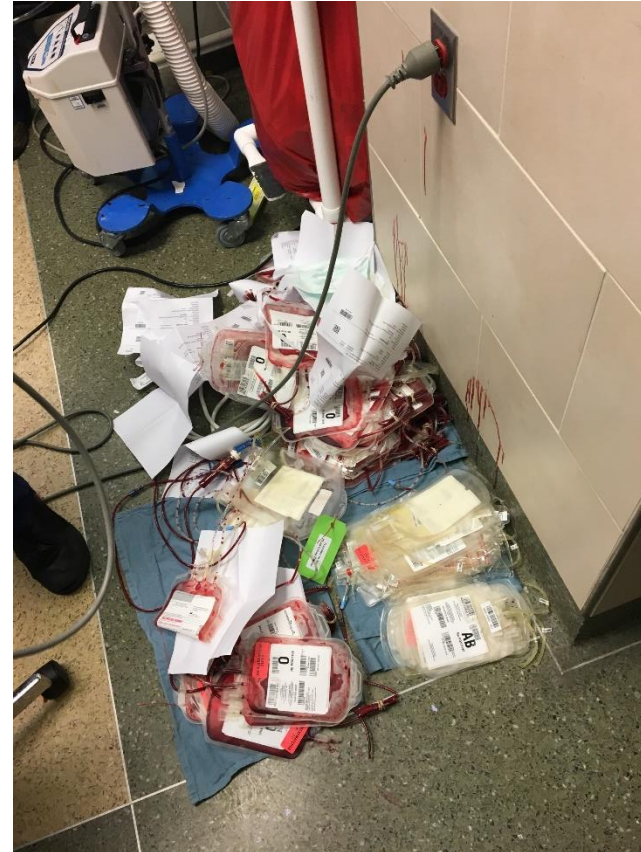
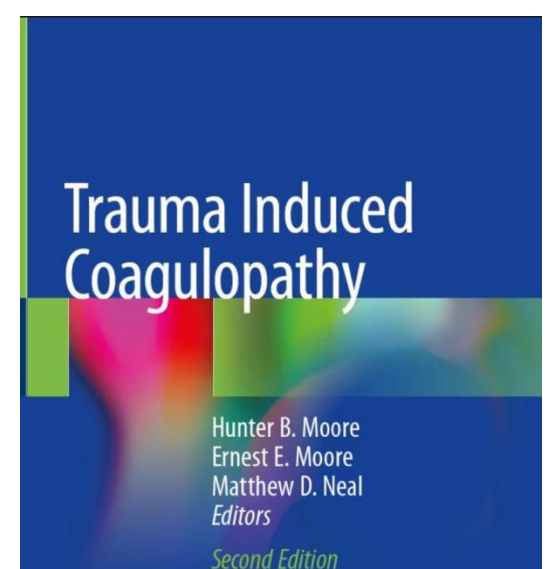
The logo for the ACTIV-4 study, featuring a red, stylized, wavy line above the text "ACTIV-4".  
**ACTIV-4**  
Acute Inpatient Anti-Thrombotic Study

# Disclosures

- Research funding: NIH, DoD
- Research funding: Haemonetics, Instrumentation Laboratory
- Honoraria, travel support, consulting: Janssen, CSL Behring, Haemonetics, Meredian
- Scientific advisory board, equity stake: Haima Therapeutics
- US Patents: DIELECTRIC SENSING TO CHARACTERIZE HEMOSTATIC DYSFUNCTION Serial Number: 16/837,704; NOVEL TLR4 INHIBITORS FOR THE TREATMENT OF HUMAN INFECTIOUS AND INFLAMMATORY DISORDERS Serial Number: 17/174,018
- Co-PI, ACTIV4 Data and Clinical Coordinating Centers, Co-Chair, ACTIV4a clinical trial

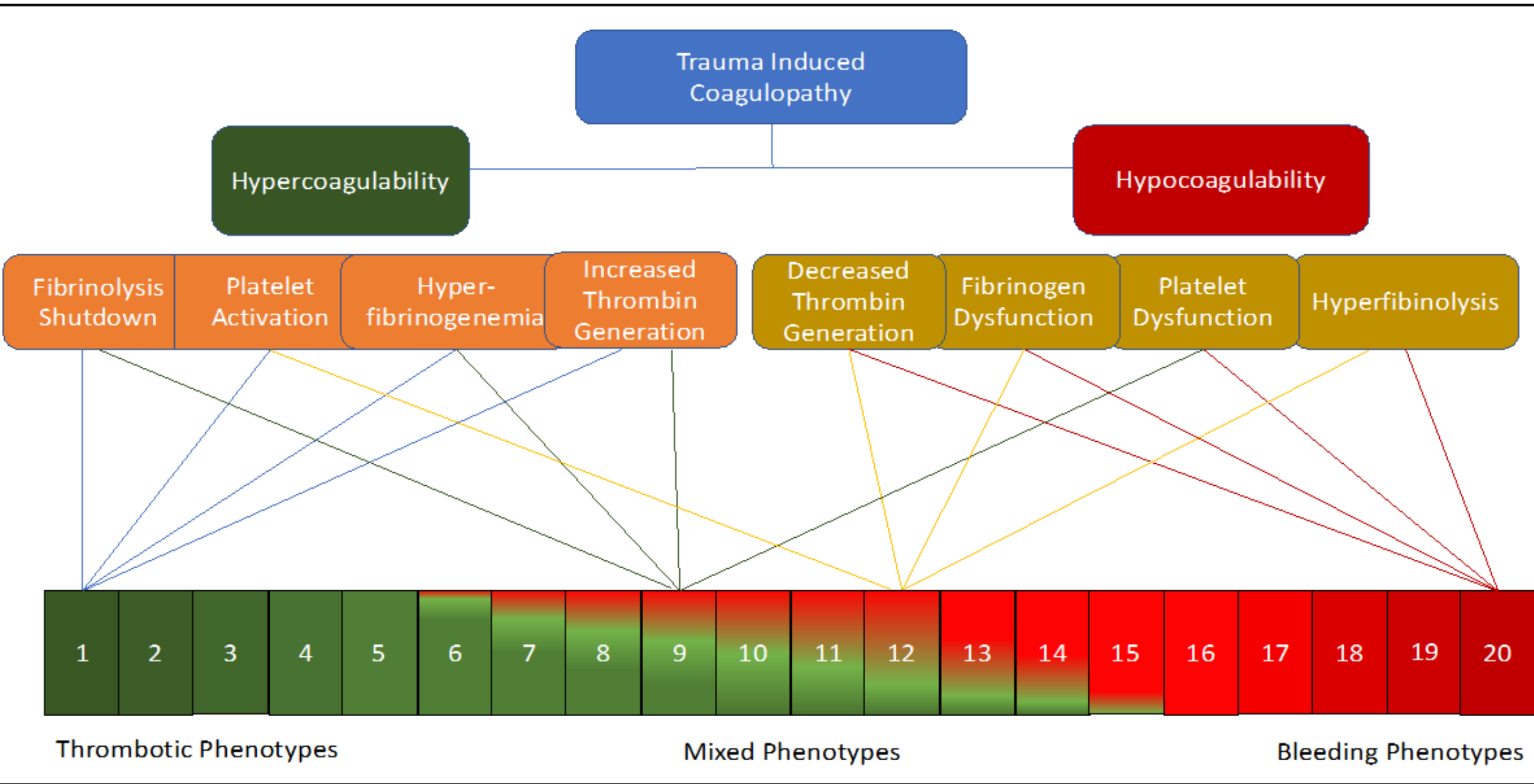
# Disclosures

- Trauma surgeon masquerading as a platelet biologist, hemostasis scientist, and now clinical trialist



# Objectives and overview

- TIC mechanisms
- Platelets and neutrophils in trauma
- Immunothrombosis
- What does any of this have to do with COVID-19?
- Mechanisms of COVID-19 associated coagulopathy
- Interventional trials – the ACTIV4 program and the mpRCT (i.e. what I did during the pandemic)
- Can trauma and TIC learn from COVID-19?



Trauma Induced Coagulopathy

Hypercoagulability

Hypocoagulability

Fibrinolysis Shutdown

Platelet Activation

Hyper-fibrinogenemia

Increased Thrombin Generation

Decreased Thrombin Generation

Fibrinogen Dysfunction

Platelet Dysfunction

Hyperfibrinolysis

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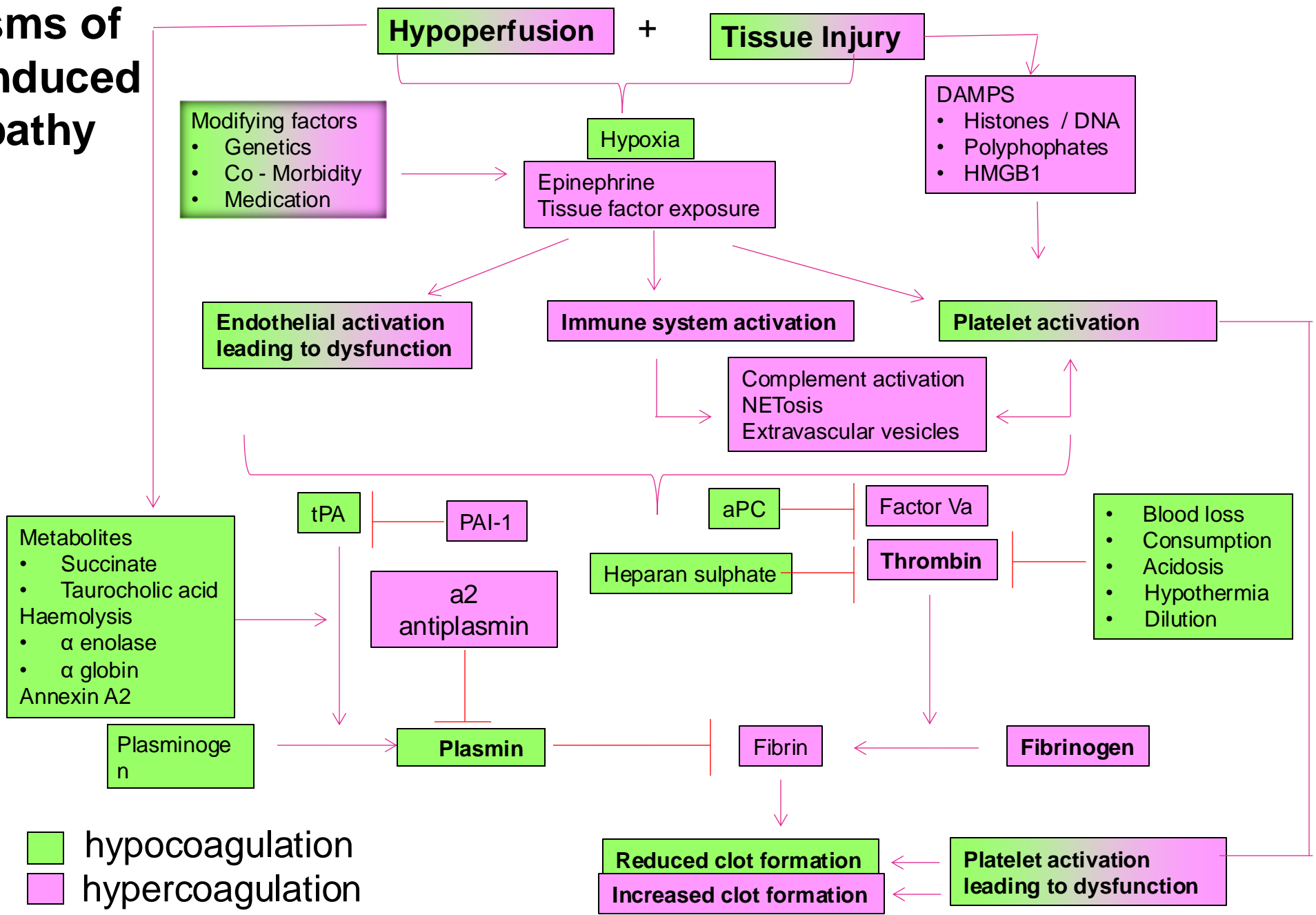
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Thrombotic Phenotypes

Mixed Phenotypes

Bleeding Phenotypes

# Mechanisms of Trauma Induced Coagulopathy



# “Irreversible Shock” Crowell 1955 Turpini 1959 Hardaway 1962 (Animals)

- Hypercoagulability = bad
  - Needs to be reversed or death via “DIC”
  - Pre Treat Heparin
  - Post Treat Fibrinolysin

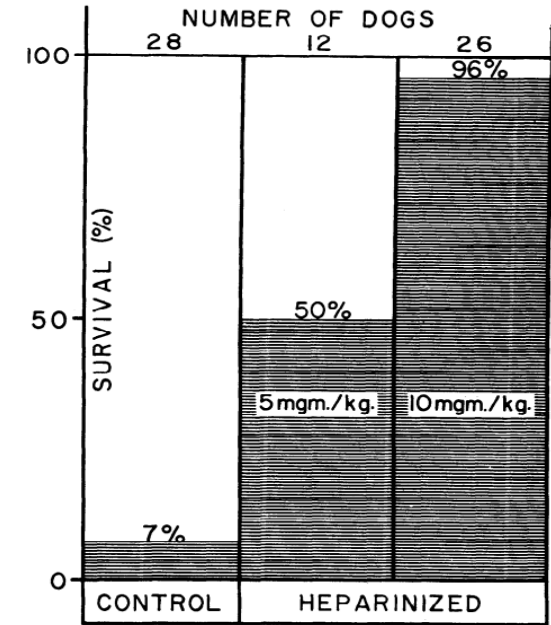
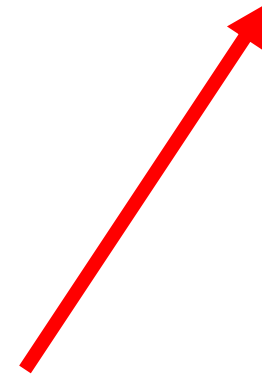
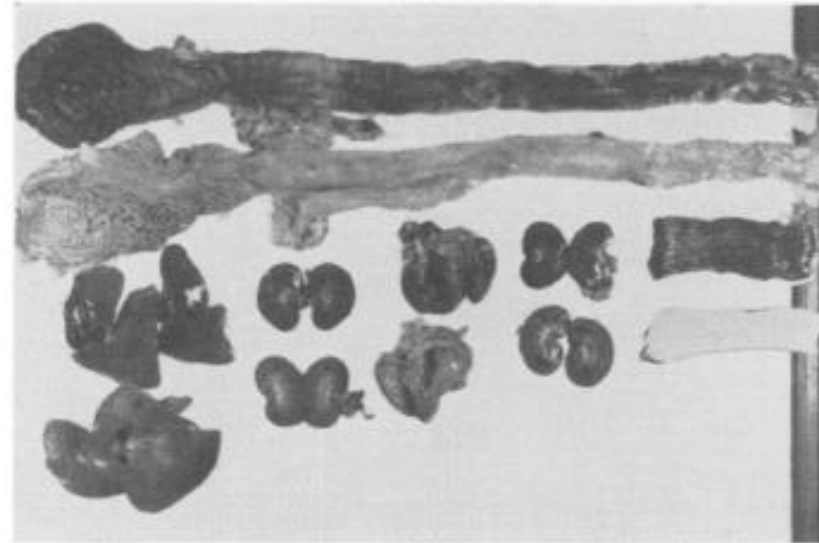
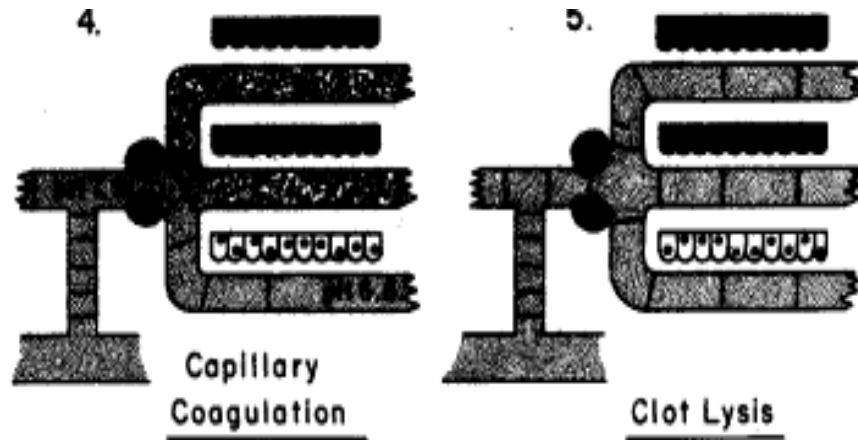


FIG. 3. Summary of all experiments grouped according to initial amount of heparin injected.



**TLR4 Regulates Platelet Function and Contributes to Coagulation Abnormality and Organ Injury in Hemorrhagic Shock and Resuscitation**  
Ning Ding, Guoqiang Chen, Rosemary Hoffman, Patricia A. Loughran, Chhinder P. Sodhi, David J. Hackam, Timothy R. Billiar and Matthew D. Neal

[www.nature.com/scientificreports](http://www.nature.com/scientificreports)

SCIENTIFIC REPORTS

**OPEN** Deep vein thrombosis in mice is regulated by platelet HMGB1 through release of neutrophil-extracellular traps and DNA

Received: 27 July 2017  
Accepted: 6 December 2017  
Published online: 01 February 2018

Mitchell R. Dyer<sup>1</sup>, Qiwei Chen<sup>1</sup>, Shannon Haldeman<sup>1</sup>, Hamza Yazdani<sup>1</sup>, Rosemary Hoffman<sup>1</sup>, Patricia Loughran<sup>1,2</sup>, Allan Tsung<sup>1</sup>, Brian S. Zuckerbraun<sup>1</sup>, Richard L. Simmons<sup>1</sup> & Matthew D. Neal<sup>1</sup>

REGULAR ARTICLE

blood advances

Platelet HMGB1 is required for efficient bacterial clearance in intra-abdominal bacterial sepsis in mice

Hui Zhou,<sup>1,2,\*</sup> Meihong Deng,<sup>1,\*</sup> Yingjie Liu,<sup>1</sup> Chenxuan Yang,<sup>1</sup> Rosemary Hoffmann,<sup>1</sup> Jingjiao Zhou,<sup>1</sup> Patricia A. Loughran,<sup>1</sup> Melanie J. Scott,<sup>1</sup> Matthew D. Neal,<sup>1</sup> and Timothy R. Billiar<sup>1</sup>

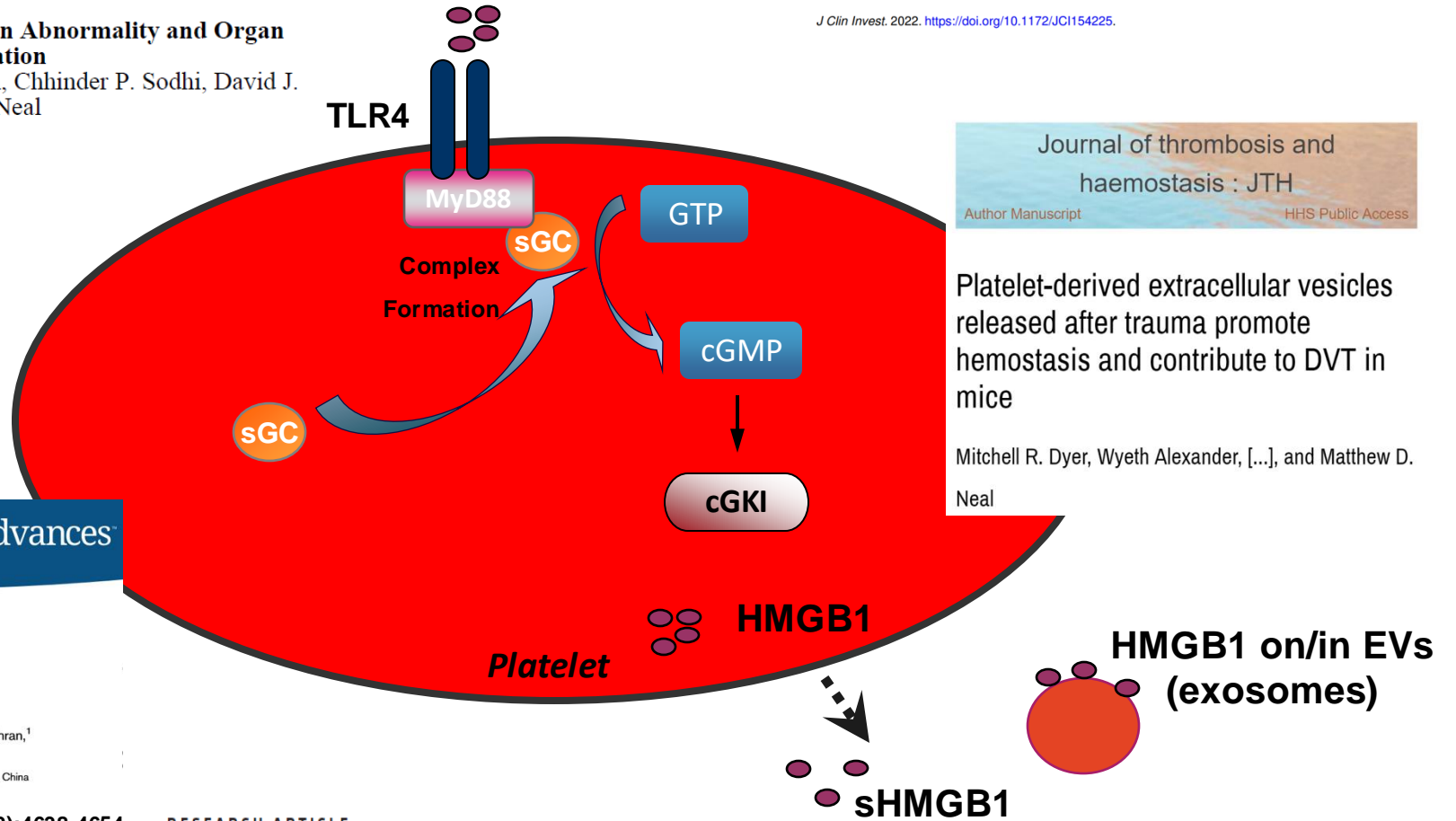
<sup>1</sup>Department of Surgery, University of Pittsburgh, Pittsburgh, PA; <sup>2</sup>Department of Intensive Care Unit, Zhongnan Hospital of Wuhan University, Wuhan, China

The Journal of Clinical Investigation *J Clin Invest* 2015;125(12):4638-4654 RESEARCH ARTICLE

**Platelet-derived HMGB1 is a critical mediator of thrombosis**

Sebastian Vogel,<sup>1</sup> Rebecca Bodenstein,<sup>1</sup> Qiwei Chen,<sup>2</sup> Susanne Feil,<sup>3</sup> Robert Feil,<sup>3</sup> Johannes Rheinlaender,<sup>4</sup> Tilman E. Schäffer,<sup>4</sup> Erwin Bohn,<sup>5</sup> Julia-Stefanie Frick,<sup>5</sup> Oliver Borst,<sup>1</sup> Patrick Münzer,<sup>1</sup> Britta Walker,<sup>1</sup> Justin Markel,<sup>2</sup> Gabor Csanyi,<sup>6</sup> Patrick J. Pagano,<sup>6</sup> Patricia Loughran,<sup>2,7</sup> Morgan E. Jessup,<sup>7</sup> Simon C. Watkins,<sup>7</sup> Grant C. Bullock,<sup>8</sup> Jason L. Sperry,<sup>2</sup> Brian S. Zuckerbraun,<sup>2</sup> Timothy R. Billiar,<sup>2</sup> Michael T. Lotze,<sup>2</sup> Meinrad Gawaz,<sup>1</sup> and Matthew D. Neal<sup>2</sup>

**Danger signals (DAMPs)**



Journal of thrombosis and haemostasis : JTH  
Author Manuscript HHS Public Access

Platelet-derived extracellular vesicles released after trauma promote hemostasis and contribute to DVT in mice

Mitchell R. Dyer, Wyeth Alexander, [...], and Matthew D. Neal

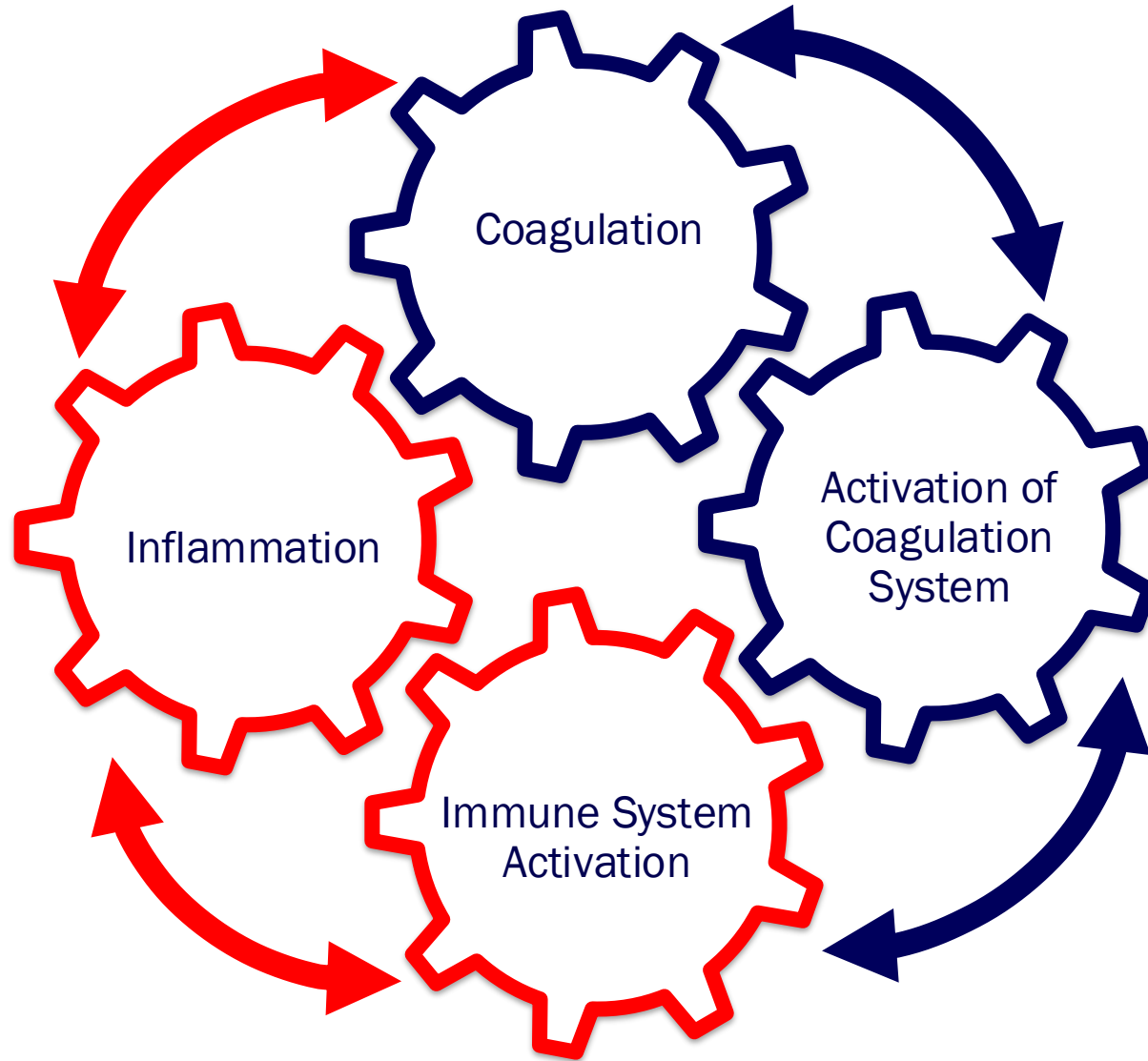
Platelet aggregation

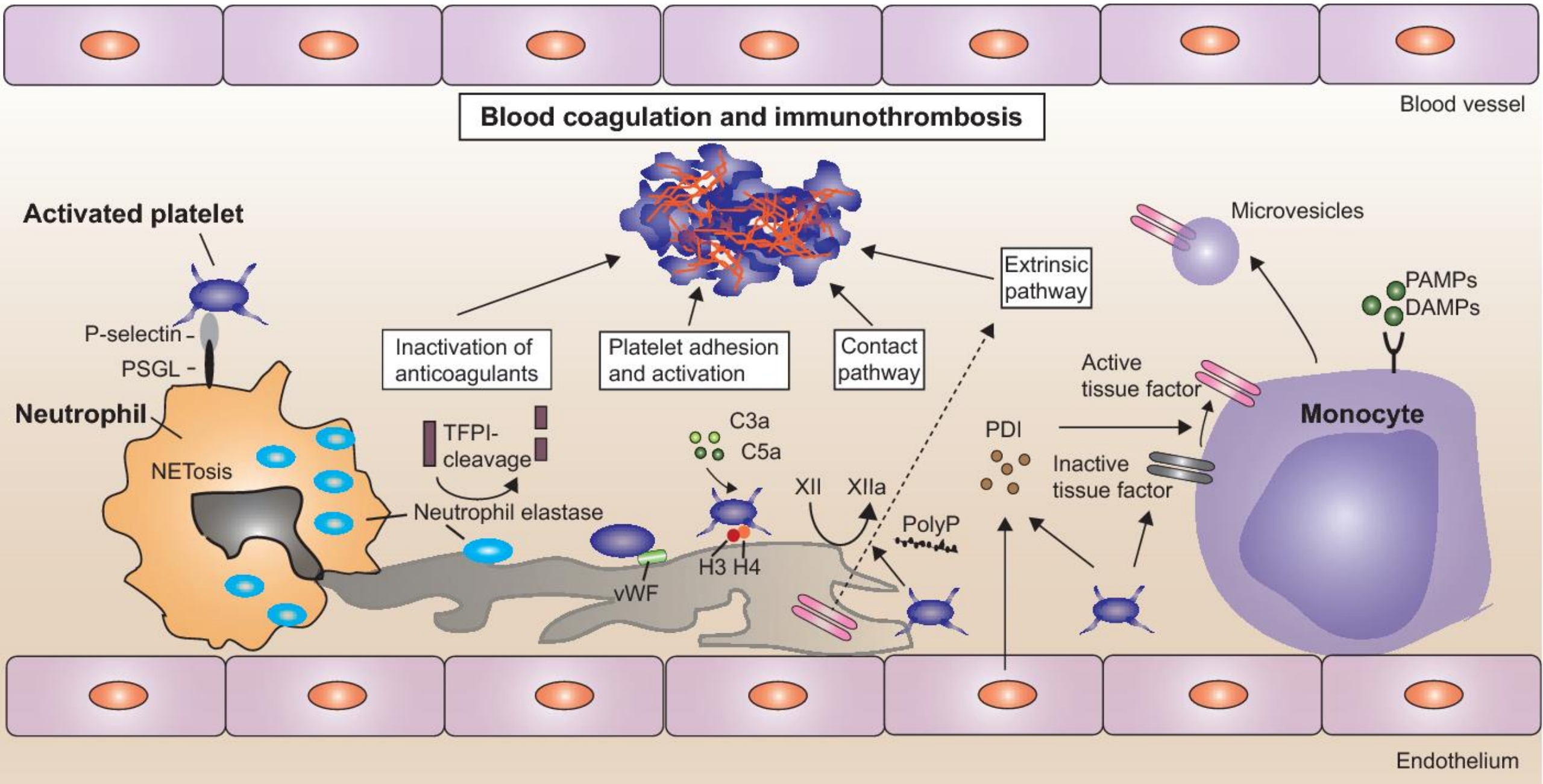
Neutrophil activation/NET

Thrombosis



# Immunothrombosis





# TACTIC: Trans-Agency Consortium for Trauma Induced Coagulopathy



The TACTIC team was  
coagulopathy (TIC) occur  
coagulation

# Covid-19

patients. Trauma induced  
"storm" of inflammatory and  
ostatic process.

TACTIC and COVID-19



[REDACTED] (NIH/NHLBI) [E] <[REDACTED]@nhlbi.nih.g

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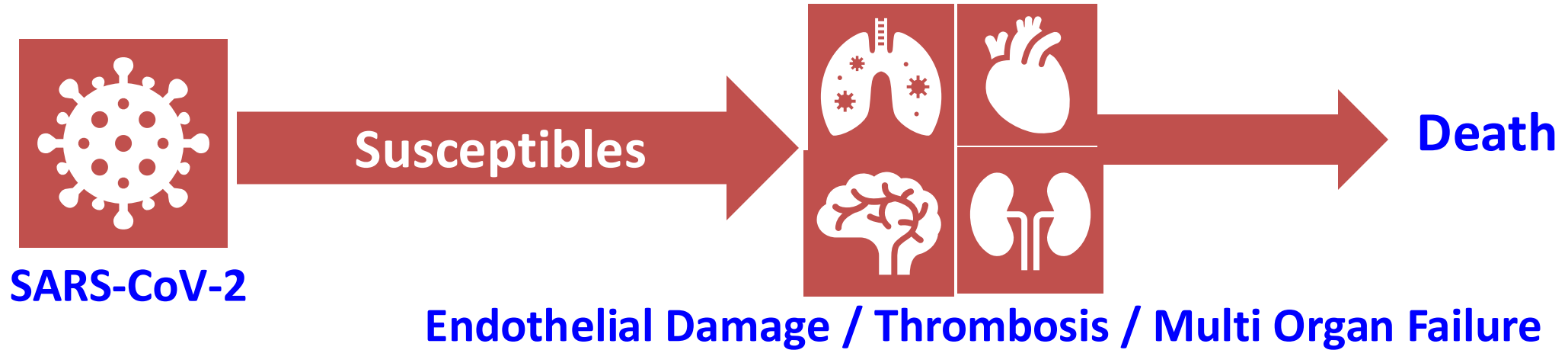
Wed 4/1/2020 12:09 PM



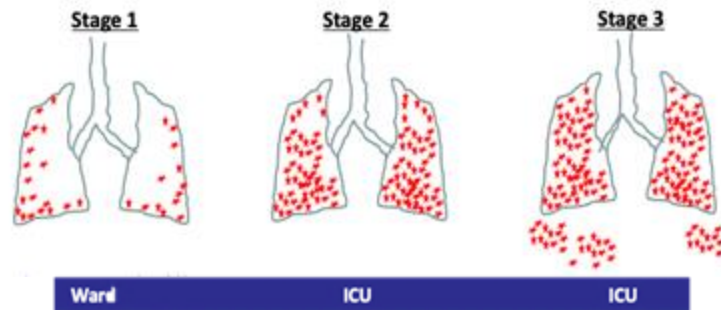
Dear Macky,

We wanted to discuss the potential of leveraging TACTIC in the NHLBI response to COVID-19...

# COVID-19 Deaths Relate to Immunothrombosis



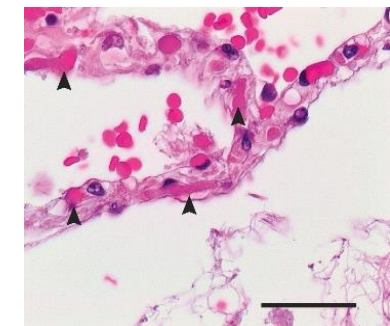
COVID-19 progresses through stages of *increased clotting and inflammation*



Macro-thrombi



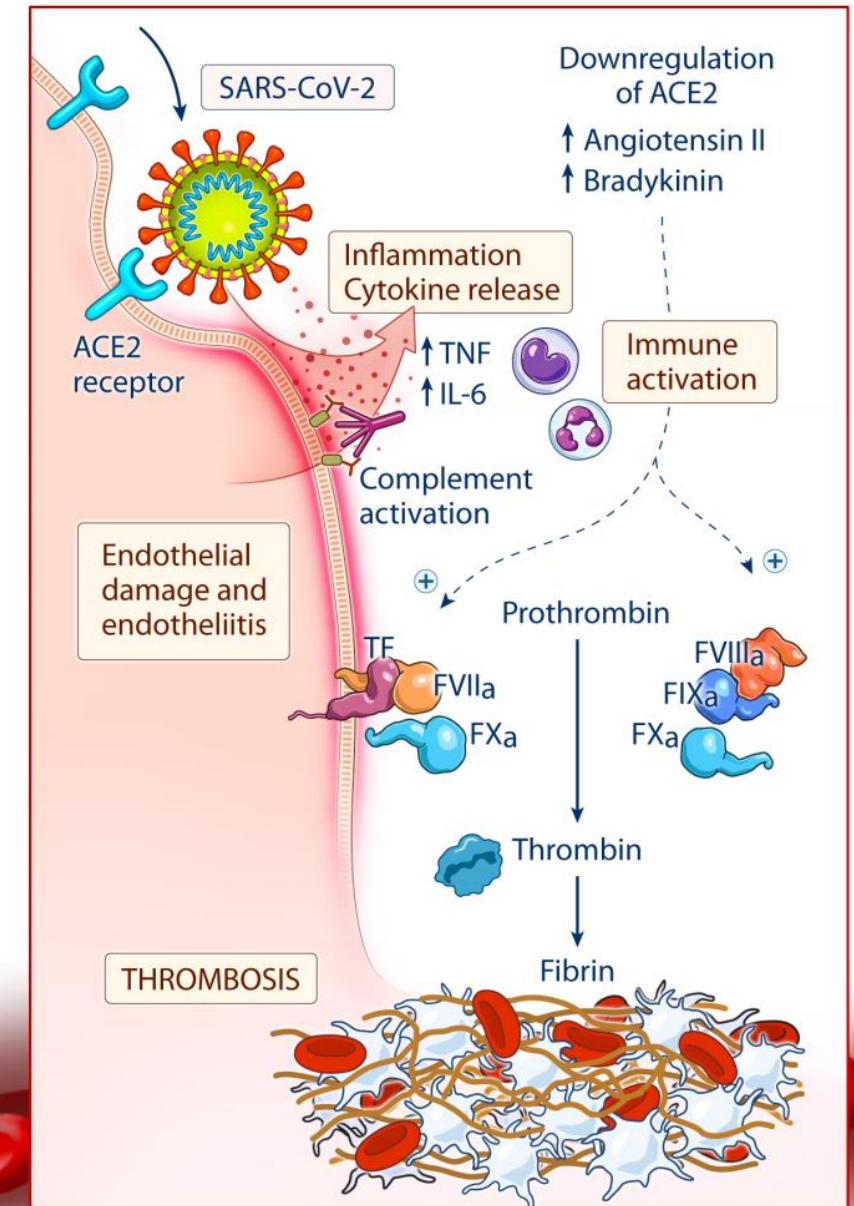
Micro-thrombi



# Mechanisms of Thrombosis in COVID-19

- Endothelial injury / tissue factor expression
- Inflammation and immune activation
- ACE-2 down regulation
- NETs/NETosis
- Platelet and macrophage activation
- Complement activation
- Increased fibrinogen; reduced fibrinolysis
- Reduced natural anticoagulants
- ? antiphospholipid antibodies

Godoy F, Goligher EC, Lawler P, Slutsky A, Zarychanski R. CMAJ. 2020



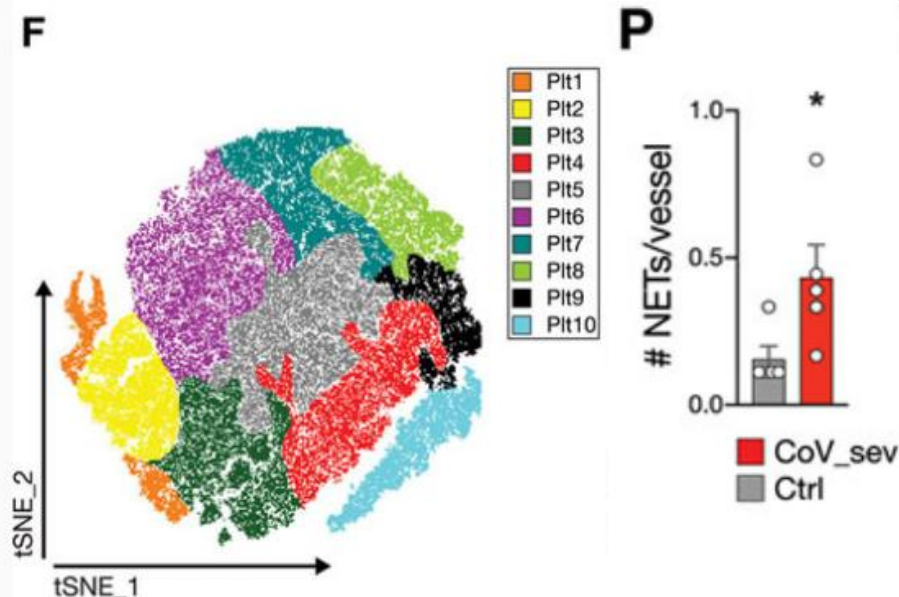
# What about Platelets?

## ORIGINAL RESEARCH ARTICLE

### Immunothrombotic Dysregulation in COVID-19 Pneumonia Is Associated With Respiratory Failure and Coagulopathy

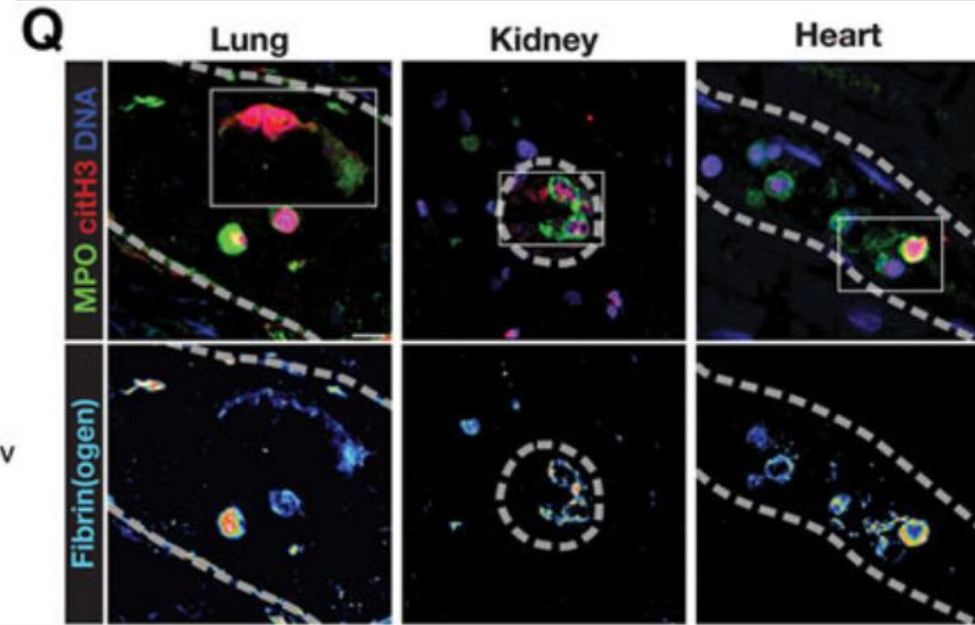
Leo Nicolai, MD <sup>\*</sup>, Alexander Leunig, BA <sup>\*</sup>, Sophia Brambs, cand med, Rainer Kaiser, MD, Tobias Weinberger, MD, Michael Weigand, MD, Maximilian Muenchhoff, MD , Johannes C. Hellmuth, MD , Stephan Ledderose, MD, Heiko Schulz, MD, Clemens Scherer, MD , Martina Rudelius, MD, Michael Zoller, MD, Dominik Höchter, MD , Oliver Keppler, MD, Daniel Teupser, MD, Bernhard Zwißler, MD, Michael von Bergwelt-Baildon, MD, Stefan Käab, MD , Steffen Massberg, MD, Kami Pekayvaz, MD <sup>†</sup>, and Konstantin Stark, MD <sup>†</sup>

- Unique platelet phenotypes
- Platelet neutrophil interactions
- Elevated NETosis



	HR (95% CI) of Outcome per SD Biomarker	
	Thrombosis	Mortality
Thromboxane B <sub>2</sub>	3.0 (1.4-7.7)	2.0 (1.1-4.1)
MPV	2.0 (1.0-4.7)	2.3 (1.3-4.7)
P-selectin	1.4 (0.7-2.5)	2.2 (1.3-4.2)

adjusted for age, sex, race, antiplatelet therapy, platelet count, and COPD



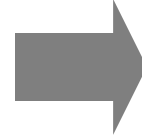


# ACTIV-4

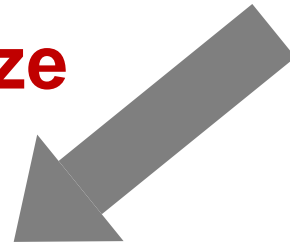
Acute Inpatient Anti-Thrombotic Study

## Pragmatic Adaptive Platform Trial

Study Entry



Randomize



Therapeutic  
Dose  
Heparin

OR

Lower  
Dose  
Heparin

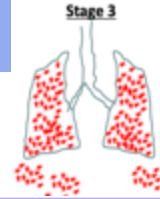
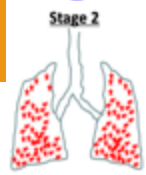
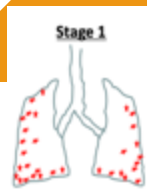
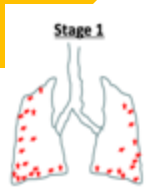


- Strata:
1. ICU Level of Care
  2. Non-ICU: Lower D-Dimer
  3. Non-ICU: Higher D-Dimer

Follow for organ  
support needs,  
death, thrombosis

Biorepository and mechanistic studies

P2Y12 inhibition  
Crizanlizumab  
SGLT2 inhibition



**Admission**

**\*\* Organ Support / Respiratory Failure**

**Death \*\***

**Thrombosis \*\***

**Bleeding**

**\*\*** Most relevant outcome in the different settings



# We had to move faster...



Harmonized and aligned:

- Primary endpoint (organ support free days)
- Data collection (electronic case report forms)
- Data safety and monitoring
- Trial governance
- Publication plan



Statistical analysis plan:

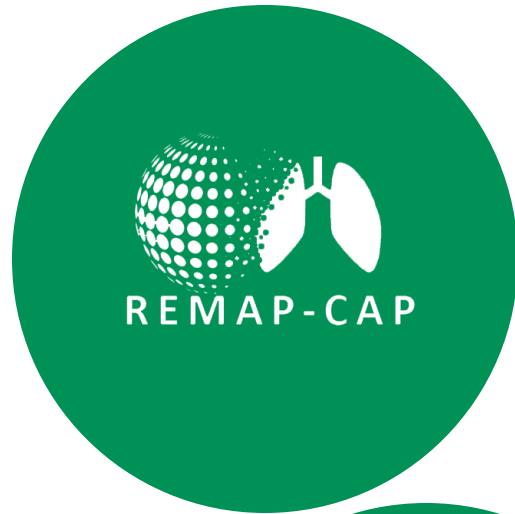
- Common statistical expertise (Berry Consulting)
- Agreed upon and pre-specified criteria for superiority, inferiority, and futility
- Bayesian adaptive design with response adaptive randomization (ATTACC, REMAP-CAP) or frequent interims (ACTIV-4)



=

**Multi-  
platform  
randomized  
clinical trial  
(mpRCT)**

# Multi-platform Randomized Control Trial (mpRCT)



**393**  
**SITES**  
**10**  
**COUNTRIES**

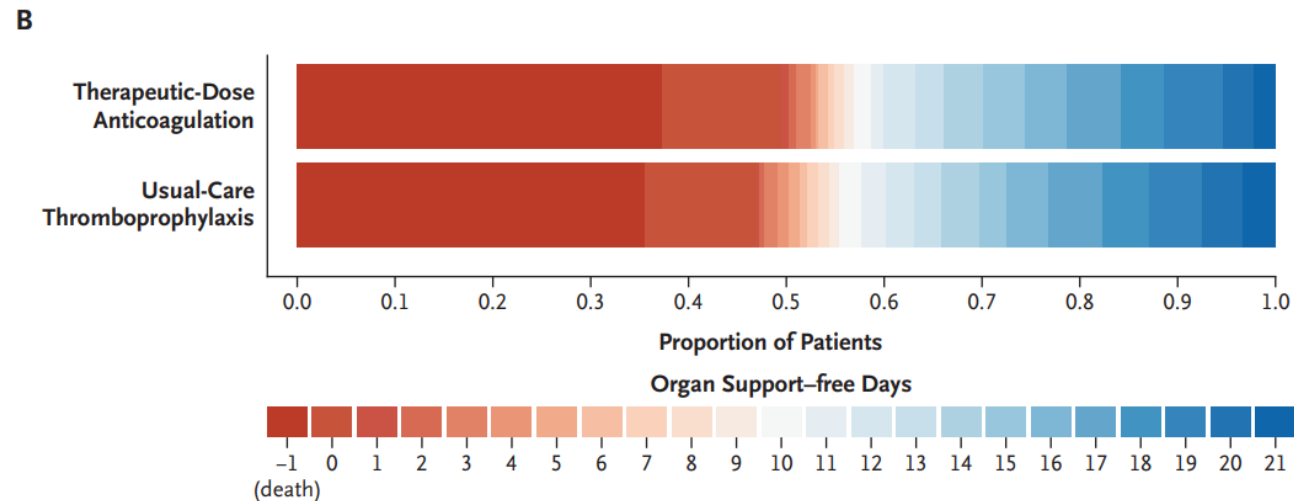
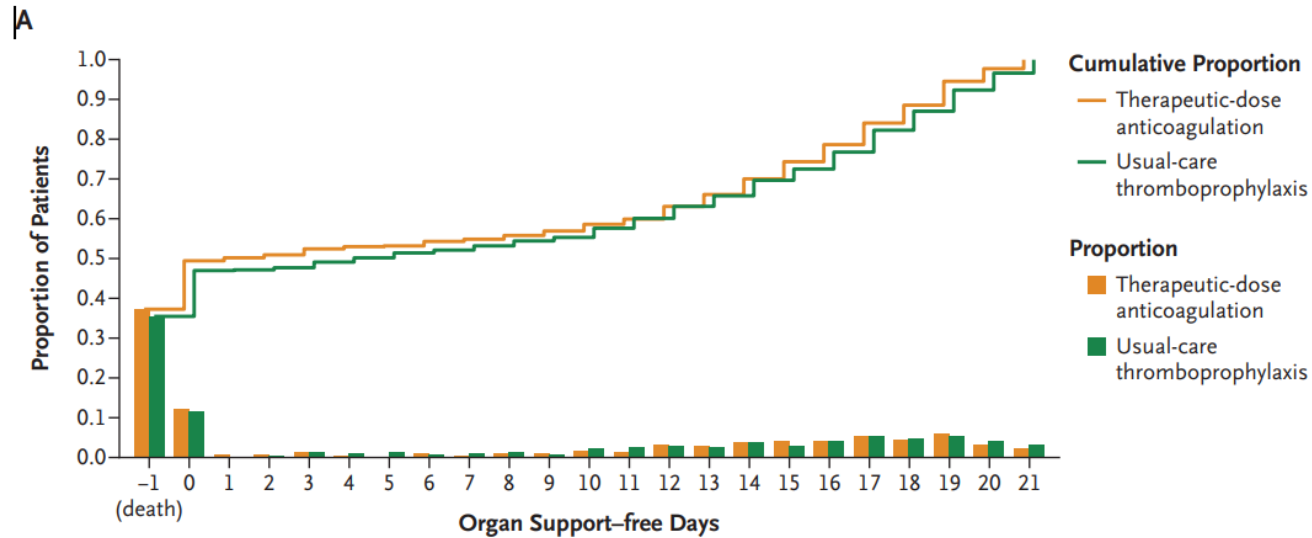
**Moderate state (ward) and Severe state (ICU level of care)**

# ACTIV 4 subsequent arms

- P2Y12 Inhibition
  - Severe state – stopped enrollment (952 as of 6/24/22)
  - Moderate state
    - Reported JAMA 2022
- SGLT2i (vascular inflammation)
  - Moderate state
  - Severe state
- Crizanlizumab (P-selectin inhibitor)
  - Moderate state
  - Severe state



# Severe state therapeutic vs proph heparin



Adjusted\* Odds Ratio

- 0.83 (95% CrI 0.67-1.03)

**Futility:** Prob(OR<1.2) = 99.9%

**Inferiority:** Prob(OR<1) = 95.0%

**Superiority:** Prob(OR>1) = 5.0%

*The* **NEW ENGLAND**  
**JOURNAL** *of* **MEDICINE**

ESTABLISHED IN 1812

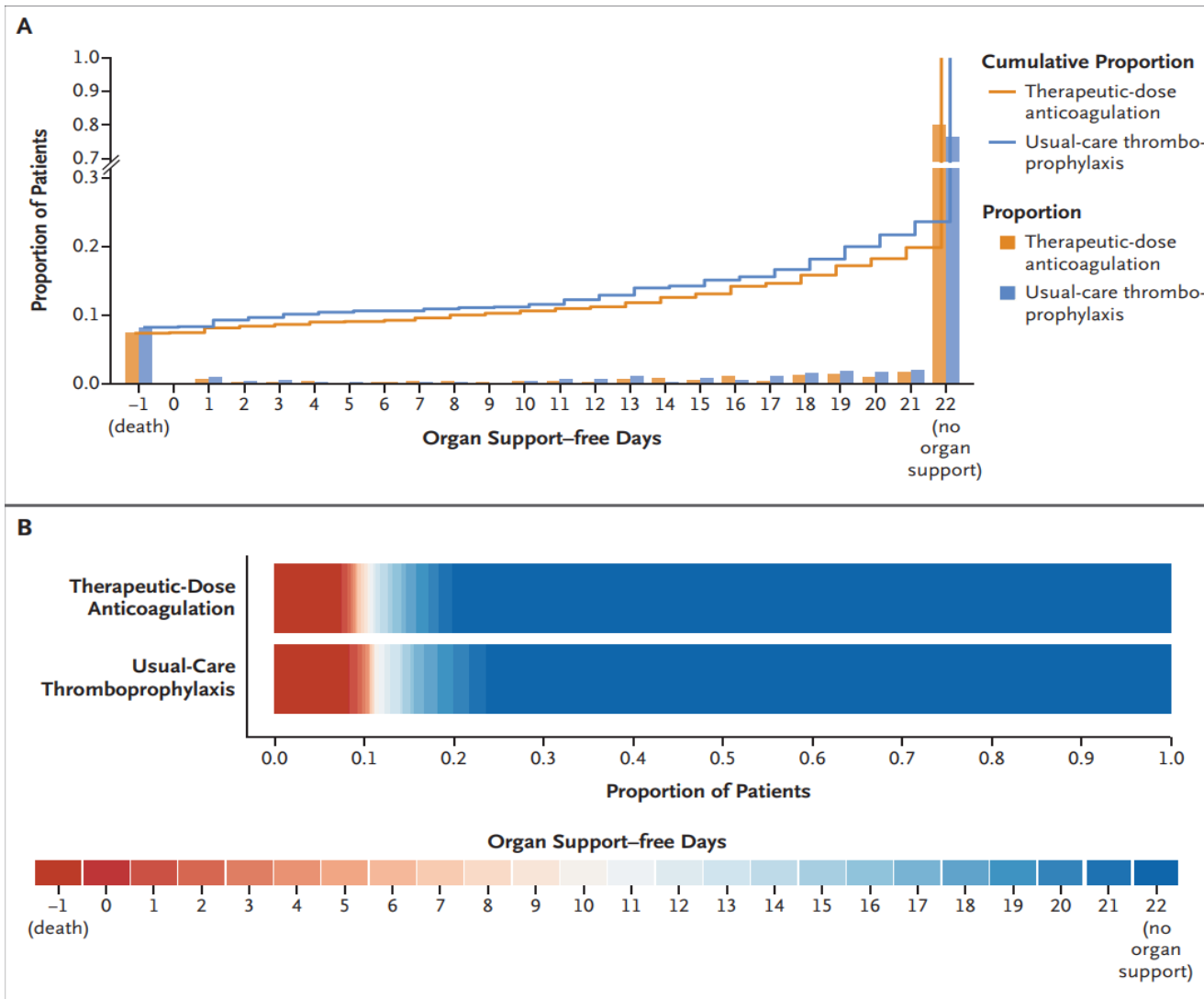
AUGUST 26, 2021

VOL. 385 NO. 9

Therapeutic Anticoagulation with Heparin in Critically Ill Patients with Covid-19

The REMAP-CAP, ACTIV-4a, and ATTACC Investigators\*

# Moderate state therapeutic vs proph heparin



Adjusted Odds Ratio

1.27 (95% CrI 1.03-1.58)

**Superiority:** Prob(OR>1) = 98.6%

Adjusted difference in risk: 4%

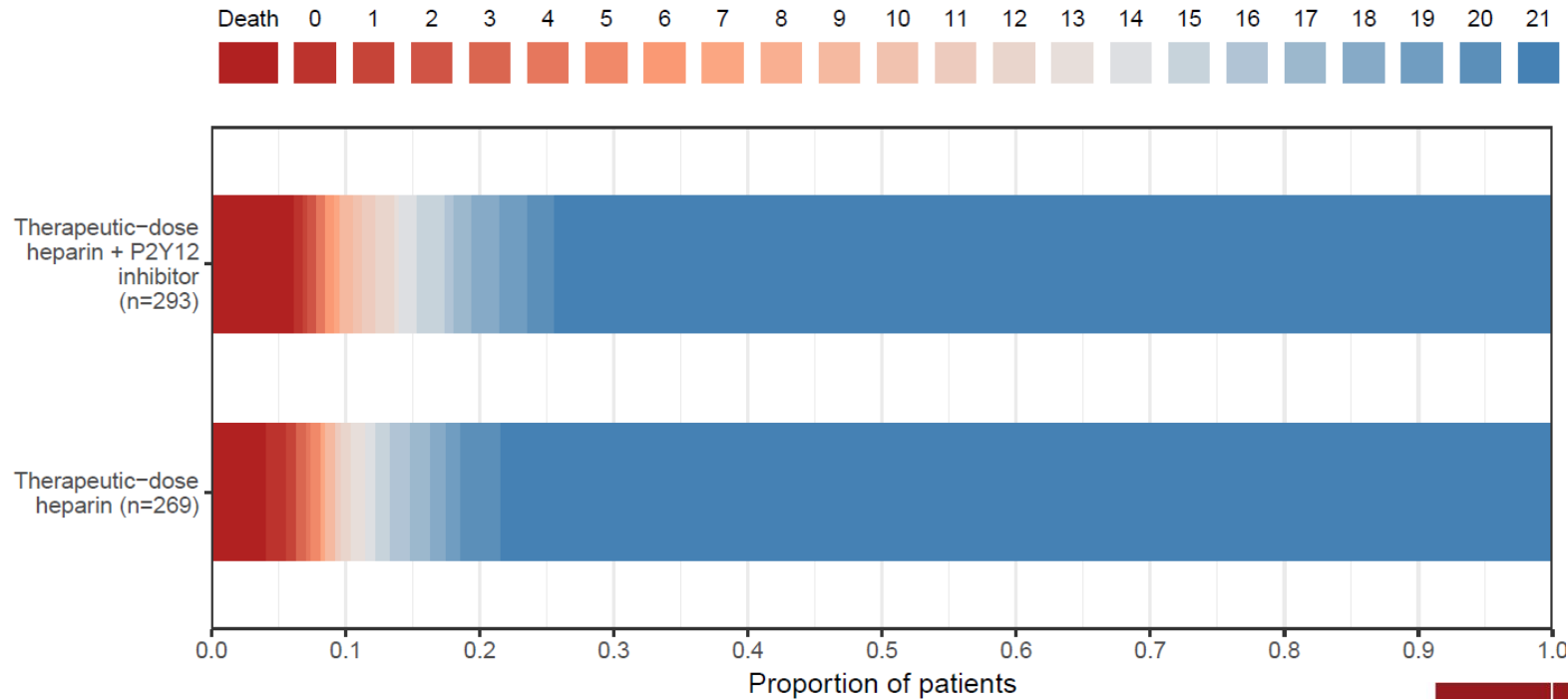
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Therapeutic Anticoagulation with Heparin in Noncritically Ill Patients with Covid-19

The ATTACC, ACTIV-4a, and REMAP-CAP Investigators\*

# P2Y12 Moderate State (P2Y12 + therapeutic heparin)



Adjusted Odds Ratio

- 0.83 (95% CrI 0.55-1.25)

**Futility:** Prob(OR<1.2) = 96.2%

**Inferiority:** Prob(OR<1) = 81.4%

**Superiority:** Prob(OR>1) = 18.6%



Research

JAMA | Original Investigation

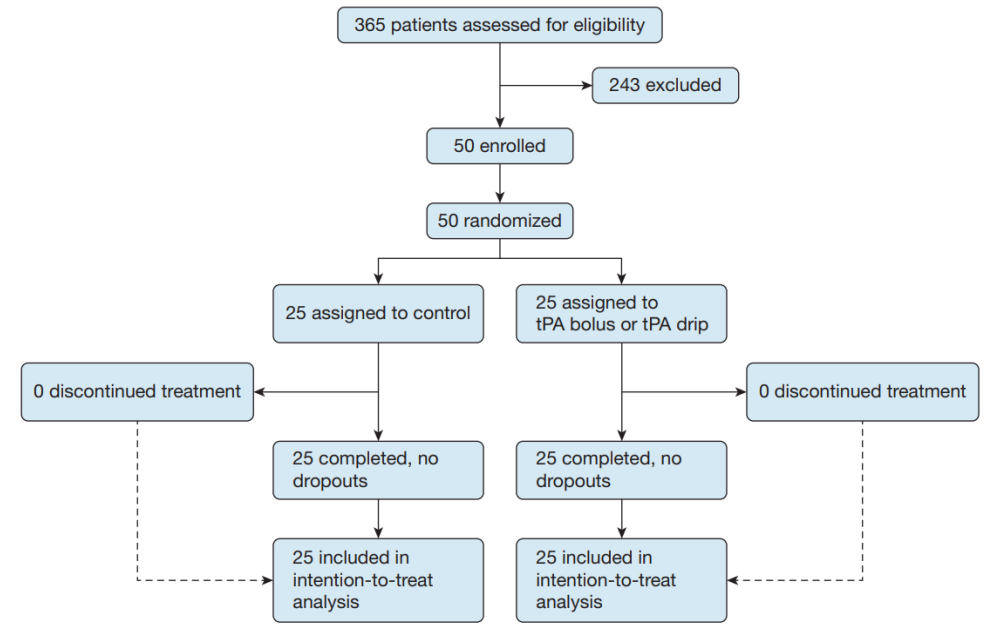
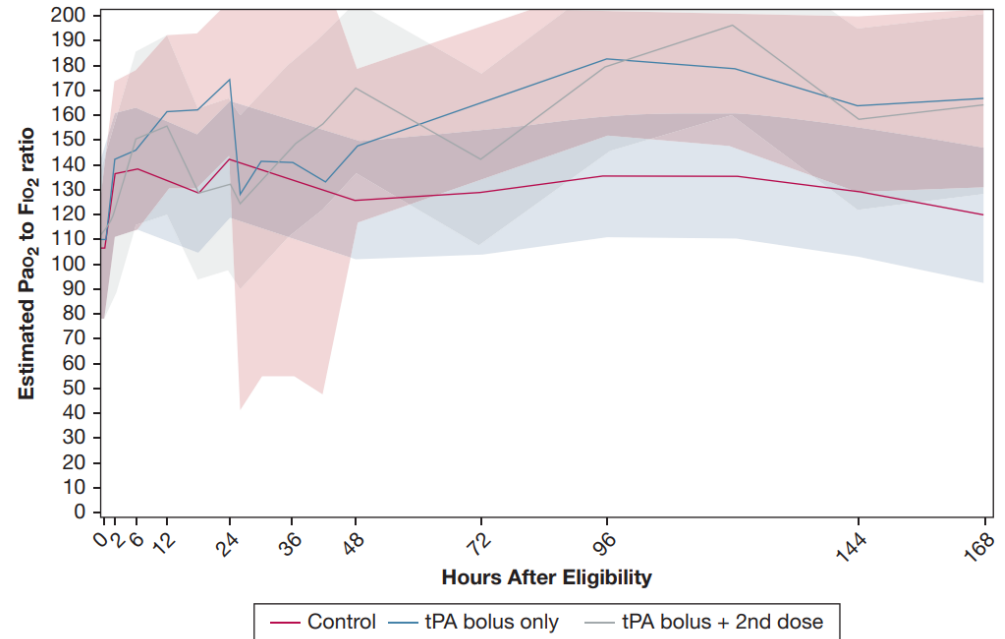
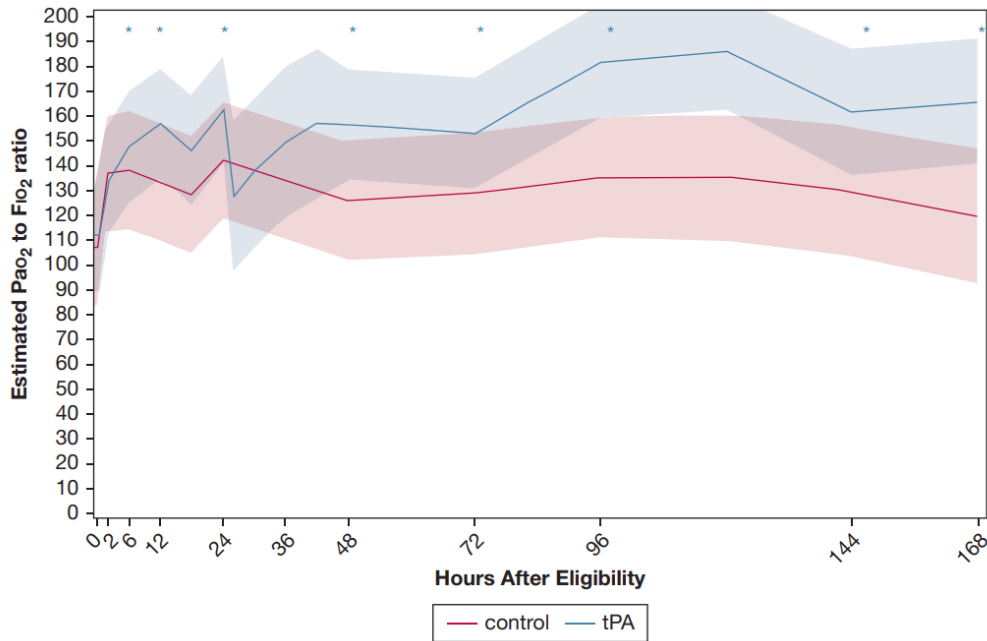
Effect of P2Y12 Inhibitors on Survival Free of Organ Support Among Non-Critically Ill Hospitalized Patients With COVID-19  
A Randomized Clinical Trial

Jeffrey S. Berger, MD, MS; Lucy Z. Kornblith, MD; Michelle N. Gong, MD; Harmony R. Reynolds, MD; Mary Cushman, MD, MSc; Yu Cheng, PhD; Bryan J. McVerry, MD; Keri S. Kim, PharmD; Renato D. Lopes, MD, PhD; Bassel Atassi, MD; Scott Berry, PhD; Grant Bochicchio, MD; Murillo de Oliveira Antunes, MD; Michael E. Farkouh, MD; Yonatan Greenstein, MD; Erinn M. Hade, PhD; Kristin Hudock, MD, MSTR; Robert Hyzy, MD; Pooja Khatri, MD; Andrei Kindzelski, MD, PhD; Bridget-Anne Kirwan, PhD; Lisa Baumann Kreuziger, MD; Patrick R. Lawler, MD, MPH; Eric Leifer, PhD; Jose Lopez-Sendon Moreno, MD; Jose Lopez-Sendon, MD; James F. Luther, MA; Lilia Nigro Maia, MD; John Quigley, MD; Robert Sherwin, MD; Lana Wahid, MD; Jennifer Wilson, MD; Judith S. Hochman, MD; Matthew D. Neal, MD; for the ACTIV-4a Investigators

# Study of Alteplase for Respiratory Failure in SARS-CoV-2 COVID-19

Check for updates

A Vanguard Multicenter, Rapidly Adaptive, Pragmatic, Randomized Controlled Trial



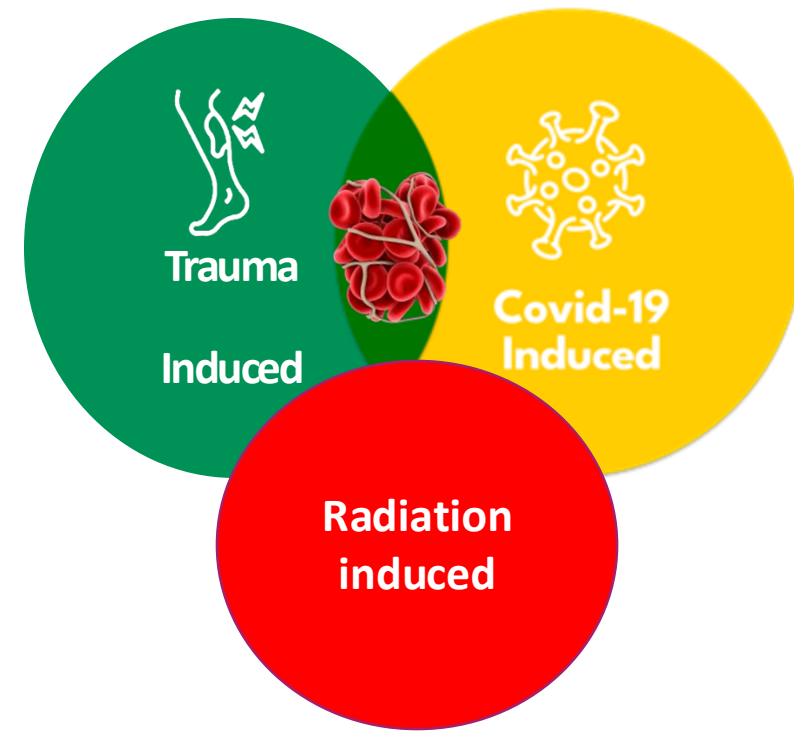
# Summary and lessons learned from COVID-19

- Heparin reduces the need for organ support in moderate (non-critically ill patients)
- No benefit if given too late
- Thrombolysis may benefit once the clot is already present
- Strong rationale for targeting platelets and neutrophils – results pending
- We tackled a complex, heterogeneous disease utilizing a Bayesian adaptive platform approach



# What will we learn?

- Emphasis on the timing of interventions
- Can we reduce MOF by targeting immunothrombosis?
- What other disease states share characteristics?
- Will history repeat itself again (like LTOWB)?
- What other mechanisms learned from COVID-19 will be relevant?
- The time is **NOW** for adaptive platform trials in trauma care



THE JOURNAL OF AABB  
**TRANSFUSION**  
transfusion.org

ADAPTIVE PLATFORM TRIAL DESIGN

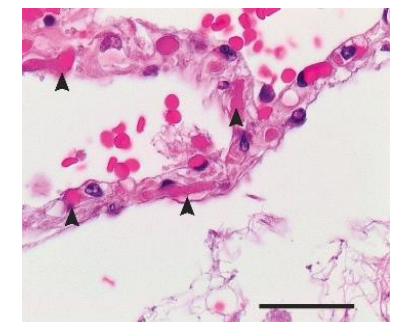
An adaptive platform trial for evaluating treatments in patients with life-threatening hemorrhage from traumatic injuries: Planning and execution

Deborah J. del Junco ✉, Matthew D. Neal, Stacy A. Shackelford, Philip C. Spinella, Francis X. Guyette, Jason L. Sperry, Roger J. Lewis, Kabir Yadav ... See fewer authors ^

**Macro-thrombi**



**Micro-thrombi**



# Acknowledgements

- TACTIC
- mpRCT partnership (ATTACC, REMAP, ACTIV-4)
- NHLBI and Operation Warp Speed
- Judith Hochman, MD
- Jeffrey Berger, MD
- Patrick Lawler, MD
- Ryan Zarychanski, MD
- Ewan Goligher, MD
- Lucy Kornblith, MD
- Gene Moore, MD





**Matthew D. Neal, MD**  
Co-Director



**Phil Spinella, MD**  
Co-Director



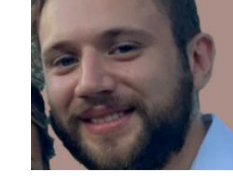
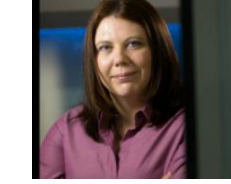
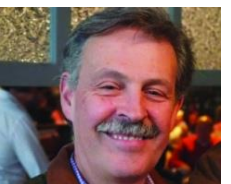
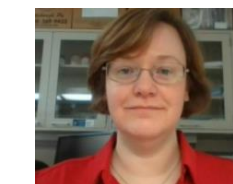
**Jason Sperry, MD**  
Associate Director



**Susan Shea, PhD**  
Associate Director



**Timothy Billiar, MD**  
Chair, Dept of Surgery



University of  
**Pittsburgh**

Trauma and Transfusion  
Medicine Research Center

# Questions?

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• [@macky\\_neal](https://www.instagram.com/macky_neal)

• Cell: 412 848 2134



## Neal lab funding

- R35GM119526-07 NIGMS
- R01HL141080-01A1 NHLBI
- OTA # 1OT2HL156812-01 NHLBI
- DM160354 Department of Defense JPC-6 Combat Casualty Care Research Program
- Department of Defense CDMRP JPC-6 Combat Casualty Care Research Program
- Department of Defense W81XWH21107810



NIH

National Institute of  
General Medical Sciences

NIH

National Heart, Lung,  
and Blood Institute