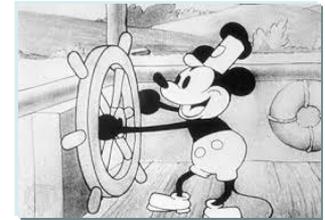


*Navigating the post-PNAS matrix with
the mouse research ship:*



A suicide or viable mission?



Marcin Osuchowski DVM, PhD

Ludwig Boltzmann Institute for Experimental and Clinical Traumatology,
AUVA Trauma Research Center, Vienna, Austria

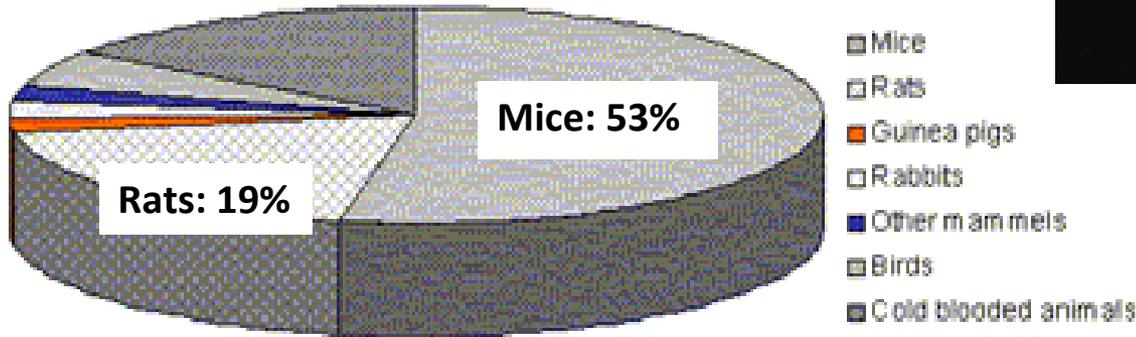
My only disclosure:

**I am totally biased in thinking that mouse models –
when appropriately used – are very useful in modeling
of human sepsis**

The Mouse...



% of Animal Species in Research - EU



Since 1.1.2014:

„mouse & sepsis“: 1358 hits (rat: 502; pig: 81)
„mouse & trauma“: 2790 hits (rat: 3536; pig: 486)
„mouse & shock“: 1653 hits (rat: 1156; pig: 219)

Over 70% of all animal sepsis studies are performed in MICE

cheap

small –
high power

minimal ethical
concerns

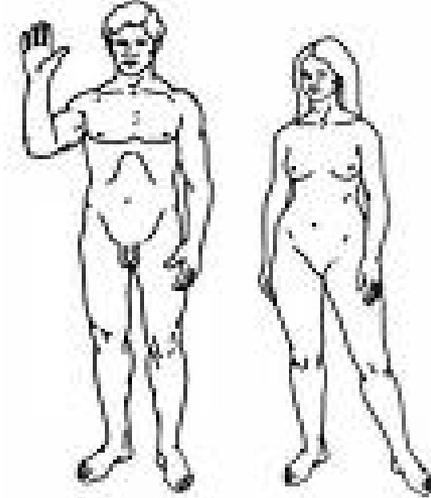
ease of
manipulation

technical
background

Mouse Physiology



Human Physiology



Far from ideal...

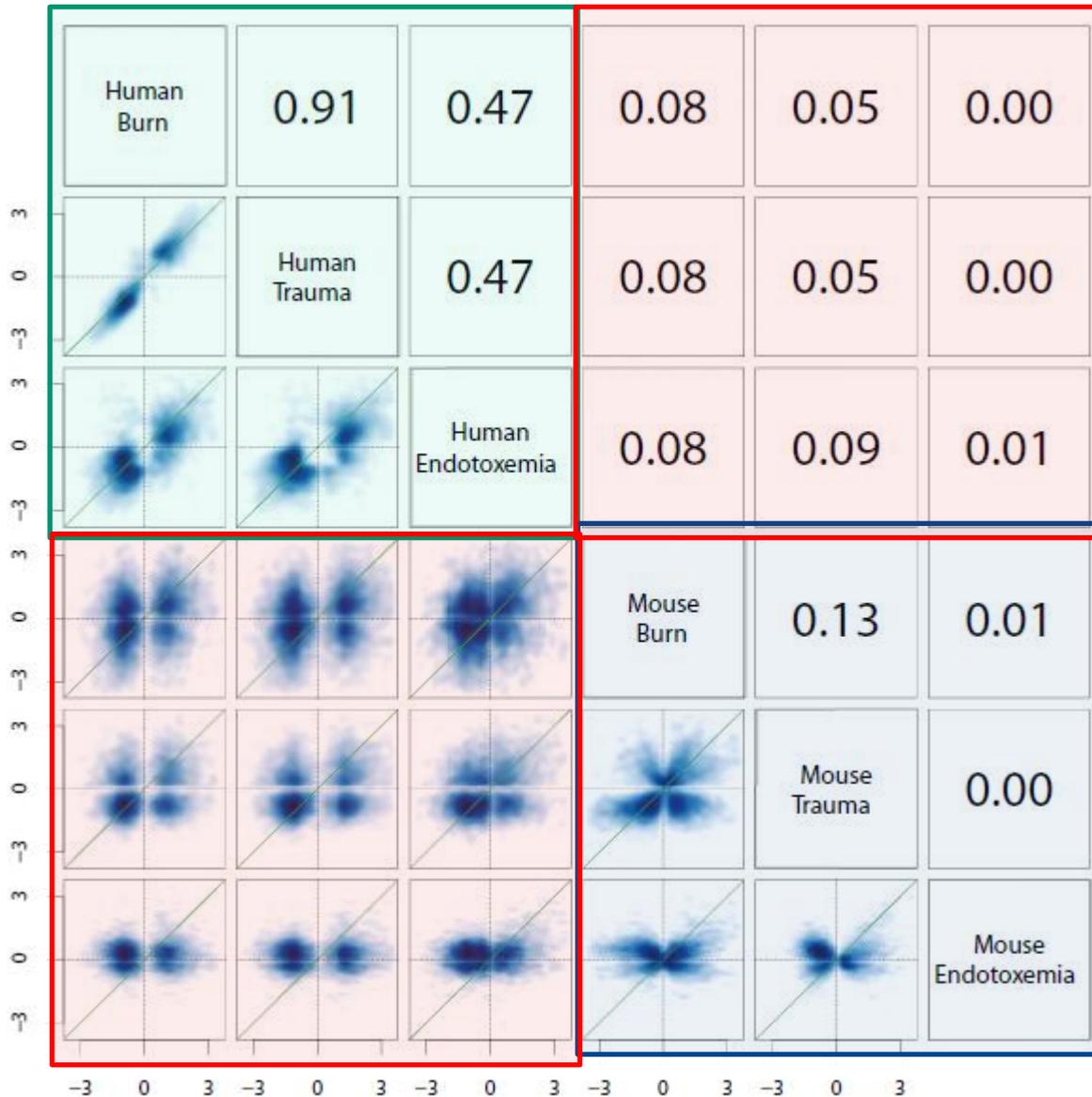
- lymphocyte rich blood
- LPS-induced NO release by macrophages - **yes**
- BALT presence - **strong**
- caspase 10 - **no**
- MHC II on T cells - **no**



- neutrophil rich blood
- LPS-induced NO release by macrophages - **no**
- BALT presence - **absent**
- caspase 10 - **yes**
- MHC II on T cells - **yes**

- different IgG and IgA isotypes/subtypes

Genomic responses in mouse models **poorly** mimic human inflammatory diseases



V. Baker^c, Weihong Xu^a,
 C. Finnerty^g, Cecilia M. López^c,
 Jeffrey L. Johnson^h, Jason Sperry^l,
 v B. Klein^l, Richard L. Gamelli^p,
 Philip H. Mason^e, J. Perren Cobb^s,
 N. Herndon^g, Ronald W. Davis^{a,3},
 o Injury, Large Scale Collaborative

Pearson's correlation: 0.02-.03
Human vs. Mouse Sepsis

Seok et al. PNAS 2013

Analysis of 4918 human genes versus their 4918 murine orthologs; Pearson's correlation (R^2)

The New York Times

By Gina Kolata

Published: February 11, 2013

“Mice Fall Test Subjects for ‘Mice of Humans’
Deadly Ills”

HUFFPOST

We Are Not Mice

PC

Sus

Pic

breast cancer

Very expensive too???

Justified or not?

We grant res plicity

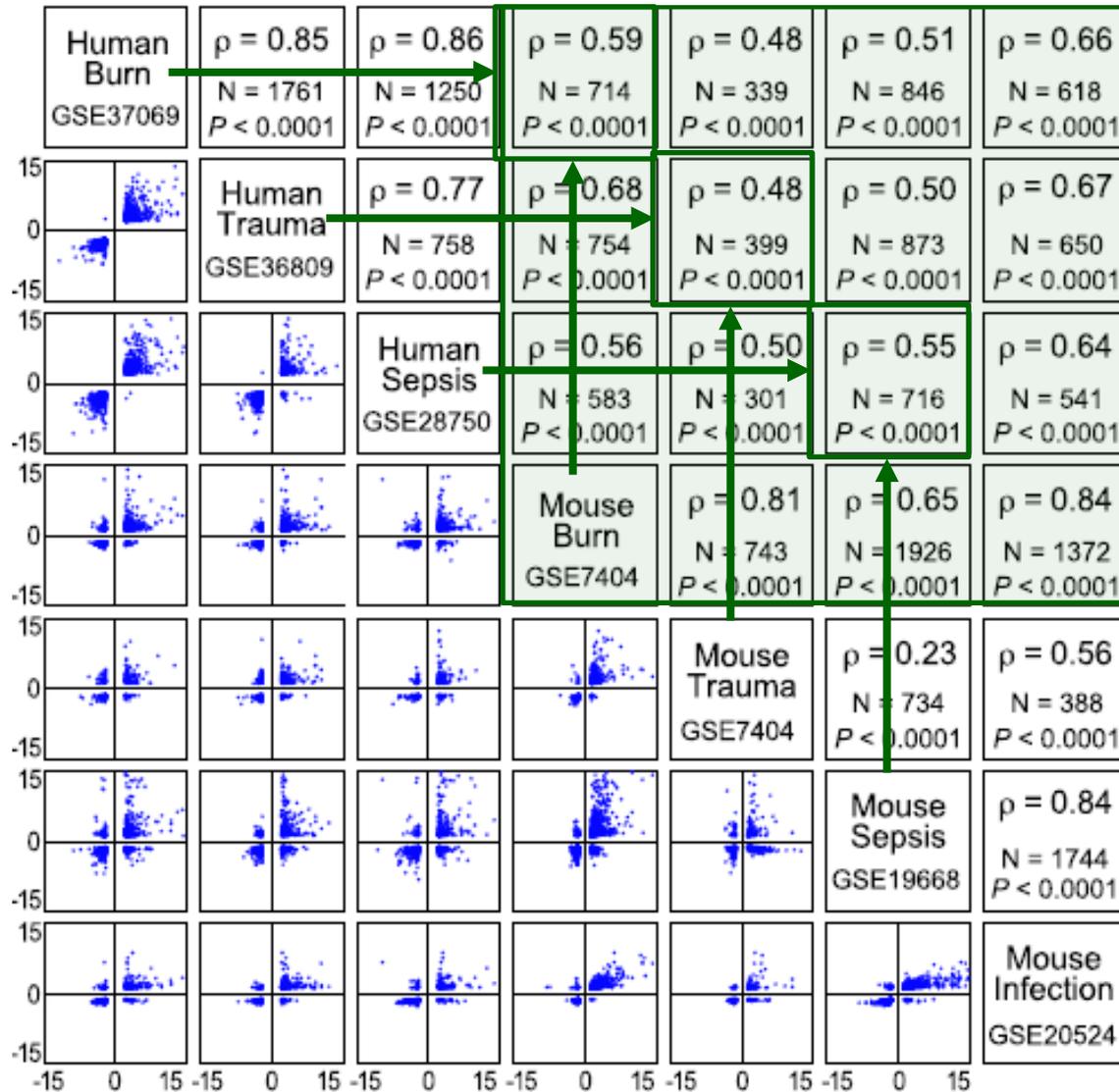
February 13, 2013

“Mouse Models of Inflammation Are Basically Worthless. Now We Know.”

Posted by Derek Low

Genomic responses in mouse models **greatly** mimic human inflammatory diseases

Keizo Takao^{a,b} and Tsuyoshi Miyakawa^{a,b,c,1}



Competition?



Differences in the analytical approach:

- fold change as cutoff
(<1.2 mouse/ <2.0 human vs. $<1.2/1.2$ (Seok et al.)) **not critical** (Fig.1 erroneous)
- Only altered genes compared **critical (?)** (ImmGen consortium: $R=0.38-0.45$)
- Spearman's correlation
(non-Gaussian/non-linear data) **not critical** (run by Seok et al.; supplement. data)
- Individual/single mouse studies compared to the matching human disease **critical**
- NextBio data mining approach: **critical**
 - a) normalized ranking approach
 - b) pair-wise comparison of gene expression signatures (*Running Fisher* algorithm)
 - c) meta-analysis of genomic data

Well, make up your own mind...

Mouse
scientists

Can We Navigate the Matrix??



ABANDON THE MOUSE RESEARCH SHIP? NOT JUST YET!

Table 1. Selected mouse-to-human translational examples (26 listed)

Shock 2014

No.	Translational phenomenon/response	Specific comments: mouse	Specific comments: human
1	Antibodies to TNF given indiscriminately fail to reduce sepsis mortality	BALB/c mice were pretreated with antibodies to TNF prior to CLP sepsis. The murine studies were published 3 y before the failed human trials (101, 116)	Anti-TNF antibodies failed to be an effective treatment strategy in a general population of septic patients (117, 118)
2	Pretreatment with an anti-TNF strategy prevents early systemic inflammatory response syndrome	Passive immunization with the antiserum to TNF- α in BALB/c mice protected them against the lethal hyperinflammation by <i>Escherichia coli</i> LPS (98)	Anti-TNF- α therapy was effective in humans with louse-borne relapsing fever when given as a pretreatment against Jarisch-Herxheimer reactions (119)
3	Low-dose steroid therapy is associated with decreased mortality in septic mice and humans	Demonstrated in C57BL/6 male mice subjected to CLP and treated with different corticoid concentrations; low but not high-dose steroids improved 21-d survival (120)	Early initiation of low-dose corticosteroid therapy decreased mortality in septic shock patients (121)
4	Regulation of chemotactic behavior of mouse and human neutrophils via purinergic signaling	Human and mouse neutrophils rely on same purinergic receptor subtypes (P2Y2, A3, and A2a receptors) for autocrine signaling (122–124)	Demonstrated <i>in vitro</i> and <i>in vivo</i> ; mice are suitable to study chemotaxis in inflammation, trauma, and sepsis (122–124; NCT01180361*)
5	Human and mouse neutrophils rely on similar signaling mechanisms for their activation during bacteria-induced acute lung injury	Increased nuclear activation of NF- κ B in pulmonary neutrophils of mice after <i>in vivo</i> administration with endotoxin (125, 126)	Increased nuclear accumulation of NF- κ B in peripheral or pulmonary neutrophils of human volunteers after <i>in vitro</i> or <i>in vivo</i> stimulation with endotoxin (127) or in peripheral neutrophils of patients with sepsis (128)
6	Sepsis always in MARS: simultaneous systemic release of both proinflammatory and anti-inflammatory cytokines in sepsis	Demonstrated in ICR/CD-1 (outbred) female mice subjected to CLP sepsis (129, 130)	Demonstrated in septic shock patients (131) and patients with postoperative abdominal sepsis (132)
7	IL-6 serves as a biomarker for sepsis mortality	IL-6 measured 6 h after the onset of CLP sepsis in BALB/c (133) and CD-1 mice (129) accurately predicts survival	Patients with high levels of IL-6 are at increased risk of dying of sepsis (134, 135)
8	Role of nicotinic receptors in inflammatory responses after endotoxemia is similar in mice and humans	Demonstrated in C57BL/6 mice and α 7 nicotinic receptor-deficient mice; endotoxin-induced response was abrogated via activation of anti-inflammatory cholinergic pathway (vagus nerve stimulation) (136)	Human volunteers were administered endotoxin and GTS-21 (α 7nAChR agonist) or placebo to study anti-inflammatory effects of cholinergic pathway (137; NCT00783068*)
9	Similar mode of pathogen-associated molecular patterns detection via Toll-like receptors (TLRs) in mice and humans	TLR-4 was identified as the receptor that senses LPS in experiments with congenic sensitive (C3H/HeN; C57BL/10ScSn) and resistant (C3H/HeJ and C57BL/10ScCr) mice (138); TLR-4 expression level determines the degree of LPS-susceptibility in mice (139)	Human volunteers administered with LPS demonstrated altered TLR-induced genes expression (140). TLR-signaling pathways are strongly modulated in septic patients (141)
10	Sepsis induces profound apoptosis of immune and gastrointestinal epithelial (GIE) cells	Demonstrated in CLP female ND4 mice (142) and <i>Pseudomonas aeruginosa</i> pneumonia-induced septic FVB/N mice (143); apoptosis in B and T lymphocytes and dendritic cells. GIE cell apoptosis in large and small intestine	Demonstrated in patients who died of sepsis and sepsis and MODS; data obtained by retrospective (rapid autopsy) and prospective (tissue resection) examination (144–146)

Critically re-checking our mouse models...

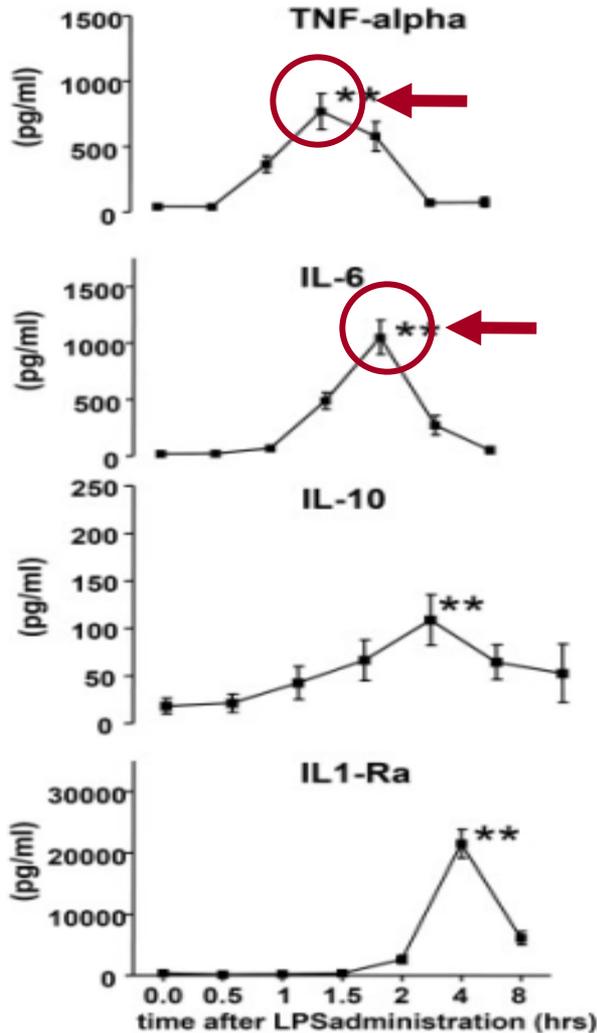
Navigation tip #1a

The Model/Disease Mismatch:
Sepsis

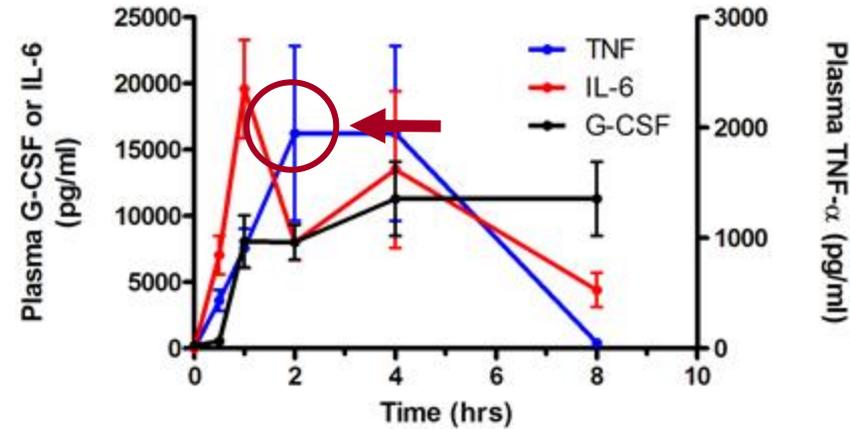


Cytokines after LPS/E.coli – mice (& all species)

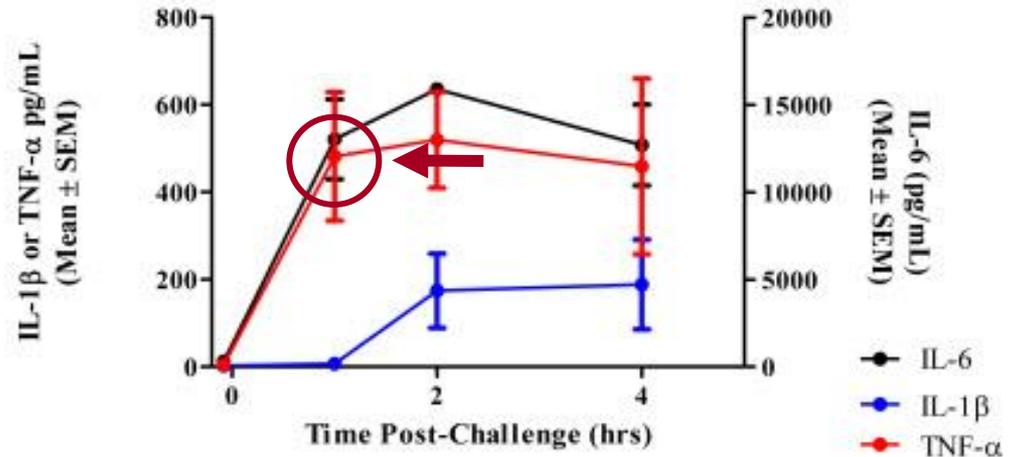
Plasma G-CSF, IL-6 and TNF Response to LPS Challenge



changes over hours



LPS-Induced Cytokine Release

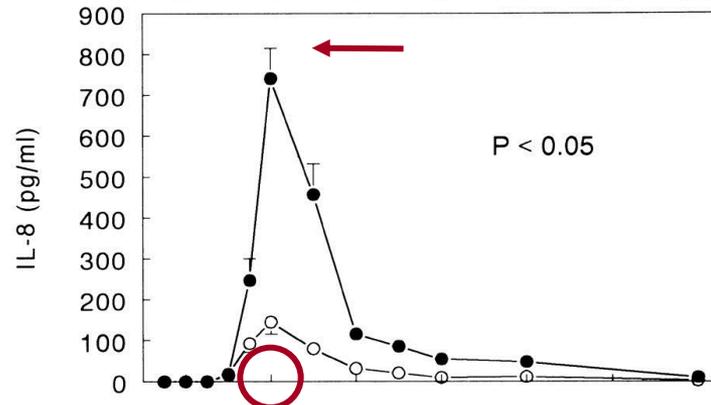
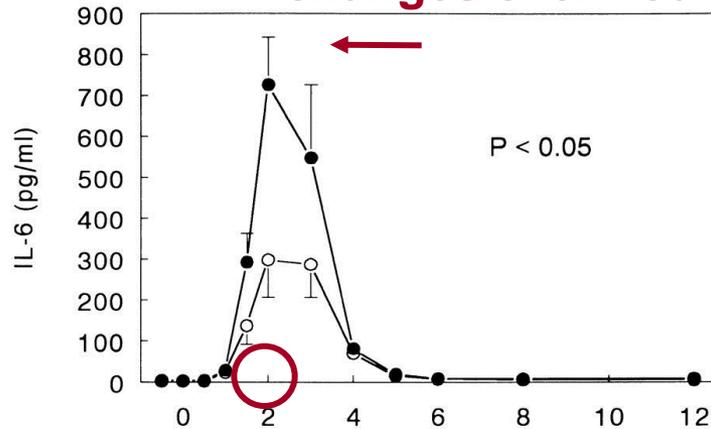
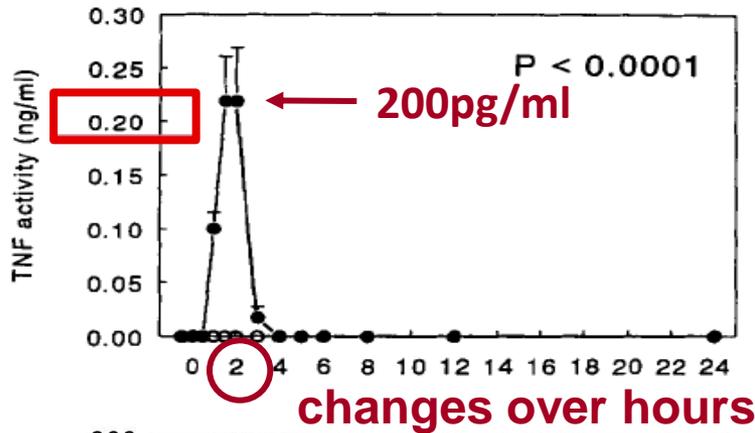


Cytokines after low-dose LPS – human

volunteers

blood

JOURNAL OF
THE AMERICAN
SOCIETY OF
HEMATOLOGY



(●) vehicle (n = 6)

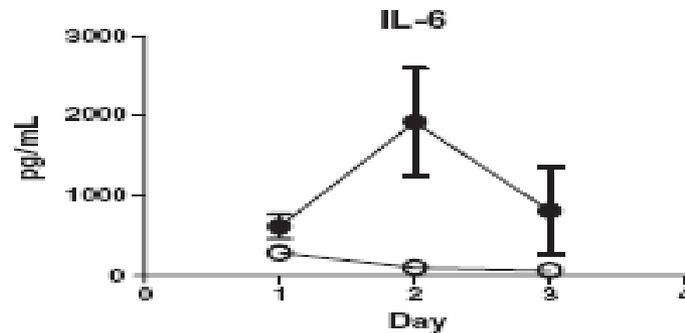
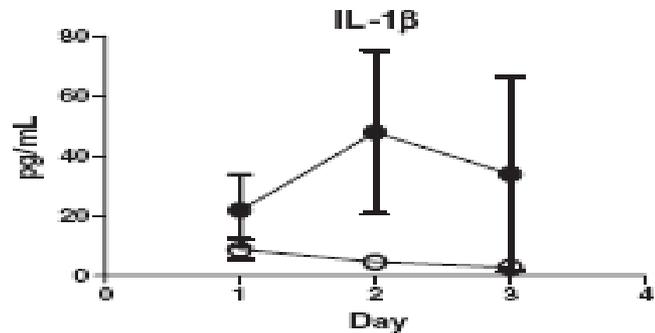
(○) TNFR:Fc (6 mg/m²; n = 6)

Multiplex cytokine profiling in patients with sepsis

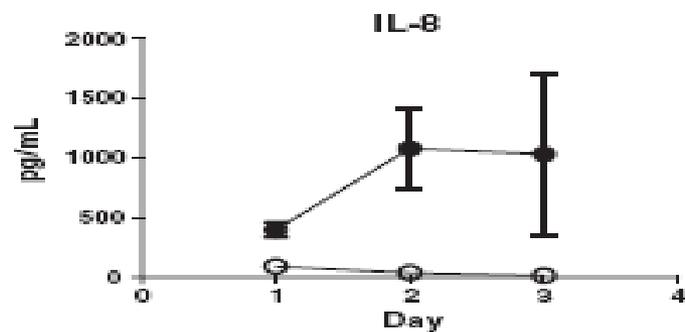
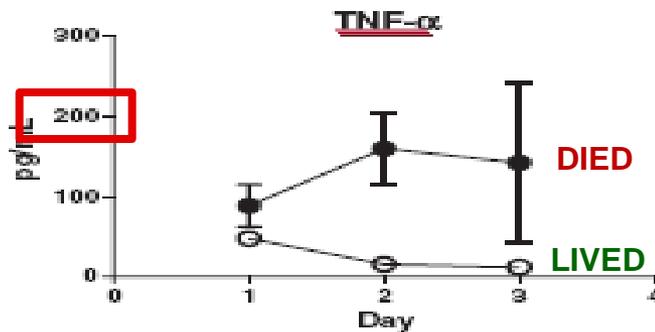
Since none of the anti-TNF α trials worked, a closer look was taken... (finally!)

SIMONA MERA,^{1,2} DOINA TATULESCU,^{1,2} CRISTINA CISMARU,^{1,2} COSMINA BONDOR,³
ADRIANA SLAVCOVICI,^{1,2} VIRGINIA ZANC,^{1,2} DUMITRU CARSTINA,² and MIHAI OLTEAN⁴

Different Temporal Dynamics & Concentrations of Circulating Cytokines



changes over DAYS



...and Their Absence in Many Patients (more than 90% in some studies)

Table 2. The cumulated daily results of the 17 cytokines measured in septic patients (n = 30)

	pg/mL	day 1	day 2	day 3	day 4	day 5	day 6	day 7
TNF- α	Median	17.295	9.31	4.735	2.54	0.325	2.66	0
	Range	402.9	354.5	283.38	111.49	68.59	58.63	55.4

Mouse sepsis models suck...

No, really?

The 1st Example from the Table

No.	Translational phenomenon/response	Specific comments: mouse	Specific comments: human
1	Antibodies to TNF given indiscriminately fail to reduce sepsis mortality	BALB/c mice were pretreated with antibodies to TNF prior to CLP sepsis. The murine studies were published 3 y before the failed human trials (101, 116)	Anti-TNF antibodies failed to be an effective treatment strategy in a general population of septic patients (117, 118)

Anti-TNF treatments are bogus...

No, really?

The 2nd Example from the Table

No.	Translational phenomenon/response	Specific comments: mouse	Specific comments: human
2	Pretreatment with an anti-TNF strategy prevents early systemic inflammatory response syndrome	Passive immunization with the antiserum to TNF- α in BALB/c mice protected them against the lethal hyperinflammation by <i>Escherichia coli</i> LPS (98)	Anti-TNF- α therapy was effective in humans with louse-borne relapsing fever when given as a pretreatment against Jarisch-Herxheimer reactions (119)

Crit Care Med. 2013 Oct;41(10):2419-29.

Antitumor necrosis factor therapy is associated with improved survival in clinical sepsis trials: a meta-analysis.

Qiu P, Cui X, Sun J, Welsh J, Natanson C, Eichacker PQ.



How many times do I
have to repeat it?!?
**LPS injection is
not sepsis!**



Re-checking our mouse models...

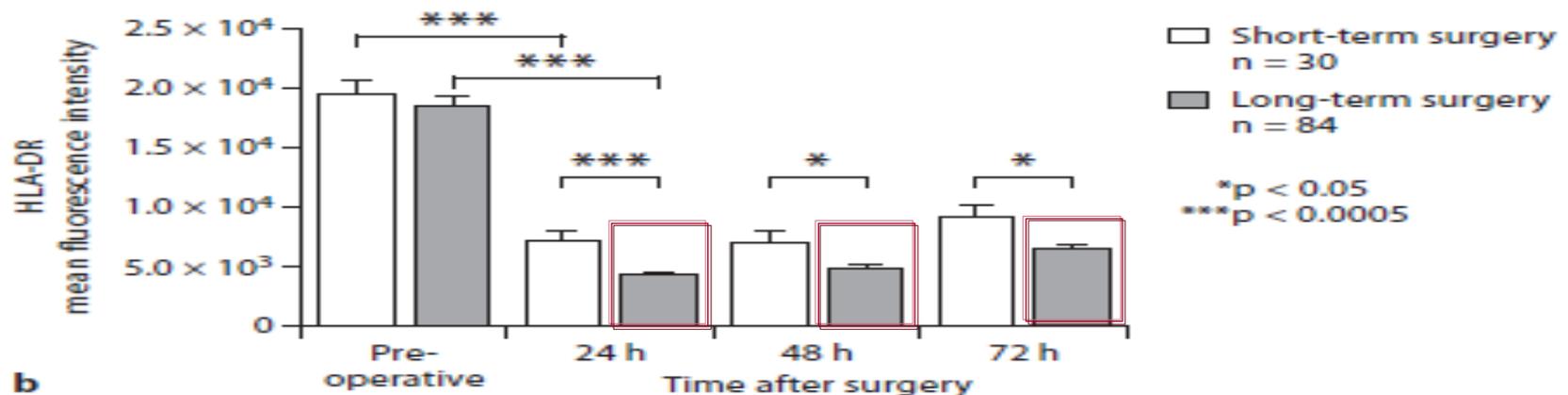
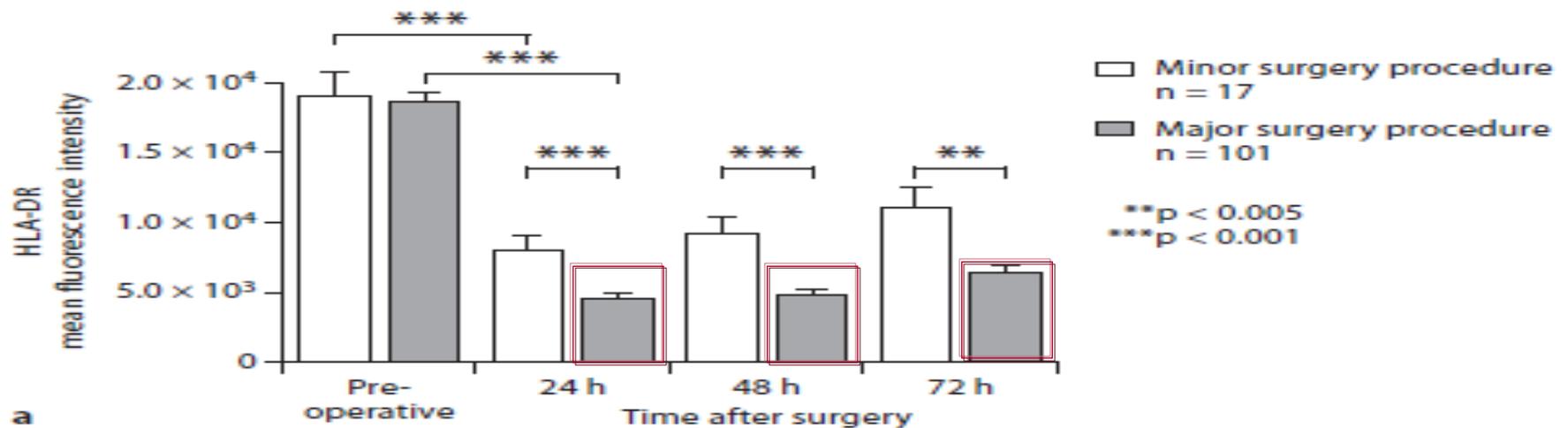
Navigation tip #1b
The Model/Disease Mismatch:
Trauma



Surgical Trauma and Postoperative Immune Dysfunction

P. Menges W. Kessler C. Kloecker M. Feuerherd S. Gaubert S. Diedrich
J. van der Linde A. Hegenbart A. Busemann T. Traeger K. Cziupka
C.-D. Heidecke S. Maier

Department of Surgery, University Hospital Greifswald, Greifswald, Germany

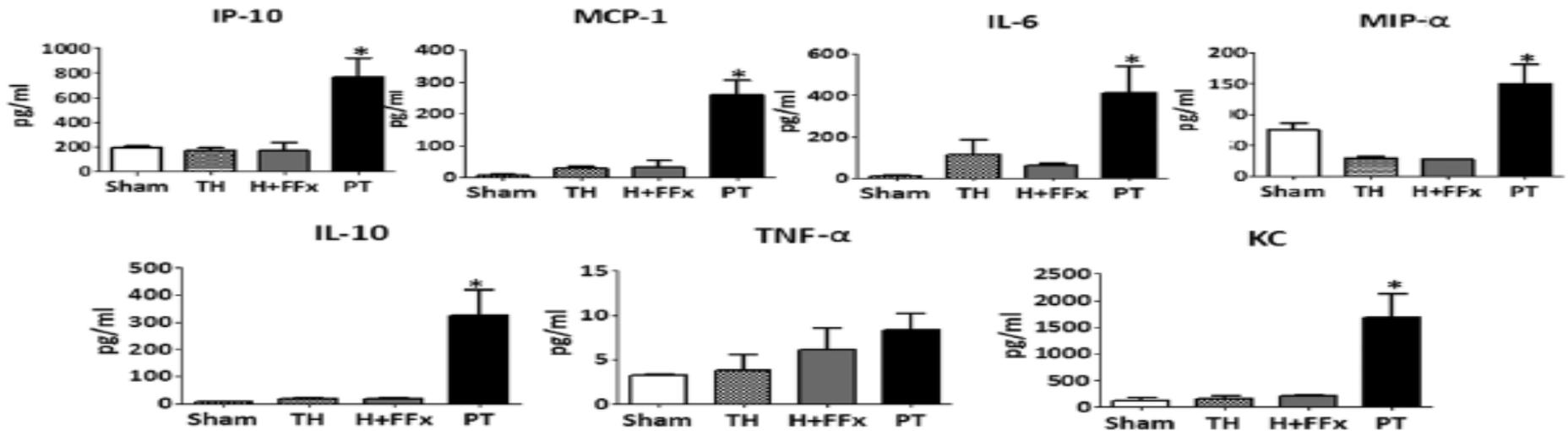


Identification and Description of A Novel Murine Model For Polytrauma and Shock

Crit Care Med. 2013

Lori F. Gentile, MD¹; Dina C. Nacionales, MD¹; Alex G. Cuenca, MD, PhD¹; Michael Armbruster, BS¹; Ricardo F. Ungaro, BS¹; Amer S. Abouhamze, MHA¹; Cecelia Lopez, MS²; Henry V. Baker, PhD²; Frederick A. Moore, MD¹; Darwin N. Ang, MD, PhD, MPH¹; Philip A. Efron, MD¹

Robuster Inflammatory Response – Day 1 after Trauma



TH=hemorrhage&laparotomy

H+FFx=hemorrhage&femur fracture

PT=hemorrhage&thigh diss.femur fracture+cecotomy

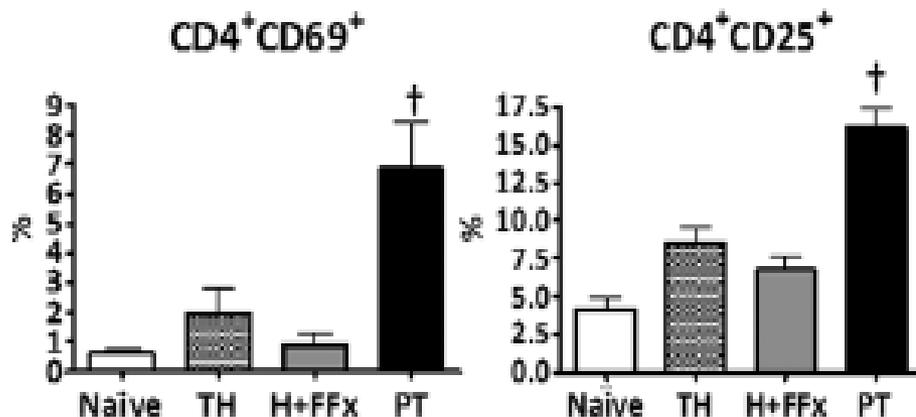
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Lori F. Gentile, MD¹; Dina C. Nacionales, MD¹; Alex G. Cuenca, MD, PhD¹; Michael Armbruster, BS¹; Ricardo F. Ungaro, BS¹; Amer S. Abouhamze, MHA¹; Cecelia Lopez, MS²; Henry V. Baker, PhD²; Frederick A. Moore, MD¹; Darwin N. Ang, MD, PhD, MPH¹; Philip A. Efron, MD¹

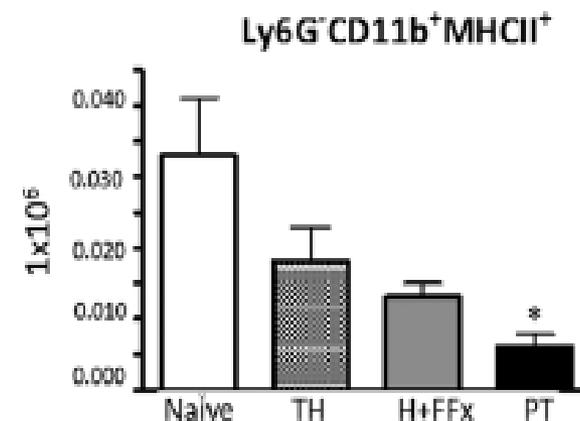
“The polytrauma model appears to be superior...(…)...in recapitulating the human response to severe injury.”

More Pronounced Immunosuppression

Day 3 – T helper lymphocytes and activation markers in blood

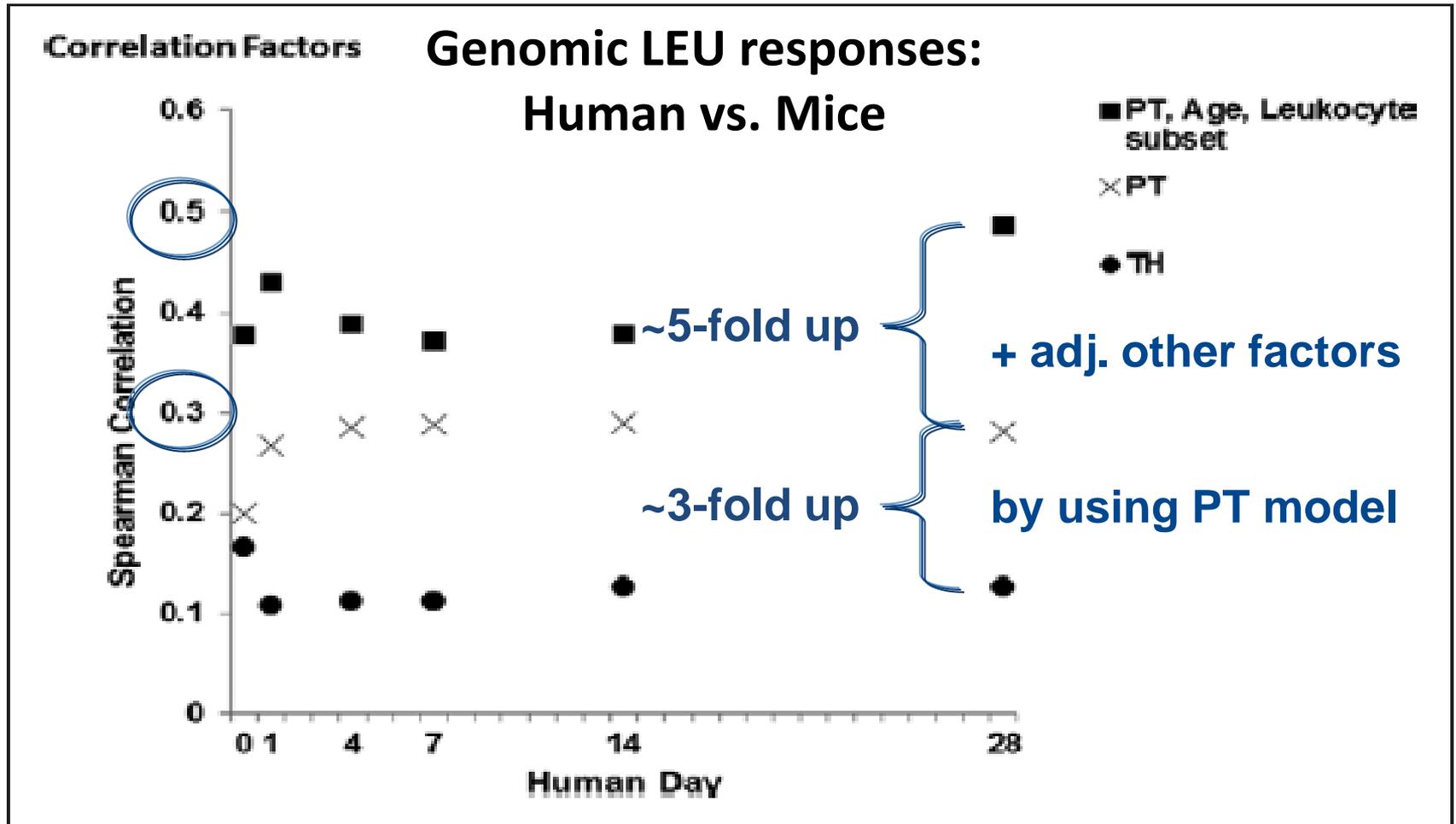


B Day 3 - Bone Marrow Monocyte MHC II Expression



A Better Understanding of Why Murine Models of Trauma Do Not Recapitulate the Human Syndrome*

Lori F. Gentile, MD¹; Dina C. Nacionales, MD¹; M. Cecilia Lopez, BS^{2,3} Crit Care Med. 2014



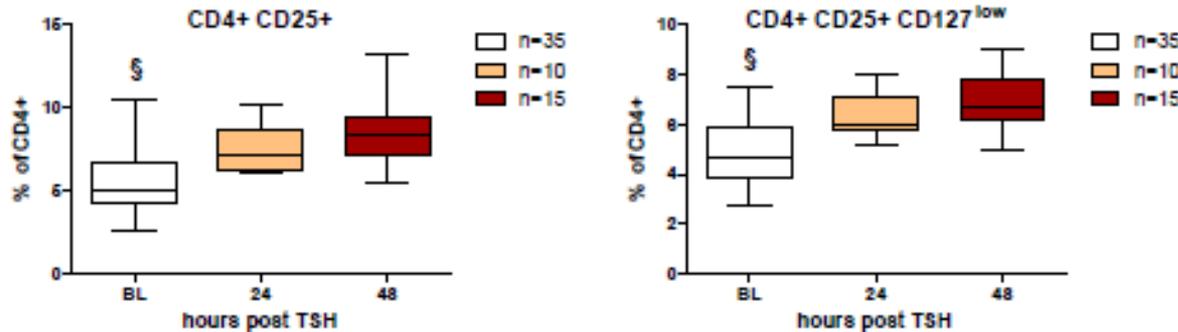
TH=hemorrhage&laparotomy PT=hemorrhage&thigh diss.femur fracture+cecotomy

Severe polytrauma simultaneously activates and impairs the innate and adaptive immune response in the blood and bone marrow compartment of young mice

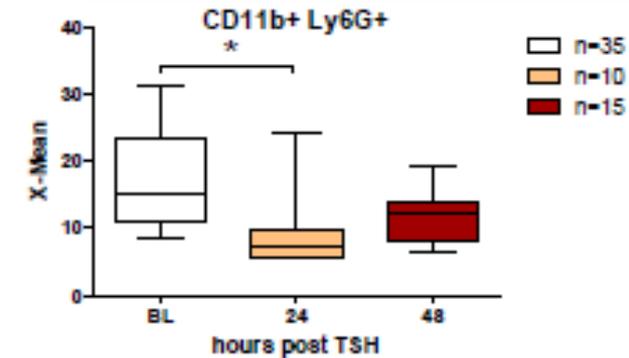
S. Drechsler, P. Rademann, J. Zipperle, M.Jafarmadar, T.Klotz, S.Bahrami, M.Osuchowski
Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Vienna, Austria

It's Great, But(t)...!

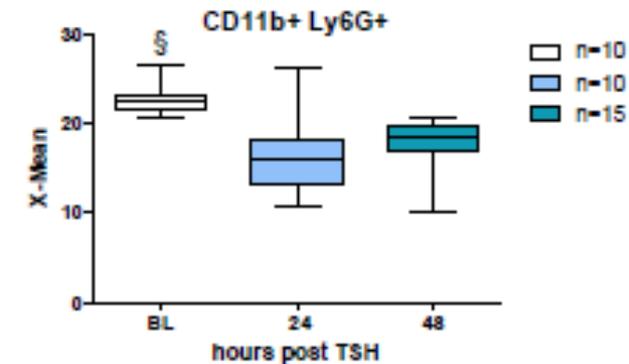
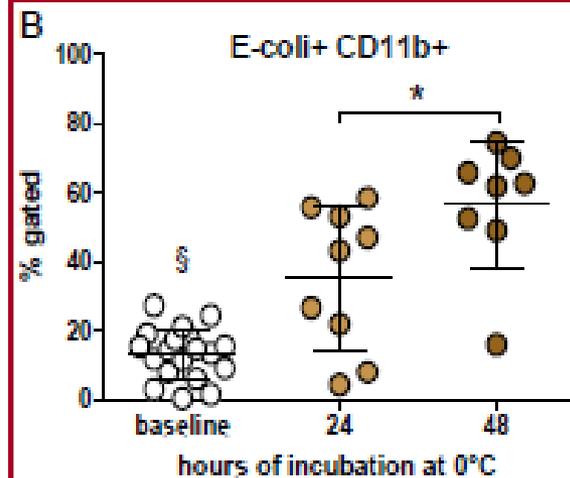
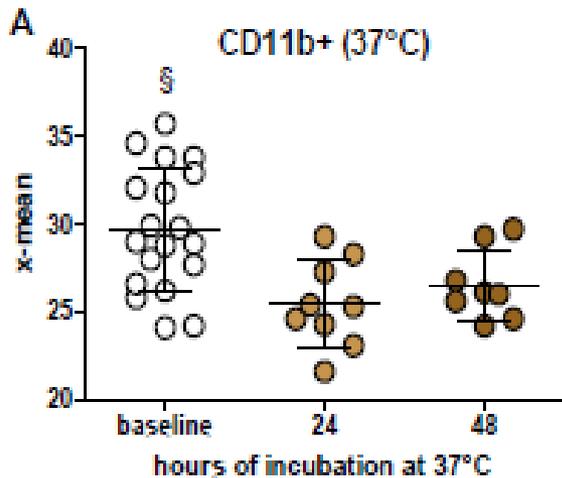
3.3 Posttraumatic increase of T_{regs}



3.4 Postraumatic drop of CD11b



3.5 Activated posttraumatic phagocytosis



The Same Great „But(t)s“ in Sepsis Modeling!

Drechsler et al. *Intensive Care Medicine Experimental* (2015) 3:12
DOI 10.1186/s40635-015-0048-z



RESEARCH

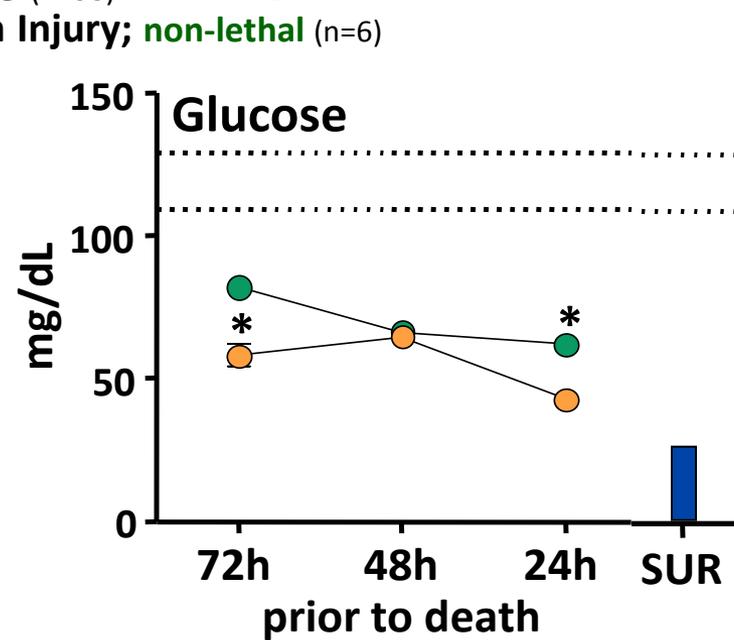
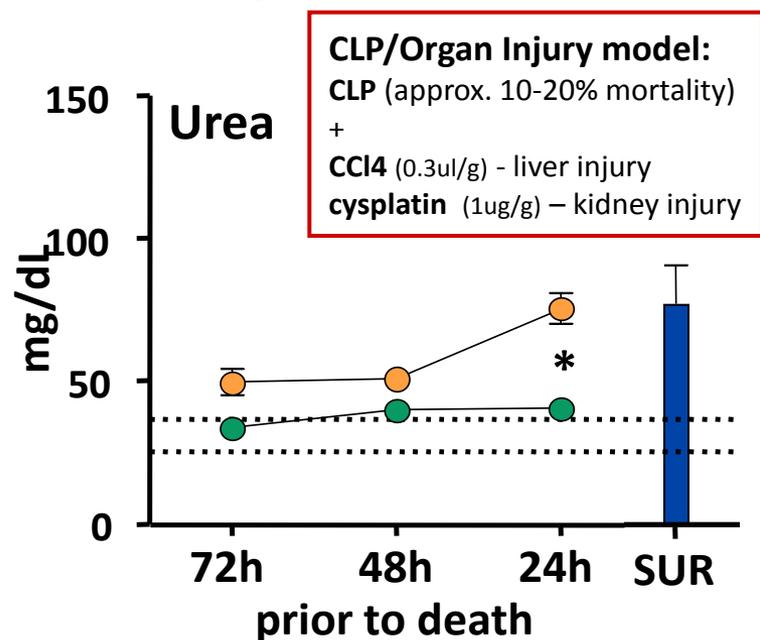
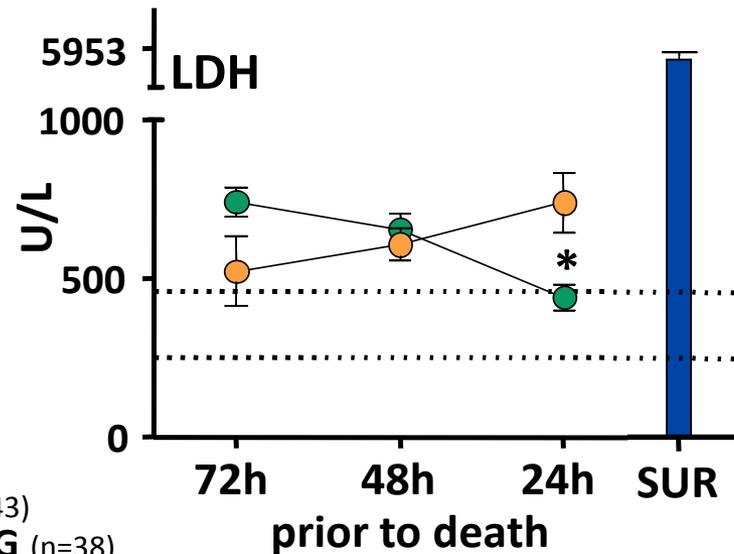
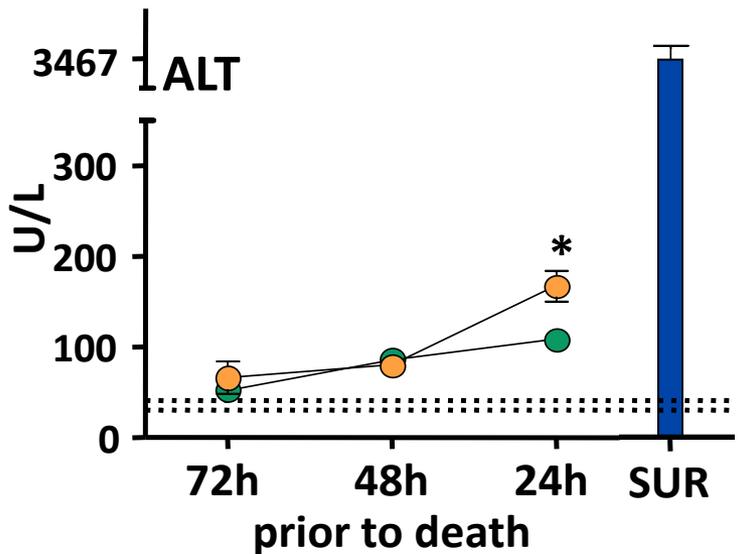
Open Access

Why do they die? Comparison of selected aspects of organ injury and dysfunction in mice surviving and dying in acute abdominal sepsis

Susanne Drechsler^{1†}, Katrin M Weixelbaumer^{1,4†}, Adelheid Weidinger¹, Pierre Raeven^{1,2}, Anna Khadem¹, Heinz Redl¹, Martijn van Griensven^{1,5}, Soheyl Bahrami¹, Daniel Remick³, Andrey Kozlov¹ and Marcin F Osuchowski^{1*}

Organ Dysfunction in Acute Sepsis (days 1-5):

Circulating Parameters Prior to Death: high ALT/LDH does not equal death

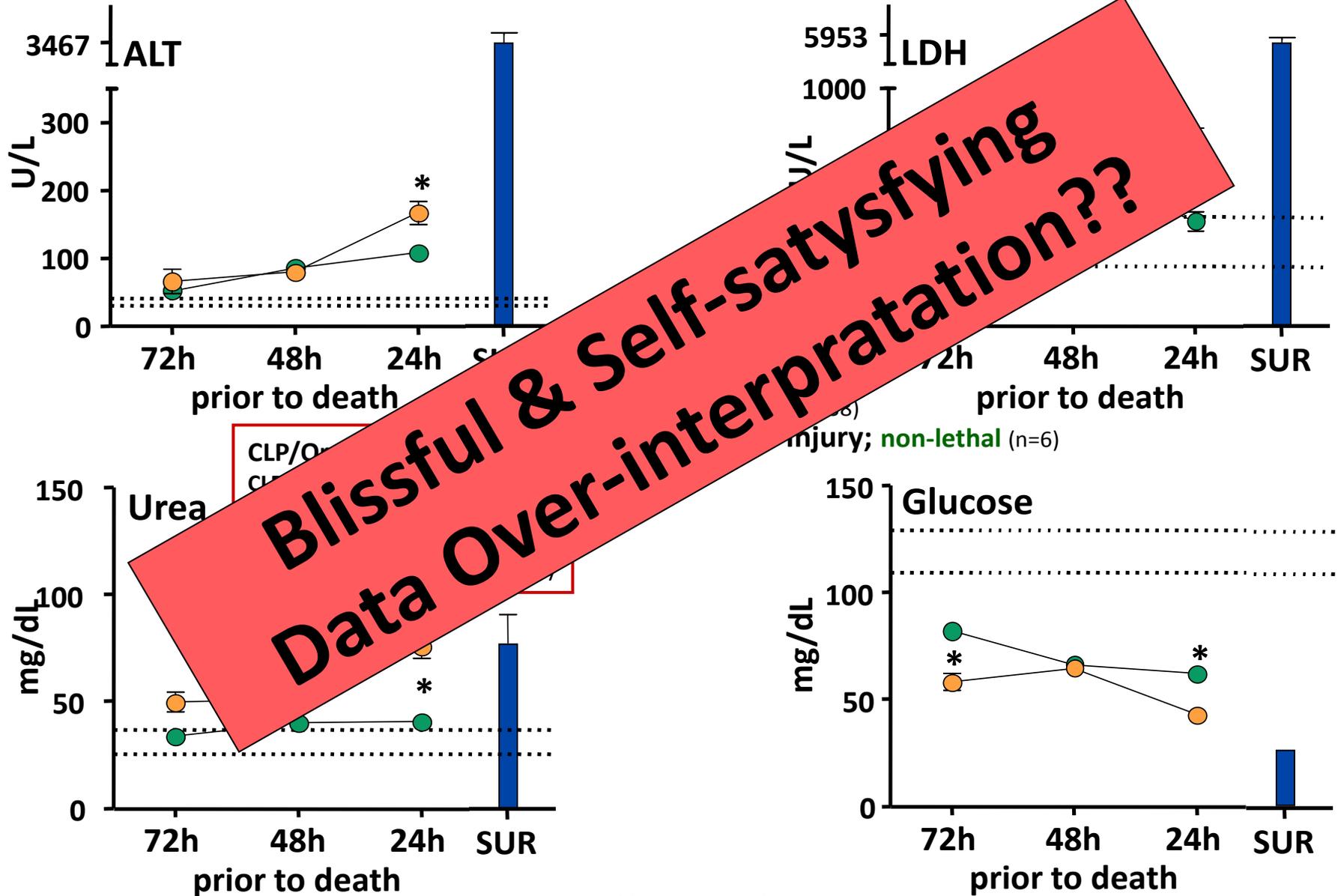


CLP/Organ Injury model:
CLP (approx. 10-20% mortality)
+
CCl4 (0.3ul/g) - liver injury
cisplatin (1ug/g) - kidney injury

- DYING (n=43)
- SURVIVING (n=38)
- CLP/Organ Injury; non-lethal (n=6)

Dotted line: normal range

Organ Dysfunction in Acute Sepsis (days 1-5): Circulating Parameters Prior to Death



**Blissful & Self-satisfying
Data Over-interpretation??**

New and Better (?) Sepsis Models Have Emerged

PERITONITIS

Intraperitoneal E.Coli injections

Infected Fibrin Clot (e.g. with E.coli)

} Monomicrobial,
better control,
reproducible

Fecal Slurry (FS)

Colon Ascendens Stent Peritonitis (CASP)

Cecal Ligation and Puncture (CLP)

} Polymicrobial,
worse control,
less reproducible

PNEUMONIA

Intratracheal application of:

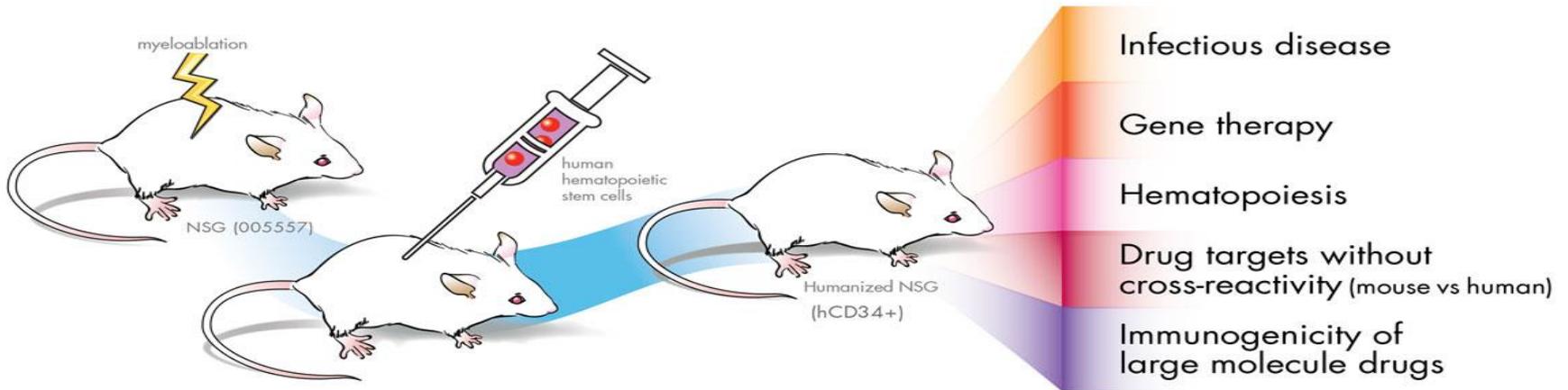
- Strept. Pneumoniae
- Pseud. Aeruginosa
- Methicillin-resistant Staph. aureus (MRSA)

1 Hit Approach: Community Acquired P. (CAP)

2 Hit Approach: Hospital Acquired P. (HAP)
CLP + Pneumonia

New and Better (?) Sepsis Models Have Emerged

Humanized Mice – New Opening?



PNEUMONIA

Intratracheal application of:

- Strept. Pneumoniae
- Pseud. Aeruginosa
- Methicillin-resistant Staph. aureus (MRSA)

1 Hit Approach: Community Acquired P. (CAP)

2 Hit Approach: Hospital Acquired P. (HAP)
CLP + Pneumonia

The most commonly used nowadays:
cecal ligation and puncture (CLP)



From Rittirsch *et al.* Nat Protoc. 2009

CLP – Major Technical Similarities to Clinic:

- Protracted spillover of LIVE microorganisms
- Gradual spread of infection that goes systemic
- Necrotic component (ligated cecum)
- persistent nidus of infection (abscess)



Mouse CLP Models Demonstrated:

Delayed clinical symptoms

Clinical-like response to antibiotics & fluids

Late (chronic) mortality with immunosuppression

Reproduction of anti-inflammatory clinical trial failures

Viewed as a serious pitfall in CLP:

- persistent nidus of infection (abscess and/or peritonitis)

(Anesth Analg 2009;108:1841-7)

Macroscopic Postmortem Findings in 235 Surgical Intensive Care Patients with Sepsis

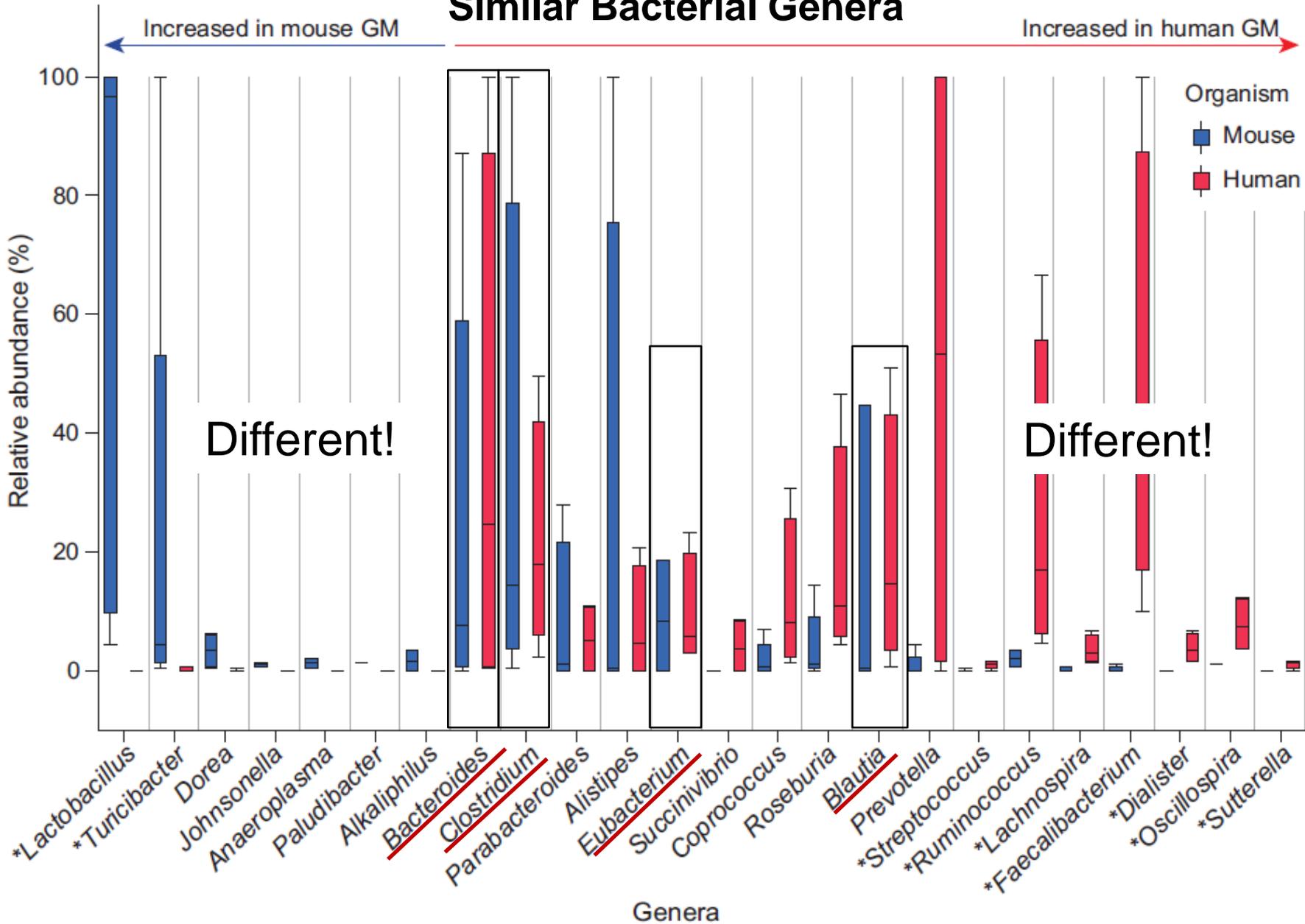
Christian Torgersen, MD* Patrizia Moser, MD† and others

autopsy revealed a continuous septic focus. The most common continuous foci were pneumonia (41.3%), tracheobronchitis (28.9%), peritonitis (23.4%), uterine/ovarial necrosis (9.8% of female patients), intraabdominal abscesses (9.1%), and pyelonephritis (6%). A

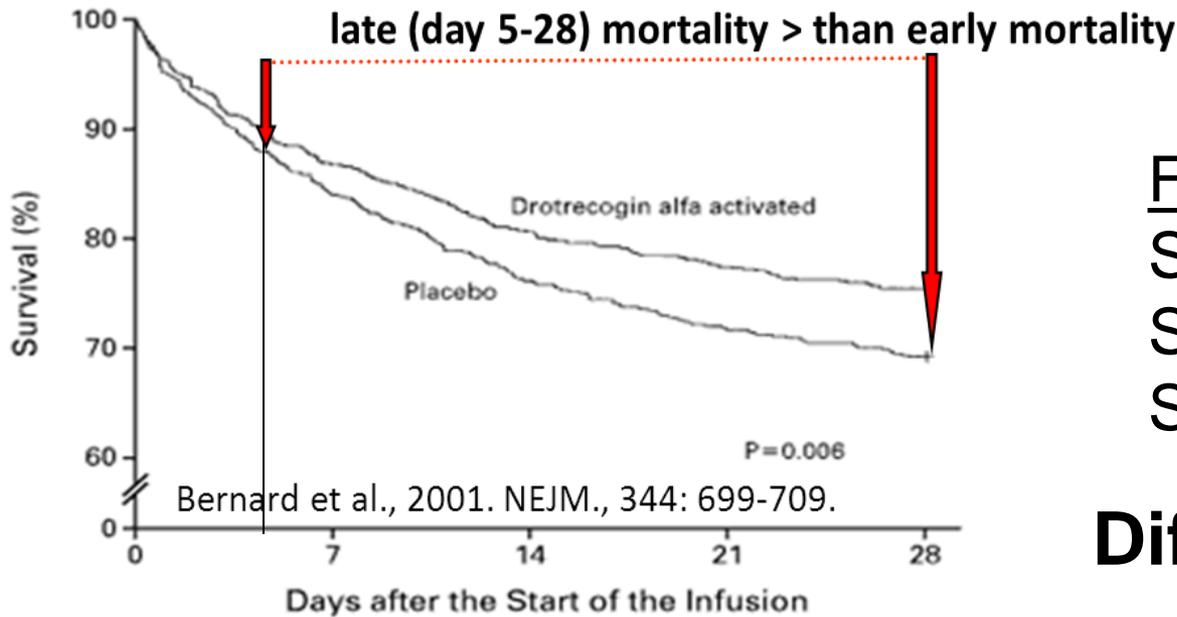
Similar Bacterial Genera

← Increased in mouse GM

→ Increased in human GM

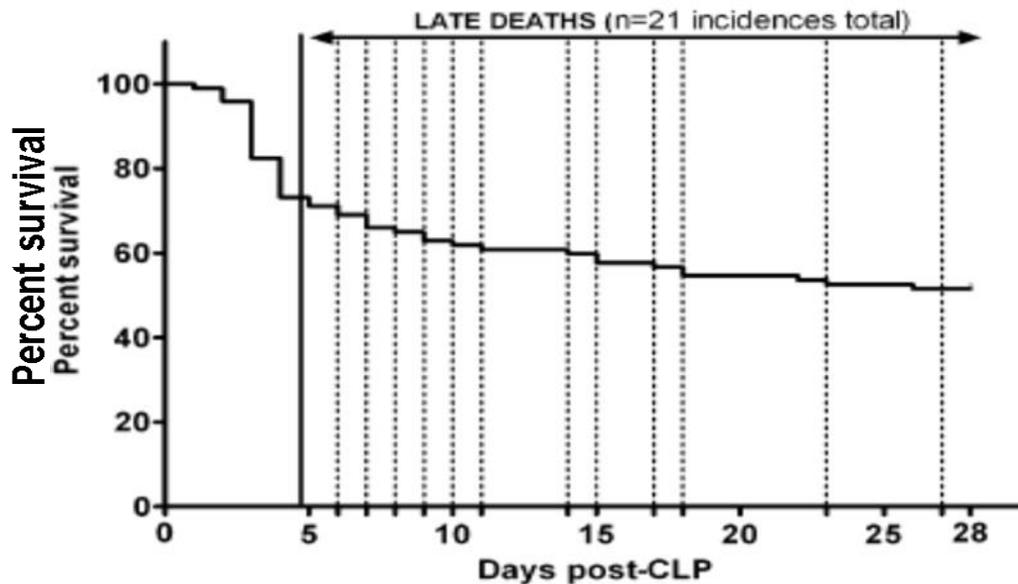


The enterotype – heavy impact (?) among same strains

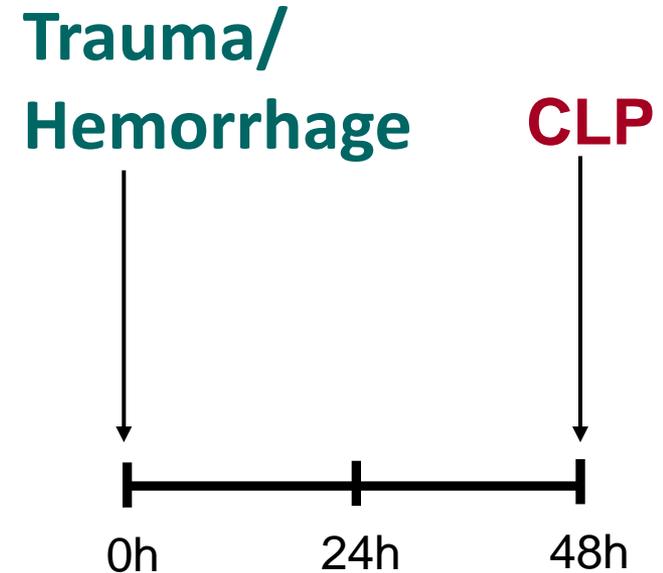
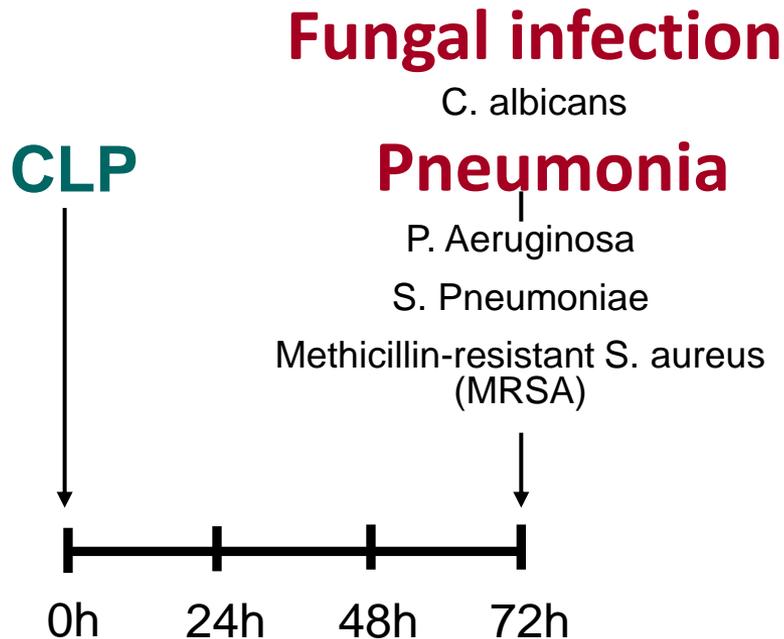


Female CD-1 mice
Same needle
Same operator
Same protocol

Different breeders



2 Hit Models as a Viable Remedy



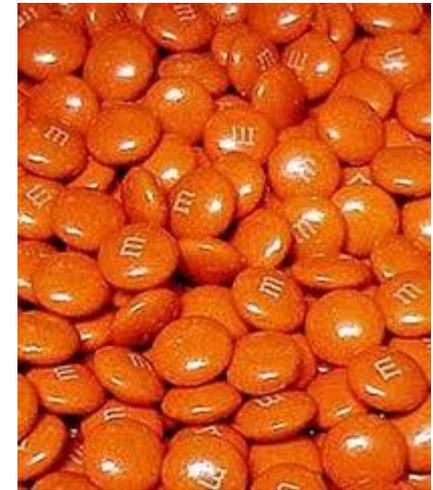
Muenzer et al., 2006, Shock
Muenzer et al., 2010, Infection and Immunity
Jung et al., 2011, Shock
Jung et al., 2012, Shock
Davis et al., 2012, The Journal of Surgical Research
Usinger et al. 2012 Journal of Infectious Disease

Chaudry /Suzuki et al., 2006, Shock & Inflammation
Van Griensvan et al. Exp Toxicol Pathol. 2002
Drechsler et al., 2011 J Biomed Biotech & 2013 PLOS ONE
Weckbach et al., 2013, J Trauma Acute Care Surg

Re-setting our mouse investigative thinking...

Navigation tip #2:

Heterogeneity versus Homogeneity



One Disease, Many Faces...

>>Differential Host Response<<

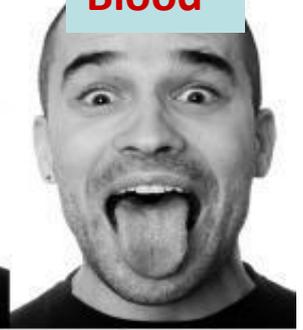
Pulmonary



SIRS



Blood



Systemic Inflammatory Response Syndrome

skin



MARS



Gastro-intestinal



Mixed Anti-inflammatory Response Syndrome

CNS



CARS



Genito-urinary



Compensatory Anti-inflammatory Response Syndrome

They are all diagnosed with sepsis...



≠



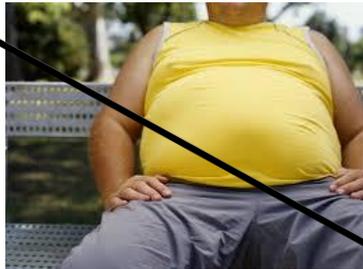
≠



1) monobacterial CAP in an elderly woman

2) Post-traumatic peritonitis in a young healthy male

3) Fulminant meningococcal sepsis in an infant



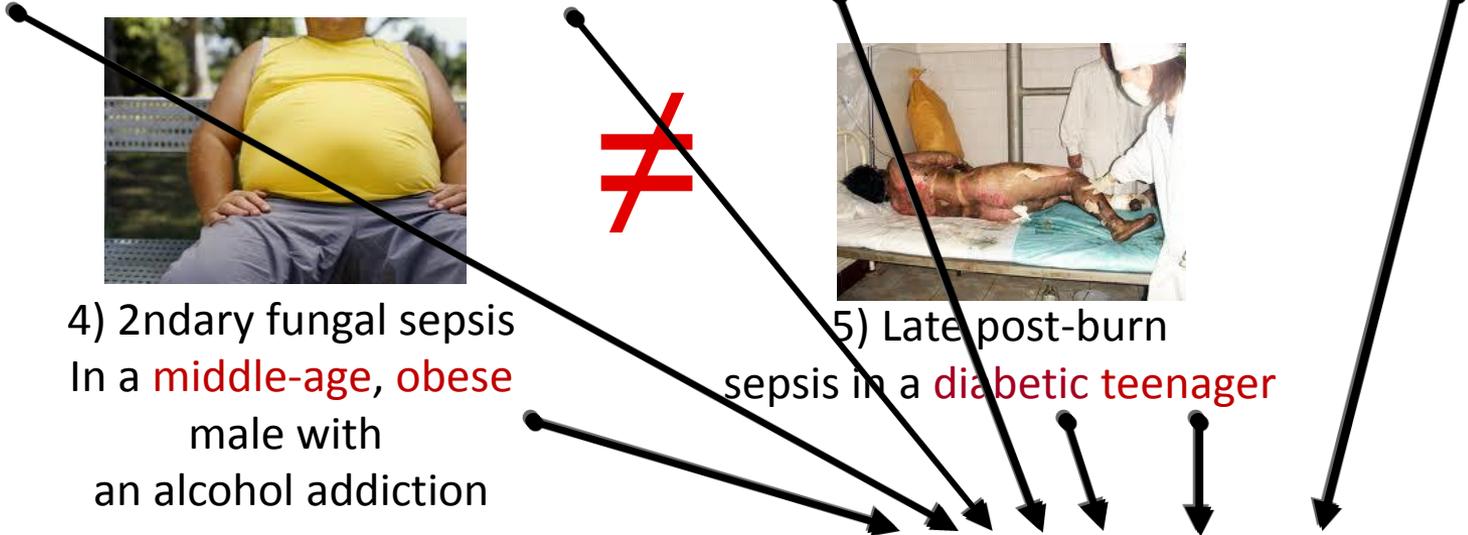
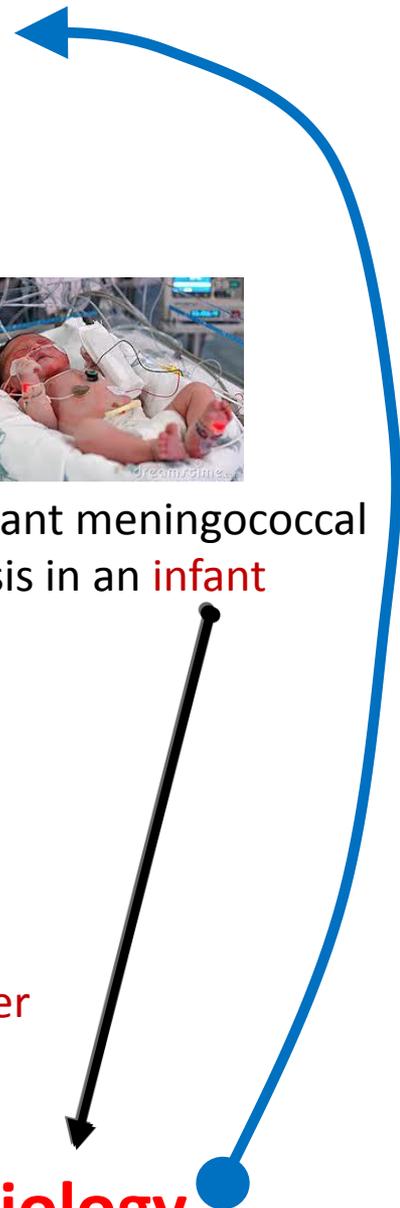
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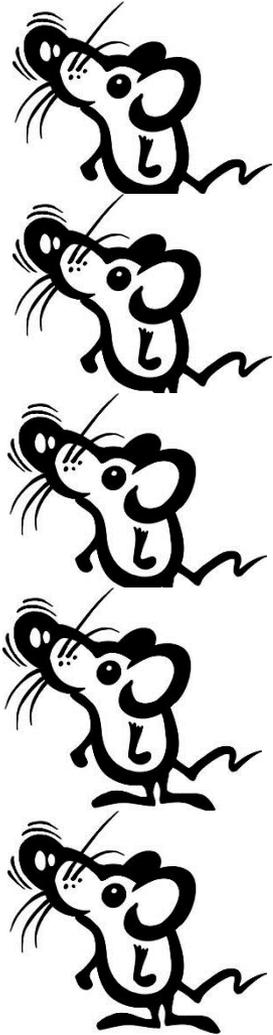
4) 2ndary fungal sepsis In a middle-age, obese male with an alcohol addiction

5) Late post-burn sepsis in a diabetic teenager

...yet the underlying pathophysiology is different in each case!



Personalize/adapt the models - - to match the real life!



sepsis –
case 1



sepsis –
case 2



sepsis –
case 3



sepsis –
case 4



sepsis –case 5



Five 3-month-old healthy,
inbred female C57BL/6 mice

Different ways to reduce heterogeneity by focusing on:

- Infectious source (e.g. abdominal vs. pneumonia)
- Type of infecting microorganism (e.g., G-pos. vs. G-neg. vs. fungus)
- Immuno-inflammatory status (i.e. robust response vs. immunosup.)
- Presence/absence of specific comorbidities
- Sepsis severity/risk of death (high vs. low)

Out of 69 Anti-sepsis Ph2/3 Human Trials listed...

Table 1. Summary of clinical trials of pharmacological interventions for the adjuvant treatment of sepsis, which have been reported since 1982

1st Author	Year	Patients (sample size)	Trial Acronym	Experimental agent	Effect on mortality*	References
Ziegler	1982	Septic shock (212)		Human antiserum to mutant <i>E. coli</i>	Benefit ^b	2
Ziegler		Sepsis and presumed or proven Gram-negative infection (543)		HA-1A, a human mAb that binds the lipid A domain of LPS	Benefit	67
McCloskey	1994	Septic shock and Gram-negative bacteremia (621)	CHESS	HA-1A, a human mAb that binds the lipid A domain of LPS	No effect ^c	5
Greenman,	1991	Gram-negative sepsis (486)		E5, a murine mAb that binds the lipid A domain of LPS	No effect	68
Bone	1995	Gram-negative sepsis with organ dysfunction (847)		E5, a murine mAb that binds the lipid A domain of LPS	No effect	69

Experimental agent	Effect on mortality*	References
BB-882, a small molecule PAF receptor antagonist	No effect	86
TCV-309, a small molecule PAF receptor antagonist	No effect	87
TCV-309, a small molecule PAF receptor antagonist	No effect	88
afasif, recombinant human platelet activator	Benefit	10

Experimental agent	Effect on mortality*	References
rogin alfa, recombinant human activated protein C	No effect	17
en, small molecule isoform unselective cyclooxygenase inhibitor	No effect	95

2 used advanced (IL-6-based) treatment targeting

Reinhart	2001	Severe sepsis and high serum concentration of IL-6 (446)	RAMSES	Afelimomab, the F(ab') ₂ fragment of a murine anti-TNF mAb	No effect
Panacek	2004	Severe sepsis and high serum concentration of IL-6 (998)	MONARCS	Afelimomab, the F(ab') ₂ fragment of a murine anti-TNF mAb	Benefit

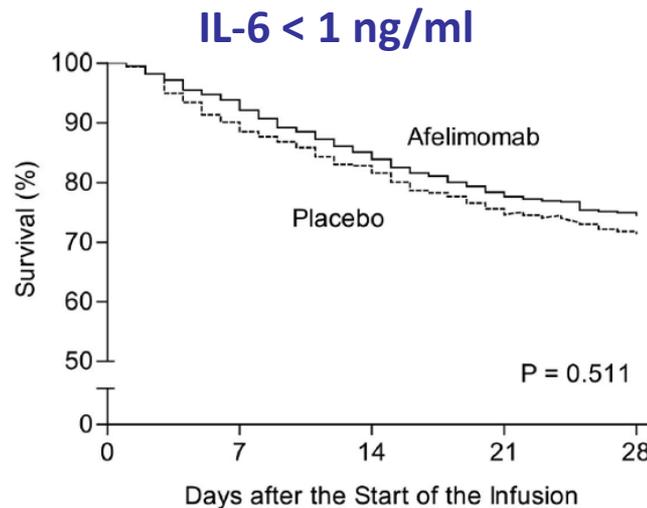
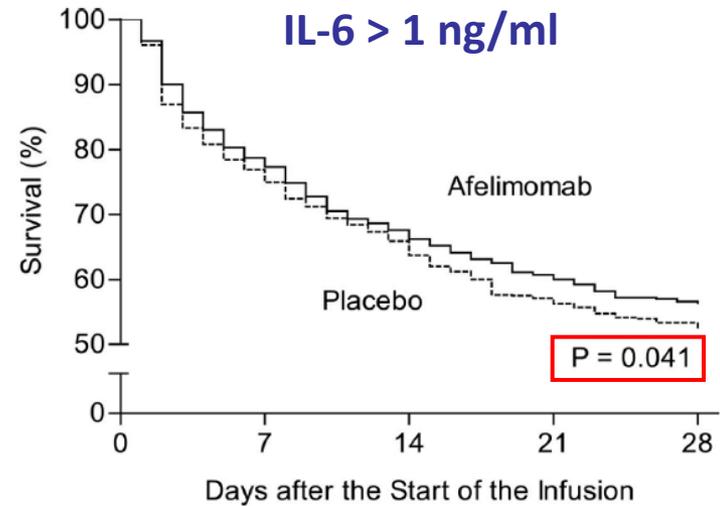
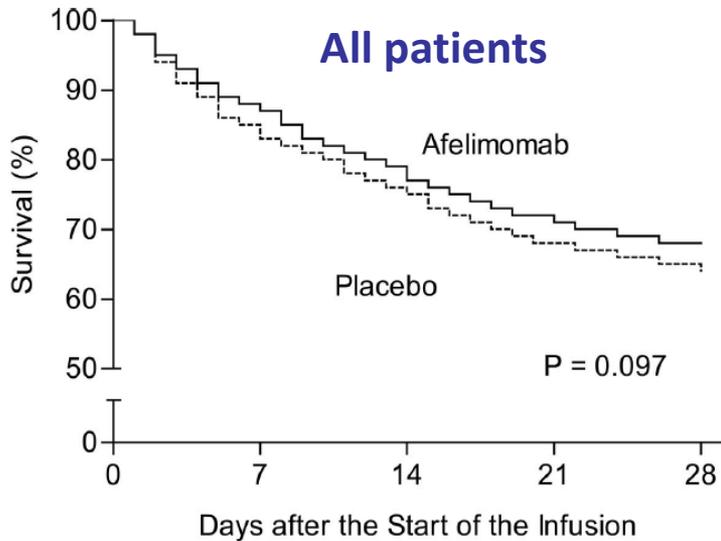
Fisher	1996	Septic shock (141)		Etanercept, a recombinant fusion protein that is a dimer of the extracellular portion of the human p75 TNF receptor and the Fc portion of IgG1; it binds and neutralizes TNF	Harm	20
Abraham	1995	Sepsis (994)	NORASEPT I	BAY x 1351, a murine anti-TNF mAb	No effect	75
Cohen	1996	Sepsis (564)	INTERSEPT	BAY x 1351, a murine anti-TNF mAb	No effect	76
Abraham	1998	Septic shock (1878)	NORASEPT II	BAY x 1351, a murine anti-TNF mAb	No effect	77
Rice	2006	Severe sepsis or septic shock (81)		Cyclosporin (ab) fragments of an ovine polyclonal antibody to TNF	No effect	78
Reinhart	1996	Severe sepsis or septic shock (122)		Afelimomab, the F(ab') ₂ fragment of a murine anti-TNF mAb	No effect	79
Reinhart	2001	Severe sepsis and high serum concentration of IL-6 (446)	RAMSES	Afelimomab, the F(ab') ₂ fragment of a murine anti-TNF mAb	No effect	80
Panacek	2004	Severe sepsis and high serum concentration of IL-6 (998)	MONARCS	Afelimomab, the F(ab') ₂ fragment of a murine anti-TNF mAb	Benefit	9
Dhainaut	1995	Septic shock (42)		CDPS71, a humanized anti-TNF mAb	No effect	81
Fisher	1993	Severe sepsis or septic shock (80)		CB0006, a murine anti-TNF mAb	No effect	82
Dhainaut	1994	Sepsis (262)		BN 52021, a small molecule PAF receptor antagonist	No effect	83
Dhainaut	1998	Severe sepsis suspected to be caused by Gram-negative infection (609)		BN 52021, a small molecule PAF receptor antagonist	No effect	84
Vincent	2000	Clinical suspicion of infection and APACHE II score between 15 and 35 (152)		BB-882, a small molecule PAF receptor antagonist	No effect	85

GR270773, a phospholipids emulsion	No effect	93
ifacogin, recombinant human tissue factor pathway inhibitor	No effect	61
ifacogin, recombinant human tissue factor pathway inhibitor	No effect	60
ifacogin, recombinant human tissue factor pathway inhibitor	No effect	62
nakinra, recombinant human interleukin-1 receptor antagonist	Benefit	6
nakinra, recombinant human interleukin-1 receptor antagonist	No effect	7
nakinra, recombinant human interleukin-1 receptor antagonist	No effect	8
P-0127, a small molecule bradykinin receptor antagonist	No effect	94
P-0127, a small molecule bradykinin receptor antagonist	No effect	
lotrecogin alfa, recombinant human activated protein C	Benefit	14
lotrecogin alfa, recombinant human activated protein C	No effect	16
lotrecogin alfa, recombinant human activated protein C	No effect	15

Hydrocortisone	No effect	103
Hydrocortisone	No effect	104
rocortisone and fludrocortisone	Benefit	18
Hydrocortisone	No effect	19
Hydrocortisone	No effect	105
Hydrocortisone	No effect	106
m, recombinant human granulocyte colony stimulating factor	No effect	107
nostim, recombinant human granulocyte macrophage colony stimulating factor	No effect	108
nostim, recombinant human granulocyte macrophage colony stimulating factor	No effect	109
nostim, recombinant human granulocyte macrophage colony stimulating factor	No effect	110
Unfractionated heparin	No effect	111
Pentoxifylline	No effect	112
, small molecule isoform unselective nitric oxide synthase inhibitor	No effect	113
, small molecule isoform unselective nitric oxide synthase inhibitor	Harm	21

IL-6 – based Risk-Stratification for Sepsis Treatment

Clinical: anti-TNF (afelimomab) in severely septic patients



Creating Homogenous Cohorts in Mouse Sepsis

Denominators: risk of death & excessive inflammation

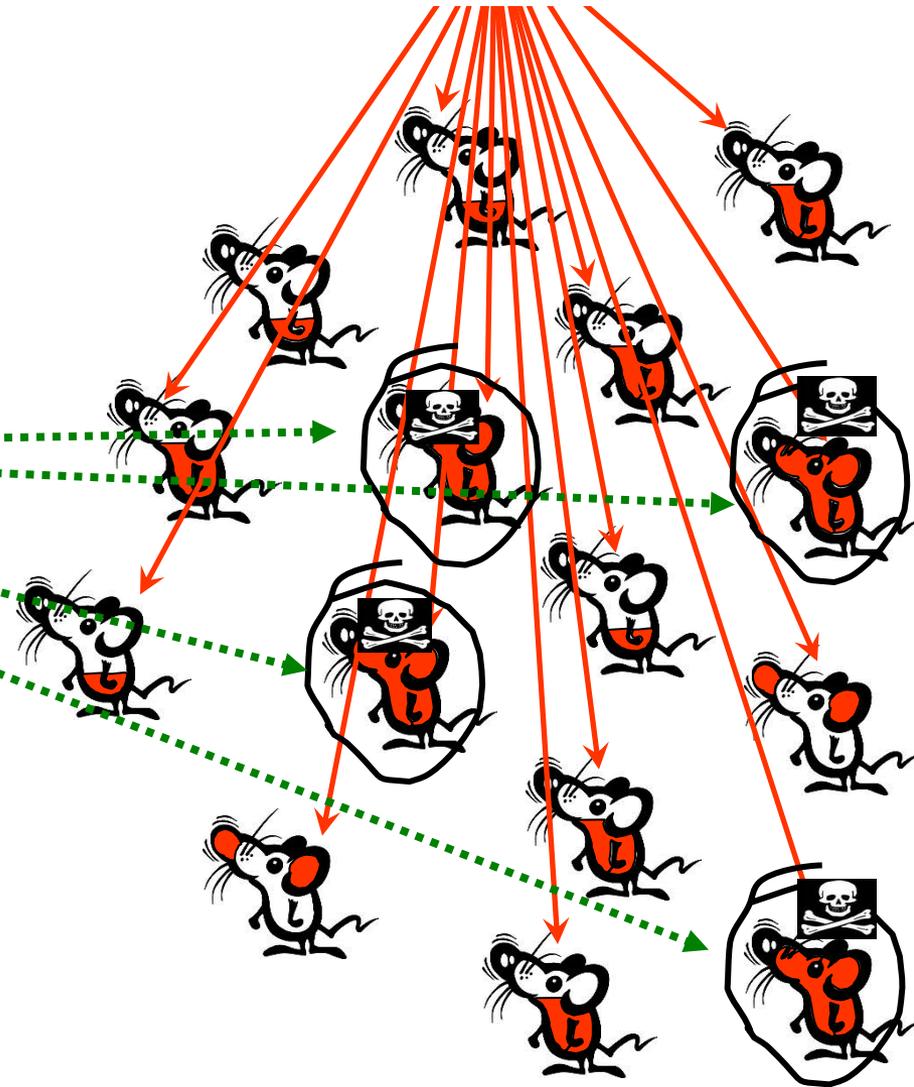
Example 7 from the Table:

IL-6 serves as a biomarker for sepsis mortality

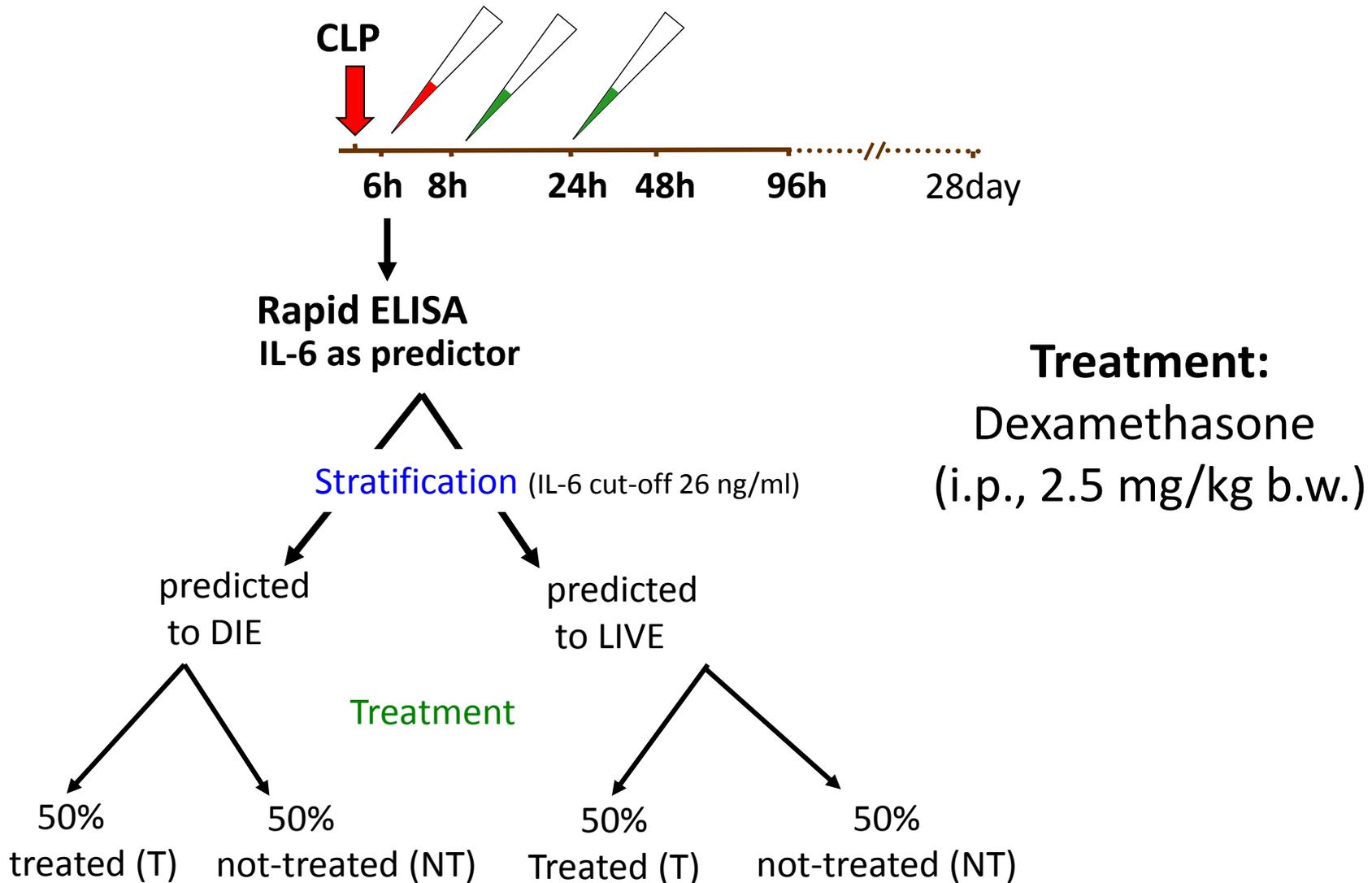
Infl: CLP tory
Response

TARGETED
THERAPY

A perfect niche for
mouse studies
to aid in clinical
trial design!

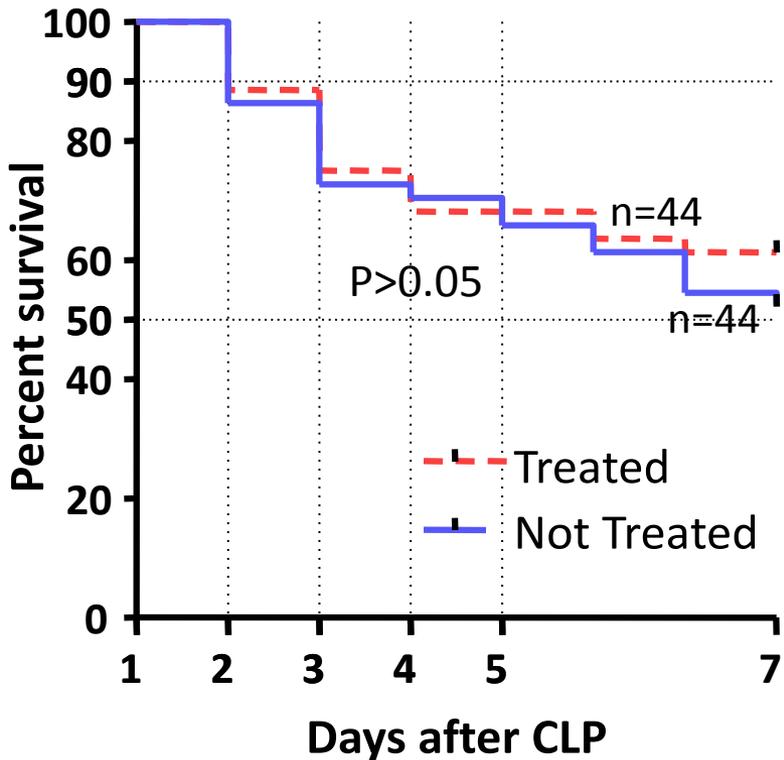


Targeted treatment in a Mouse Model of Acute Sepsis

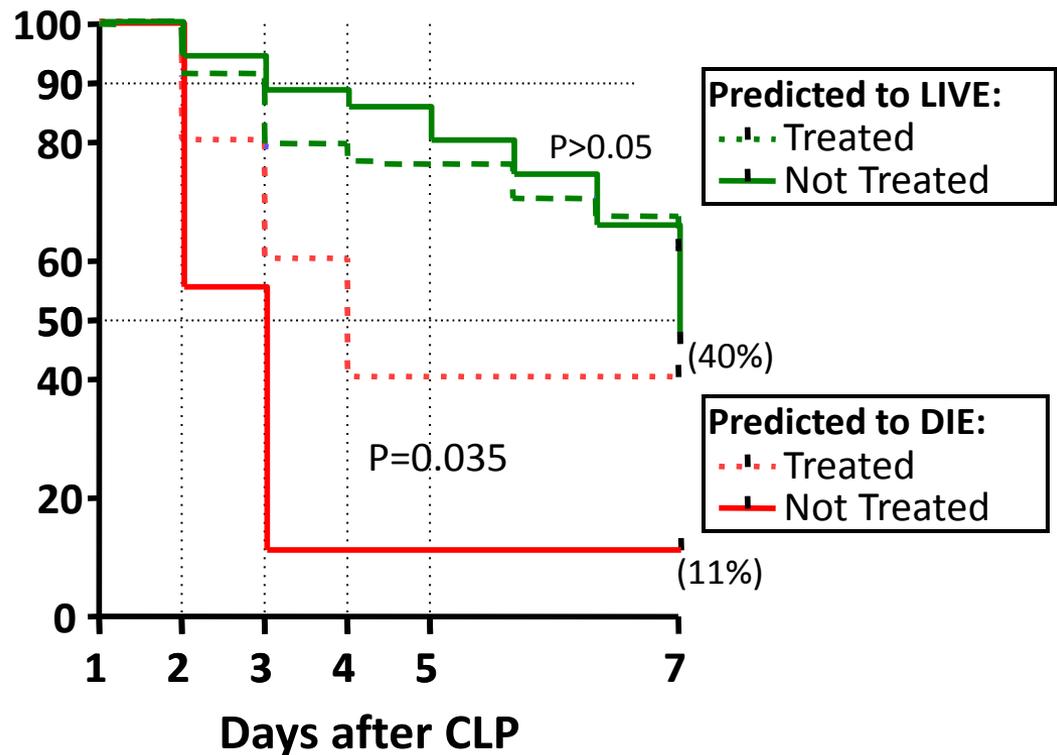


Targeted Treatment in Mouse Sepsis: looking for potential Benefits

dexamethasone treatment without
stratification: days 1-7



dexameth. treatment based on
stratification: days 1-7



Targeted Treatment in Mouse Sepsis: Identifying potential Risks

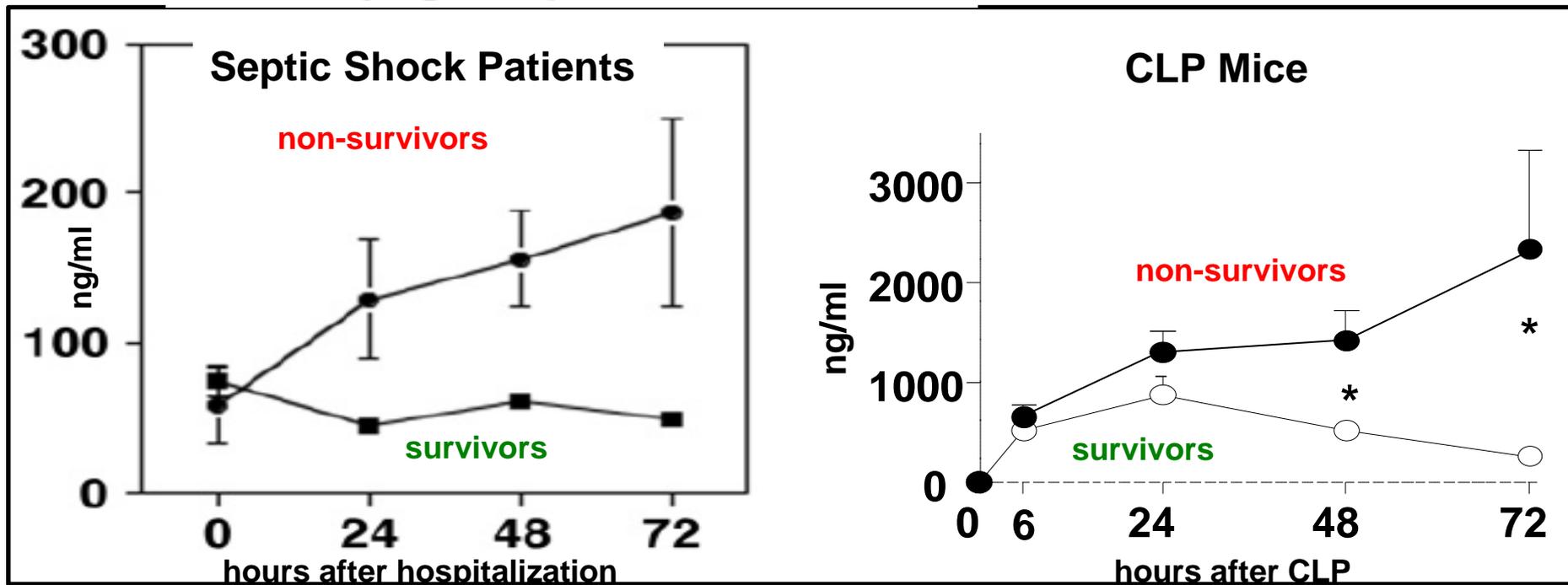
J Thromb Haemost 2014; 12: 958–69.

ORIGINAL ARTICLE

Systemic inhibition and liver-specific over-expression of PAI-1 failed to improve survival in all-inclusive populations or homogenous cohorts of CLP mice

Example 23: Similar dynamics of circulating plasminogen activator inhibitor (PAI) 1 in subjects surviving and dying of sepsis

ICA,† F. PEIRETTI,†
‡SSI,† P. J. DECLERCK‡ and



Shapiro et al. Crit Care 2010

Raeven et al. PLoS ONE 2013

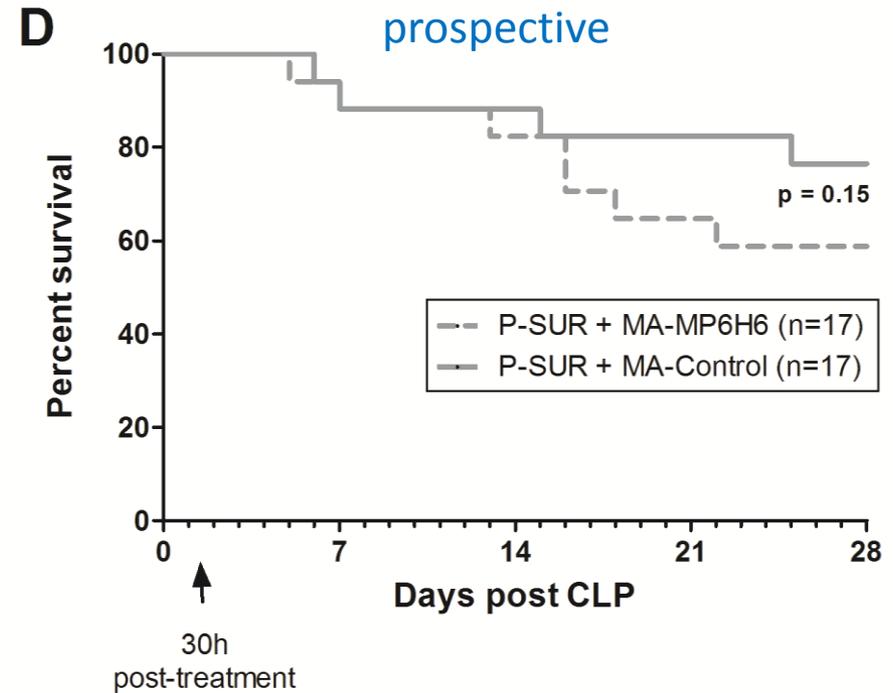
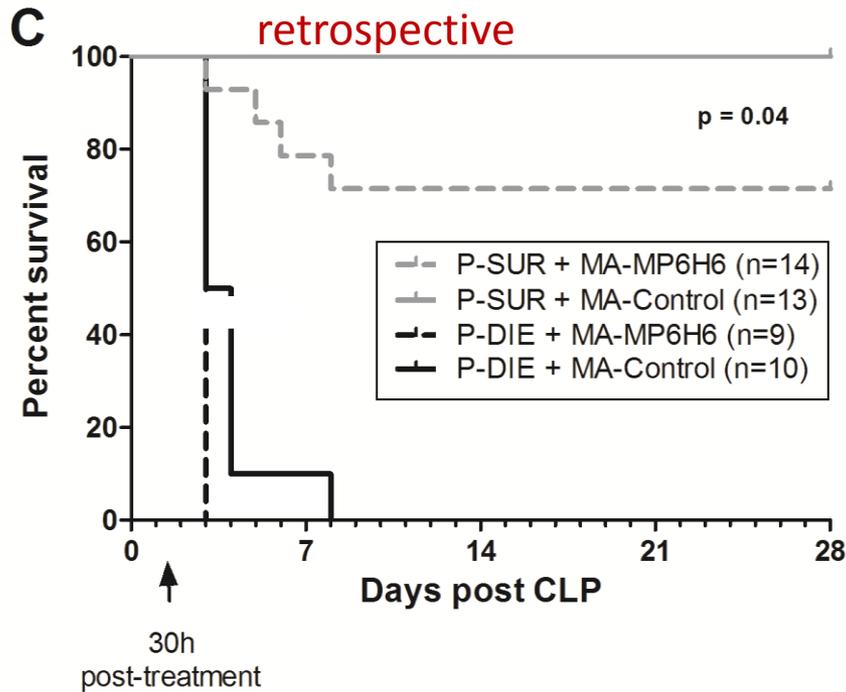
Possible **Detrimental Treatment Effect** in Predicted-to-Live Cohort

J Thromb Haemost 2014; 12: 958–69.

ORIGINAL ARTICLE

Systemic inhibition and liver-specific over-expression of PAI-1 failed to improve survival in all-inclusive populations or homogenous cohorts of CLP mice

P. RAEVEN,* § S. DRECHSLER,* K. M. WEIXELBAUMER,* D. BASTELICA,† F. PEIRETTI,†
A. KLOTZ,* M. JAFARMADAR,* H. REDL,* S. BAHRAMI,* M. C. ALESSI,† P. J. DECLERCK‡ and
M. F. OSUCHOWSKI*



**Targeting homogenous cohorts will save lives:
Smart mouse modeling can pave the way!**



Producing IF instead of useful data...

Navigation tip # 3:

To Do or Not to Do? Compromising the study design





The "hourglass" notion of research

Phenomenon
Big picture / question

Theory

"100% of all disasters are failures of design, not analysis."

-- Ron Marks, Toronto, August 16, 1994

Study design

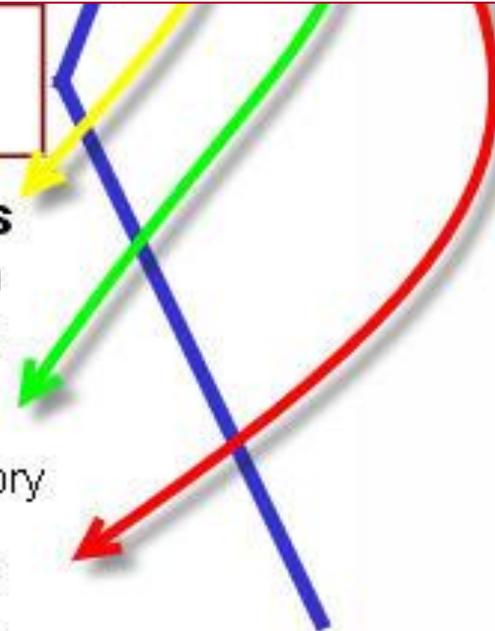
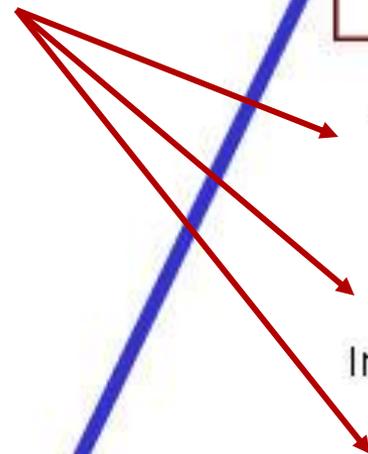


Methods
Measurement v. experimental

Data / Results
• Descriptive data
• Test hypothesis

Discussion
Implications for theory

Conclusions
Future research?



Who's Afraid of Peer Review?

John Bohannon, Science 2013;342:60-65



Two of the most valuable things
a researcher can possess are:

1. knowledge of the principles of good study design
2. the courage to refuse to cut corners
(and publish fewer papers?)

Current reality:

You will ALWAYS publish your stuff somewhere

If you want to publish in Critical Care Medicine:

Mechanism not novel enough to be clinically important

Intervention works only as pre-treatment

The effect is of doubtful clinical significant

Paper on a rarely encountered clinical care

A fitting mouse model is not enough...

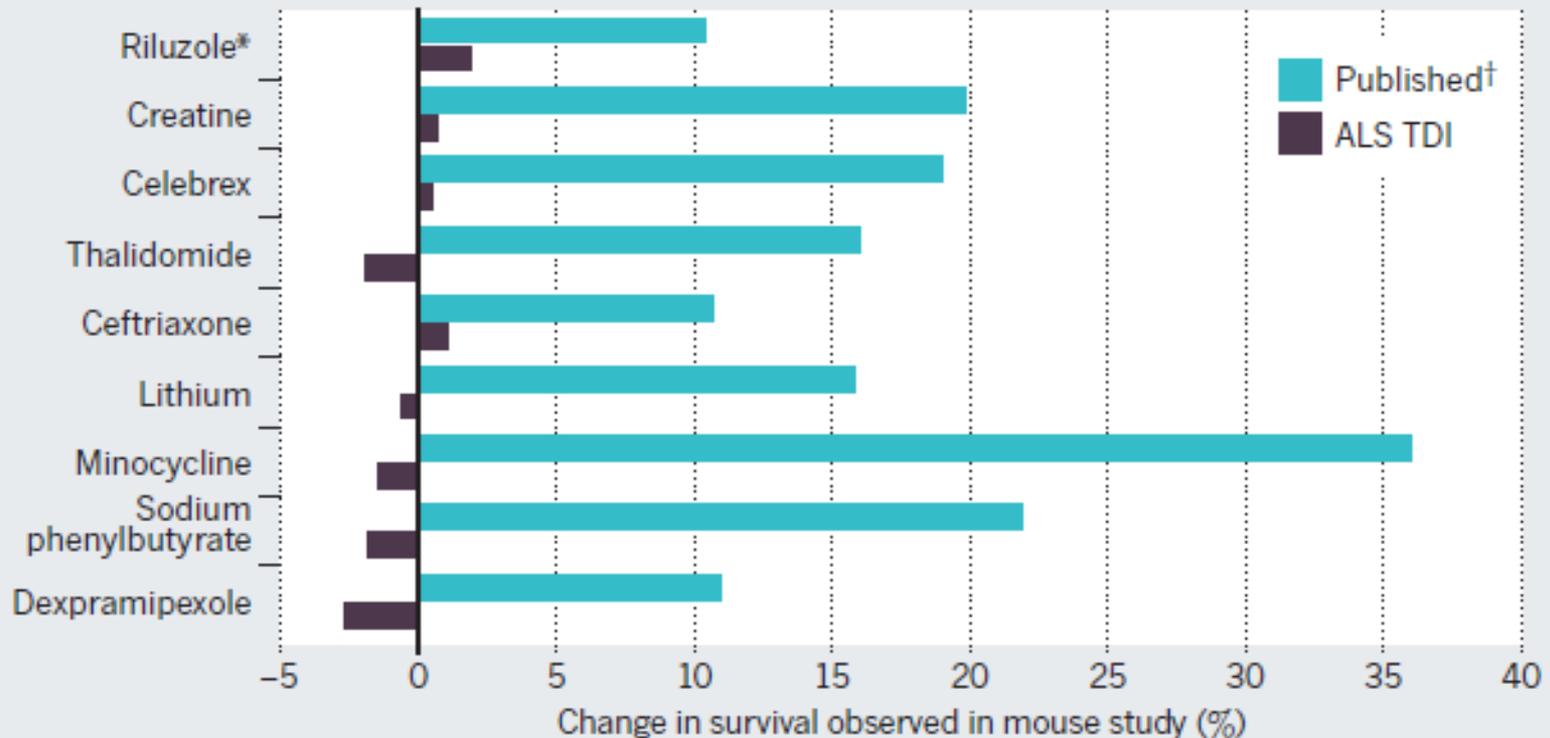
- **Allocation concealment:** Concealing the allocation sequence from those assigning animals to intervention groups, until the moment of assignment.
- **Bias:** Systematic distortion of the estimated intervention effect away from the "truth," caused by inadequacies in the design, conduct, or analysis of an experiment.
- **Blinding (masking):** Keeping the persons who perform the experiment, collect data, and assess outcome unaware of the treatment allocation.
- **Eligibility criteria:** Inclusion and exclusion criteria: the characteristics that define which animals are eligible to be enrolled in a study.
- **External validity:** The extent to which the results of an animal experiment provide a correct basis for generalisations to the human condition.
- **Intention-to-treat analysis:** Analysis of data of all animals included in the group to which they were assigned, regardless of whether they completed the intervention.
- **Internal validity:** The extent to which the design and conduct of the trial eliminate the possibility of bias.
- **Power:** The probability that a study will detect a statistically significant effect of a specified size.
- **Randomisation:** Randomly allocating the intervention under study across the comparison groups, to ensure that group assignment cannot be predicted.
- **Sample size:** The number of animals in the study

Make mouse studies work

by Steve Perrin 27 MARCH 2014 | VOL 507 | NATURE | 423

DUE DILIGENCE, OVERDUE

Results of rigorous animal tests by the Amyotrophic Lateral Sclerosis Therapy Development Institute (ALS TDI) are less promising than those published. All these compounds have disappointed in human testing.



*Although riluzole is the only drug currently approved by the US Food and Drug Administration for ALS, our work showed no survival benefit.
†References for published studies can be found in supplementary information at go.nature.com/hf4jf6.

Sloppy design falsifies data...

Other Hazards You Must Consider in Your Mouse Modeling to Navigate the MATRIX:

Co-morbidities (absent in models, present in patients)

Use of Antibiotics (infrequent in models, always in patients)

Tech ICU Capability (problematic in the mouse, standard in patients)

Gender (survival advantage in female mice/rats, unclear in patients)

Survival Follow-up (short/medium in models, long in patients)

Age (young animals but aged patients)

Long-term/Late sepsis studies (early sepsis in mice, late in patients)

Take Home Navigation Tips

Adapt models to patients (not vice versa)

Choose wisely (not because you have it)

Realize its flaws (but also strenghts)

Be critical (as mouse is just the beginning)

The Mouse Research Ship

A very bad habit



Abandon the bad habits!

Do Not Abandon the Mouse Ship -

Special thanks to



- M741, M742, M743, M744,
M745, M746, M747, M748,
M749, M750, M751, M752,
M753, M754, M755, M756,
M757, M758 M759, M760,
M761, M762, M763, M764,
M765, M786, M787, M788,
M789, M790, M791, M792,
M793, M794, M795, M796,
M797, M798, M799, M800,
M801, M802, M803, M804,
M805, M806, M807, M808,
M809, M810, M811, Ect...
-



Soheyl Bahrami
IC Dept. Head



other
LBI
employees



Christina



Kathrin



Suzy



Pierre



Andrey



Heinz Redl –
LBI Head



Dan Remick



Paul

Thank you!



Mohammad



Pia



Johannes



Tony



Anna