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

Mice: 53%
Rats: 19%

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
Mouse Physiology

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

Human Physiology

- 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

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

Seok et al. PNAS 2013
Genomic responses in mouse models greatly mimic human inflammatory diseases

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

<table>
<thead>
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<th>Human Burn</th>
<th>Human Trauma</th>
<th>Human Sepsis</th>
<th>Mouse Burn</th>
<th>Mouse Trauma</th>
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</tbody>
</table>
How Come?!!
Differences in the analytical approach:

- fold change as cutoff  
  (not critical)  
  (<1.2 mouse/<2.0 human vs. <1.2/1.2 (Seok et al.))  
  (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...
Can We Navigate the Matrix??

Mouse scientists
<table>
<thead>
<tr>
<th>No.</th>
<th>Translational phenomenon/response</th>
<th>Specific comments: mouse</th>
<th>Specific comments: human</th>
<th>Shock 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Antibodies to TNF given indiscriminately fail to reduce sepsis mortality</td>
<td>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)</td>
<td>Anti-TNF antibodies failed to be an effective treatment strategy in a general population of septic patients (117, 118)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Pretreatment with an anti-TNF strategy prevents early systemic inflammatory response syndrome</td>
<td>Passive immunization with the antisera to TNF-α in BALB/c mice protected them against the lethal hyperinflammation by <em>Escherichia coli</em> LPS (98)</td>
<td>Anti-TNF-α therapy was effective in human with house-borne relapsing fever when given as a pretreatment against Jarisch-Herxheimer reactions (119)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Low-dose steroid therapy is associated with decreased mortality in septic mice and humans</td>
<td>Demonstrated in C57BL/6 male mice subjected to CLP and treated with different corticosteroid concentrations; low but not high-dose steroids improved 21-d survival (120)</td>
<td>Early initiation of low-dose corticosteroid therapy decreased mortality in septic shock patients (121)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Regulation of chemotactic behavior of mouse and human neutrophils via purinergic signaling</td>
<td>Human and mouse neutrophils rely on same purinergic receptor subtypes (P2Y2, A2a, and A2a receptors) for autocrine signaling (122–124)</td>
<td>Demonstrated <em>in vitro</em> and <em>in vivo</em>; mouse are suitable to study chemotaxis in inflammation, trauma, and sepsis (122–124; NCT01 180361*1)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Human and mouse neutrophils rely on similar signaling mechanisms for their activation during bacteria-induced acute lung injury</td>
<td>Increased nuclear activation of NF-κB in pulmonary neutrophils of mice after <em>in vivo</em> administration with endotoxin (125, 126)</td>
<td>Increased nuclear accumulation of NF-κB in peripheral or pulmonary neutrophils of human volunteers after <em>in vitro</em> or <em>in vivo</em> stimulation with endotoxin (127) or in peripheral neutrophils of patients with sepsis (128)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Sepsis always in MARS: simultaneous systemic release of both proinflammatory and anti-inflammatory cytokines in sepsis</td>
<td>Demonstrated in ICR/CD-1 (outbred) female mice subjected to CLP sepsis (129, 130)</td>
<td>Demonstrated in septic shock patients (131) and patients with postoperative abdominal sepsis (132)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>IL-6 serves as a biomarker for sepsis mortality</td>
<td>IL-6 measured 6 h after the onset of CLP sepsis in BALB/c (133) and CD-1 mice (129) accurately predicts survival</td>
<td>Patients with high levels of IL-6 are at increased risk of dying of sepsis (134, 135)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Role of nicotinic receptors in inflammatory responses after endotoxemia is similar in mice and humans</td>
<td>Demonstrated in C57BL/6 mice and α7 nicotinic receptor–deficient mice; endotoxin–induced response was abrogated via activation of anti-inflammatory cholinergic pathway (vagus nerve stimulation) (136)</td>
<td>Human volunteers were administered endotoxin and GTS-21 (α7nACHR agonist) or placebo to study anti-inflammatory effects of cholinergic pathway (137; NCT00783068*)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Similar mode of pathogen-associated molecular patterns detection via Toll-like receptors (TLRs) in mice and humans</td>
<td>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)</td>
<td>Human volunteers administered with LPS demonstrated altered TLR-induced genes expression (140). TLR-signaling pathways are strongly modulated in septic patients (141)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Sepsis induces profound apoptosis of immune and gastrointestinal epithelial (GIE) cells</td>
<td>Demonstrated in CLP female ND4 mice (142) and <em>Pseudomonas aeruginosa</em> pneumonia-induced septic FVB/N mice (143); apoptosis in B and T lymphocytes and dendritic cells. GIE cell apoptosis in large and small intestine</td>
<td>Demonstrated in patients who died of sepsis and sepsis and MODS; data obtained by retrospective (rapid autopsy) and prospective (tissue resection) examination (144–146)</td>
<td></td>
</tr>
</tbody>
</table>
Critically re-checking our mouse models...

Navigation tip #1a
The Model/Disease Mismatch: Sepsis
Cytokines after LPS/E.coli – mice (& all species)

van den Boogaard M et al. 2010 Critical Care
Cytokines after low-dose LPS – human volunteers

van der Poll T et al. *Blood* 1997;89:3727-3734

(*) vehicle (n = 6)

(○) TNFR:Fc (6 mg/m2; n = 6)
Since none of the anti-TNFα trials worked, a closer look was taken...(finally!)

Different Temporal Dynamics & Concentrations of Circulating Cytokines

...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)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>pg/mL</th>
<th>day 1</th>
<th>day 2</th>
<th>day 3</th>
<th>day 4</th>
<th>day 5</th>
<th>day 6</th>
<th>day 7</th>
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<tbody>
<tr>
<td>TNF-α</td>
<td>Median</td>
<td>17.295</td>
<td>9.31</td>
<td>4.735</td>
<td>2.54</td>
<td>0.325</td>
<td>2.66</td>
<td>0</td>
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<tr>
<td></td>
<td>Range</td>
<td>402.9</td>
<td>354.5</td>
<td>283.38</td>
<td>111.49</td>
<td>68.59</td>
<td>58.63</td>
<td>55.4</td>
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</table>

Mouse sepsis models suck...

No, really?

The 1st Example from the Table

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Anti-TNF treatments are bogus...
No, really?

The 2nd Example from the Table

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**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


Department of Surgery, University Hospital Greifswald, Greifswald, Germany
Identification and Description of A Novel Murine Model For Polytrauma and Shock

Lori F. Gentile, MD; Dina C. Nacionales, MD; Alex G. Cuenca, MD, PhD; Michael Armbruster, BS; Ricardo F. Ungaro, BS; Amer S. Abouhamze, MHA; Cecelia Lopez, MS; Henry V. Baker, PhD; Frederick A. Moore, MD; Darwin N. Ang, MD, PhD, MPH; Philip A. Efron, MD

Robuster Inflammatory Response – Day 1 after Trauma

TH = hemorrhage & laparotomy
H+FFx = hemorrhage & femur fracture
PT = hemorrhage & thigh diss. femur fracture + cecotony

Crit Care Med. 2013
"The polytrauma model appears to be superior...(...)...in recapitulating the human response to severe injury."

More Pronounced Immunosuppression
Genomic LEU responses: Human vs. Mice

TH = hemorrhage & laparotomy  PT = hemorrhage & thigh dissection, 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 Tregs

3.4 Posttraumatic drop of CD11b

3.5 Activated posttraumatic phagocytosis
The Same Great „But(t)s“ in Sepsis Modeling!

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

Susanne Drechsler, Katrin M Weixelbaumer, Adelheid Weidinger, Pierre Raeven, Anna Khadem, Heinz Redl, Martijn van Griensven, Soheyl Bahrami, Daniel Remick, Andrey Kozlov and Marcin F Osuchowski
Organ Dysfunction in Acute Sepsis (days 1-5):
Circulating Parameters Prior to Death: high ALT/LDH does not equal death
Organ Dysfunction in Acute Sepsis (days 1-5): Circulating Parameters Prior to Death

Dotted line: normal range

Blissful & Self-satisfying Data Over-interpretation??

ALT

LDH

Urea

Glucose

CLP/Organ Injury; non-lethal (n=6)
New and Better (?) Sepsis Models Have Emerged

**PERITONITIS**
Intraperitoneal E.Coli injections
Infected Fibrin Clot (e.g. with E.coli)
Fecal Slurry (FS)
Colon Ascendens Stent Peritonitis (CASP)
Cecal Ligation and Puncture (CLP)

Monomicrobial, better control, reproducible
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 nowdays: cecal ligation and puncture (CLP)

From Rittirsch et al. Nat Protoc. 2009
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

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)
Viewed as a serious pitfall in CLP:

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


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/ovarian 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**

Relative abundance (%)

**Different!**

*Organism*
- Mouse
- Human

**Genera**
- Lactobacillus
- Turicibacter
- Dorea
- Anaeroplasm
- Paludibacter
- Alkaliphilus
- Bacteroides
- Clostridium
- Parabacteroides
- Alistipes
- Eubacterium
- Succinivibrio
- Coprococcus
- Roseburia
- Blautia
- Prevotella
- Ruminococcus
- Lachnospira
- Dialister
- Oscillospira
- Sutterella

Nguyen et al. Disease Models and Mech. 2015
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

Fungal infection
C. albicans

Pneumonia
P. Aeruginosa
S. Pneumoniae
Methicillin-resistant S. aureus (MRSA)

Trauma/
Hemorrhage

CLP
0h 24h 48h 72h

CLP
0h 24h 48h

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 (Systemic Inflammatory Response Syndrome)
- Blood
- Skin
- MARS (Mixed Anti-inflammatory Response Syndrome)
- Gastro-intestinal
- CNS
- CARS (Compensatory Anti-inflammatory Response Syndrome)
- Genito-urinary
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

4) Secondary 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!

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

sepsis – case 1

sepsis – case 2

sepsis – case 3

sepsis – case 4

sepsis – case 5
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>
<thead>
<tr>
<th>1st Author</th>
<th>Year</th>
<th>Patients (sample size)</th>
<th>Trial Acronym</th>
<th>Experimental agent</th>
<th>Effect on mortality</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ziegler</td>
<td>1982</td>
<td>Septic shock (212)</td>
<td></td>
<td>Human antiserum to mutant E. coli</td>
<td>Benefit*</td>
<td>2</td>
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<tr>
<td>Ziegler</td>
<td>1982</td>
<td>Septic shock and presumed or proven Gram-negative infection (543)</td>
<td></td>
<td>HA-1A, a human mAb that binds the lipid A domain of LPS</td>
<td>Benefit</td>
<td>67</td>
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<tr>
<td>McCloskey</td>
<td>1994</td>
<td>Septic shock and Gram-negative bacteraemia (621)</td>
<td>CHESIS I</td>
<td>HA-1A, a human mAb that binds the lipid A domain of LPS</td>
<td>No effect</td>
<td>5</td>
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<tr>
<td>Greenman</td>
<td>1991</td>
<td>Gram-negative sepsis (486)</td>
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<td>ES, a murine mAb that binds the lipid A domain of LPS</td>
<td>No effect</td>
<td>68</td>
</tr>
<tr>
<td>Bone</td>
<td>1995</td>
<td>Gram-negative sepsis with organ dysfunction (647)</td>
<td></td>
<td>ES, a murine mAb that binds the lipid A domain of LPS</td>
<td>No effect</td>
<td>69</td>
</tr>
</tbody>
</table>

2 used advanced (IL-6-based) treatment targeting...
IL-6 – based Risk-Stratification for Sepsis Treatment

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

Panacek et al. Crit Care Med. 2004
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

A perfect niche for mouse studies to aid in clinical trial design!
Targeted treatment in a Mouse Model of Acute Sepsis

CLP

6h 8h 24h 48h 96h 28day

Rapid ELISA
IL-6 as predictor

Stratification (IL-6 cut-off 26 ng/ml)

predicted to DIE
predicted to LIVE

Treatment:
Dexamethasone
(i.p., 2.5 mg/kg b.w.)

50% treated (T) 50% not-treated (NT)
50% Treated (T) 50% not-treated (NT)

Osuchowski et al. Critical Care Med. 2009
Targeted Treatment in Mouse Sepsis: looking for potential Benefits

dexamethasone treatment without stratification: days 1-7

dexameth. treatment based on stratification: days 1-7

Percent survival

Days after CLP

Osuchowski et al., Crit. Care Med. 2009
Targeted Treatment in Mouse Sepsis: Identifying potential Risks

Systemic inhibition and liver-specific over-expression of PAI-1 failed to improve survival in all-inclusive populations or homogenous cohorts of CLP mice. Similar dynamics of circulating plasminogen activator inhibitor (PAI) 1 in subjects surviving and dying of sepsis.

Example 23:

**Septic Shock Patients**
- **Survivors**
- **Non-survivors**

**CLP Mice**
- **Survivors**
- **Non-survivors**

Hours after hospitalization

Shapiro et al. Crit Care 2010
Raeven et al. PLoS ONE 2013
Possible *Detrimental Treatment Effect* in *Predicted-to-Live Cohort*

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


**C** retrospective

**D** prospective

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 GIGO concept:
The "hourglass" notion of research

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

-- Ron Marks, Toronto, August 16, 1994
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

Modified from T. Buchmann’s slide from today’s talk (ICCAI Session)
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.

Van der Worp...Malcolm R. MacLoeod. PLoS Medicine 2010
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.

*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 strengths)

Be critical (as mouse is just the beginning)
Do Not Abandon the Mouse 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...
Thank you!