Rabbit Models of Pseudomonas Pneumonia

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Disclosures

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Why rabbits?

![Tree diagram](image)

Table 5 | Comparison of the LPS dose required to induce physiological changes across species, relative to the LPS dose required in humans.

<table>
<thead>
<tr>
<th>Species</th>
<th>Threshold of physiological changes</th>
<th>Severe sepsis or lethal dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humans</td>
<td>1–5 ng/kg (i.v.)</td>
<td></td>
</tr>
<tr>
<td>Chimpanzees</td>
<td>1–5 ng/kg (i.v.)</td>
<td></td>
</tr>
<tr>
<td>Baboons and old world monkeys</td>
<td>0.1–6 mg/kg (i.v.)</td>
<td></td>
</tr>
<tr>
<td>Rabbits</td>
<td>2–4 ng/kg (i.v.)</td>
<td>10 μg/kg (i.v.) (100% of animals)</td>
</tr>
<tr>
<td>Swine</td>
<td></td>
<td>10 μg/kg (i.v.) (30% of animals)</td>
</tr>
<tr>
<td>Mice</td>
<td>0.5 mg/kg (i.p.)</td>
<td>8 mg/kg (i.p.)</td>
</tr>
<tr>
<td>Rats</td>
<td></td>
<td>15 mg/kg (i.p.) to 40 mg/kg (i.v.)</td>
</tr>
</tbody>
</table>

Mathison, JCI 1988; Vaure, Liu, FII 2014
Preclinical Rabbit Models For Drug Development

• Initial Lead Candidate Selection/Validation in Higher-Throughput Models
  • Rabbit Model of Acute Pneumonia (PNAS 2010, STM 2016, AAC 2016/17)
  • Rabbit Model of Severe Sepsis (PLoS ONE 2008)
  • Rabbit Model of Acute Bacterial Skin and Skin Structure Infection (AAC 2016)

• Further Efficacy Testing/MOA Study in Lower-Throughput Surgical Models
  • Rabbit Model of Aortic Valve Endocarditis (AAC 2008, AAC 2010)
  • Rabbit Model of Prosthetic Joint Infection (ISSSI 2016)
  • Rabbit Model of Ventilator-Associated Pneumonia and Septic Shock
Evaluation of MEDI3902, a Multifunctional Bispecific Antibody, in Rabbit Models of Acute Pneumonia and Ventilator-Associated Pneumonia and Septic Shock

Fab = anti-PcrV
scFv = anti-Psl
Hinge-Fc

Rabbit Study Endpoints: Reduction in Mortality and/or Prevention of Major Morbidity

DiGiandomenico et al. Sci Trans Med 2014
Rabbit Model of Acute Pneumonia

• Rabbits were randomized for prophylaxis (24hbi) or treatment (1hpi) with
  • 15, 5, 1, 0.3 mpk MEDI3902
  • 15 mpk c-IgG

• Rabbits were challenged with 9e7 CFU Pa6077 endobronchially

• Rabbits were monitored q2h for first 36 hpi, and then 3x daily thereafter
  • Humane criteria for euthanasia: respiratory rate > 90, cyanosis, and cough
Rabbit Acute Pneumonia Model: MEDI3902 vs. c-IgG prophylaxis

A

15 mg/kg MEDI3902 @ 24 hbi
5 mg/kg MEDI3902 @ 24 hbi
1 mg/kg MEDI3902 @ 24 hbi
0.3 mg/kg MEDI3902 @ 24 hbi
15 mg/kg c-IgG @ 24 hbi

\[ \text{percent survival} \]

\[ \text{hpi with PA6077 (8.8e7 CFU)} \]

\[ P < 0.001 \]

\[ P = 0.003 \]

B

\[ \text{LW/BW (x 10^3)} \]

\[ \text{mg/kg MEDI3902} \]

\[ \text{c-IgG} \]

\[ P = 0.002 \]

\[ P = 0.009 \]

\[ P = 0.022 \]

\[ P = 0.12 \]
Rabbit Acute Pneumonia Model: MEDI3902 vs. c-IgG treatment

A

- 15 mg/kg MEDI3902 @ 1 hpi
- 5 mg/kg MEDI3902 @ 1 hpi
- 1 mg/kg MEDI3902 @ 1 hpi
- 15 mg/kg c-IgG @ 1 hpi

Percent survival

0 12 24 36 48 60 72 84 96

hpi with PA6077 (9.0e7 CFU)

P = 0.001
P < 0.001

B

LW/BW (x 10^3)

0 4 8 12 16 20 24

15 5 1 15

mg/kg MEDI3902 c-IgG

P = 0.018
P = 0.009
P = 0.010
Rabbit Acute Pneumonia Model: MEDI3902 vs. c-IgG

**Prophylaxis**

- **MEDI3902**
  - Doses: 15, 5, 1, 0.3, 15 mg/kg
  - Log_{10} CFU/lung

- **c-IgG**
  - Doses: 15, 5, 1, 0.3, 15 mg/kg
  - Log_{10} CFU/lung

- **P-values**:
  - P < 0.001
  - P = 0.11
  - P = 0.062
  - P = 0.37

**Treatment**

- **MEDI3902**
  - Doses: 15, 5, 1, 0.3, 15 mg/kg
  - Log_{10} CFU/lung

- **c-IgG**
  - Doses: 15, 5, 1, 0.3, 15 mg/kg
  - Log_{10} CFU/lung

- **P-values**:
  - P = 0.014
  - P = 0.018
  - P = 1.00
Rabbit Model of Ventilator-Associated Pneumonia and Septic Shock

- Rabbits were randomized for prophylaxis at 24 h before challenge
  - 15 mpk R347 (4 rabbits)
  - 15 mpk MEDI3902 (4 rabbits)

- Rabbits were mechanically ventilated with lung-protective low-tidal volume (6-7 ml/kg) for 2 h, then challenged with 2.5e7 CFU Pa6077

- Rabbits were monitored continuously for 36 hpi

- Blood samples were obtained at 0, 2, 4, ..., 24, 28, 32, 36 hpi for comprehensive blood analysis
  - Veterinary Hematology Analyzer (Heska HT5)
    - 5-part WBC differential, RBC, and PLT
  - Blood Gas & Electrolyte Analyzer (Heska EPOC)
    - Blood gas: pO<sub>2</sub> and pCO<sub>2</sub>
    - Acid-base: pH, bicarbonate, TCO<sub>2</sub>, base excess, anion gap
    - Electrolytes: sodium, potassium, chloride
    - creatinine, lactate, glucose
  - Blood Chemistry Analyzer (Abaxis VetScan VS2)
    - Renal: ALB, BUN, Ca, CRE, GLU
    - Liver: ALB, ALP, ALT, BUN, TBIL
THE BASIC SET-UP

Anesthesia Ventilator

- **FiO2**: 35%
- **Isoflurane**: 1.5%
- **PIP**: 15
- **PEEP**: 6
- **I:E**: 1:2
- **Tidal Volume**: 6-7 ml/kg
- **EtCO₂**: 35-45 mmHg

**HR/ECG**

**temp**

**SpO₂**

**ABP**

**0.5 ml blood sampling q2h**
- *CBC with differential*
- *blood gas & electrolyte & chemistry*

**Water Heat Pad**

**IV pump**

5% dextrose

**carotid artery**

**Patient Monitor**

**5% dextrose**

**blood gas & electrolyte & chemistry**
Rabbit VAP/SS Model: MEDI3902 vs. c-IgG prophylaxis

- 15 mpk MEDI3902 @ 24hpi (4 rabbits)
- 15 mpk c-IgG @ 24hpi (4 rabbits)

LW/BW (x 10^3) vs. hours post infection

P = 0.029

Image showing comparison of c-IgG and MEDI3902 treatments.
Rabbit VAP/SS Model: MEDI3902 vs. c-IgG prophylaxis – BACTERIAL COUNTS

- Blood bacterial counts over time post infection
- Comparison of bacterial counts in organs (lungs, spleen, kidneys, liver)
- Log CFUs/mL blood and log CFUs/g of organs
- P-values: P=0.015, P=0.42, P=0.23, P=0.97
What causes death in the c-IgG (1-4) rabbits?

Although MEDI3902 (4) rabbit survived, what are the effects of persistent bacteremia on the host?
Rabbit VAP/SS Model: MEDI3902 vs. c-IgG prophylaxis – WBCs & platelets

WBCs (x 10^3/μl) over hours post infection

Platelets (x 10^3/μl) over hours post infection
Rabbit VAP/SS Model: MEDI3902 vs. c-IgG prophylaxis – neutrophils & monocytes

**Neutrophils (x 10^3/μl)**

- **c-IgG (1)**
- **c-IgG (2)**
- **c-IgG (3)**
- **c-IgG (4)**
- **MEDI3902 (1-3)**
- **MEDI3902 (4)**

**Monocytes (x 10^3/μl)**

- **c-IgG (1)**
- **c-IgG (2)**
- **c-IgG (3)**
- **c-IgG (4)**
- **MEDI3902 (1-3)**
- **MEDI3902 (4)**

Hours post infection:

- 0
- 2
- 4
- 6
- 8
- 10
- 12
- 14
- 16
- 18
- 20
- 22
- 24
- 28
- 32
- 36
Rabbit VAP/SS Model: MEDI3902 vs. c-IgG prophylaxis – $pO_2/FiO_2$ and $pCO_2$

*Graphs showing changes in $pO_2/FiO_2$ and $pCO_2$ over hours post infection for different prophylaxis groups.*
Rabbit VAP/SS Model: MEDI3902 vs. c-IgG prophylaxis – base excess and lactate
Rabbit VAP/SS Model: MEDI3902 vs. c-IgG prophylaxis – K+ and creatinine
Rabbit VAP/SS Model: MEDI3902 vs. c-IgG prophylaxis – ALT & AMY

Graphs showing the alanine aminotransferase (ALT) and amylase (AMY) levels over hours post infection for different groups:
- c-IgG (1)
- c-IgG (2)
- c-IgG (3)
- c-IgG (4)
- MEDI3902 (1-3)
- MEDI3902 (4)
## SUMMARY

<table>
<thead>
<tr>
<th></th>
<th>c-IgG (1)</th>
<th>c-IgG (2)</th>
<th>c-IgG (3)</th>
<th>c-IgG (4)</th>
<th>MEDI3902(4)</th>
<th>MEDI3902(1-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>blood counts</strong></td>
<td>↓ WBC</td>
<td>↓ WBC</td>
<td>↓ WBC</td>
<td>↓ WBC</td>
<td>↑ EOS</td>
<td>↑ BAS</td>
</tr>
<tr>
<td></td>
<td>↓ NEU</td>
<td>↓ NEU</td>
<td>↓ NEU</td>
<td>↓ NEU</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>pO₂/FiO₂</strong></td>
<td>89</td>
<td>321</td>
<td>55</td>
<td>111</td>
<td>491</td>
<td>371</td>
</tr>
<tr>
<td><strong>pCO₂</strong></td>
<td>100</td>
<td>12</td>
<td>94</td>
<td>93</td>
<td>37</td>
<td>44</td>
</tr>
<tr>
<td><strong>BE</strong></td>
<td>-12.6</td>
<td>-14.9</td>
<td>-2.4</td>
<td>-19</td>
<td>-3.3</td>
<td>-1.9</td>
</tr>
<tr>
<td><strong>lactate</strong></td>
<td>16.8</td>
<td>19.4</td>
<td>12.9</td>
<td>19.2</td>
<td>2.1</td>
<td>0.8</td>
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<tr>
<td><strong>K⁺</strong></td>
<td>8.6</td>
<td>9.6</td>
<td>5.3</td>
<td>8.0</td>
<td>3.7</td>
<td>3.7</td>
</tr>
<tr>
<td><strong>creatinine</strong></td>
<td>3.9</td>
<td>5.9</td>
<td>1.5</td>
<td>3.0</td>
<td>1.1</td>
<td>2.3</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>--</td>
<td>↑ 3x</td>
<td>--</td>
<td>--</td>
<td>↑ 6x</td>
<td>--</td>
</tr>
<tr>
<td><strong>AMY</strong></td>
<td>--</td>
<td>↑ 2.5x</td>
<td>--</td>
<td>--</td>
<td>↑ 4x</td>
<td>--</td>
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