Development and Challenges of a Rabbit Model System of Pneumonia

FDA Public Workshop
Advancing Animal Models for Antibacterial Drug Development

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Challenges of MDR Gram-Negative Pneumonias in Our Critically Ill Patients

- Therapeutically ineffective or toxic antimicrobial agents
- Immune Impairment
- Delayed Diagnosis and Detection

Meeting the Challenges through Bench to Bedside Translational Research
Novel Antimicrobial Compounds: PK/PD and Safety

Augmentation of Host Defenses

Early Diagnosis, Biomarkers, and Therapeutic Monitoring

In Vitro Systems

Laboratory Animal Models

Phase I-II Clinical Trials

Phase III Clinical Trials
Novel Antimicrobial Compounds: PK/PD and Safety

• We then investigate lead candidate compounds in one or more rabbit models of MDR GNR pneumonia.

• Central silastic venous catheter permits atraumatic venous access
• Ara-C induction of profound and persistent neutropenia
• Further immunomodulation with CsA and methylprednisolone, where applicable
• Intensive supportive care with at least twice daily monitoring, and 24/7 on-call schedule
Characteristics the Rabbit Models of MDR GNR Pneumonia

- Organisms studied
  - *Pseudomonas aeruginosa*
    - Pan-susceptible
    - OPRD porin loss
    - Efflux pump expression
    - AmpC hyperexpression
  - *Acinetobacter baumannii* (MDR)
  - *Klebsiella pneumoniae*
    - *Klebsiella pneumoniae* (KPC-Kp)
    - *Klebsiella pneumoniae* (NDM-1)
  - *Stenotrophomonas maltophilia*
- Direct endotracheal inoculation of a carefully quantified inoculation under general anesthesia
- Colonization of the tracheobronchial tree
- As immune suppression progresses, colonization progresses to segmental and lobar pneumonia
- Transition within 24 hours: trigger to treat justification
- Duration of study 7-14 days
Rationale and Benefits for Selection of Rabbit Models of Multidrug Resistant Gram-Negative Pneumonia

• In comparison to conventional murine models of pneumonia, where duration is measured in 24-48h
• The rabbit models reflect the human pattern of infection more accurately over a 7-14 day period
• Each animal serves as a surrogate model for patient care: closer to bedside management
• Rabbit lung is anatomically similar to that of humans

• Vascular catheter permits serial sampling for blood cultures as well as antigenic, molecular, and proteomic biomarkers
• Reflect treatment durations of 5, 7, 10, or 14 days
• Allow assessment of emergence of antimicrobial resistance developing over the duration of therapy
• Accurately reflects degree and duration of immunosuppression of high risk patients
Limitations and Challenges for Selection of Rabbit Models of Multidrug Resistant Gram-Negative Pneumonia

• Labor intensity
  – Necessary for support and monitoring of immunocompromised large animals analogous to the intensity for immune-impaired patients

• Limited number of strains
  – Limited number but well characterized representative are chosen to address the hypothesis being tested

• High standards of Laboratory Animal Care and Welfare (IACUC/AAALAC/USDA)
  – Better laboratory animal welfare = better science

• Numbers of animals
  – Rabbit models do not replace but rather complement murine models

• Cost
  – De-risks clinical trial and strengthens predictability of outcome
  – Ultimately proving to be cost-effective in drug development and clinical trial design
Illustration of Study Designs

Ceftolozane-Tazobactam Administered in Humanized Dosing for the Treatment of Experimental *Pseudomonas aeruginosa* Pneumonia in Persistently Neutropenic Rabbits: Impact on Strains with Genetically Defined Mechanisms of Resistance

Lung Pathology of Persistently Neutropenic Rabbits with Experimental *Pseudomonas aeruginosa* Pneumonia

**Hematoxylin and eosin stain**
Severe, multifocal to coalescing, subacute, necrotizing pneumonia with thrombosis, pleuritis, marked edema and hemorrhage

**Tissue Gram stain**
Intralesional Gram-negative bacilli with large numbers of intra- and extracellular Gram-negative bacilli

Petraitis V *et al*. AAC. 2019
Strains of *Pseudomonas aeruginosa* with Genetically Defined Mechanisms of Resistance in Persistently Neutropenic Rabbits with Experimental Pneumonia

<table>
<thead>
<tr>
<th>Isolate, mechanism of resistance</th>
<th>MIC (µg/ml)</th>
<th>Ceftolozane-tazobactam</th>
<th>Ceftazidime</th>
<th>Piperacillin-tazobactam</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAE 3656, pan-susceptible</td>
<td>0.5</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>PAE 3616, OPRD porin loss</td>
<td>2</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>PAE 3647, efflux pump expression</td>
<td>4</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td></td>
</tr>
<tr>
<td>PAE 3691, AmpC hyperexpression</td>
<td>2</td>
<td>32</td>
<td>&gt;64</td>
<td></td>
</tr>
</tbody>
</table>

Source: JMI Laboratories, North Liberty, IA
[https://www.jmilabs.com/](https://www.jmilabs.com/)
Weill Cornell Medicine Infectious Diseases Translational Research Laboratory
Petraitis V *et al.* AAC.2019
Plasma Pharmacokinetics Ceftolozane-Tazobactam in the Treatment of Experimental Pseudomonas aeruginosa Pneumonia in Persistently Neutropenic Rabbits

Plasma pharmacokinetics of ceftolozane from 26, 53, and 106 mg/kg

Dose proportionality of ceftolozane from 26 to 106 mg/kg

Petraitis V et al. AAC.2019
Plasma Pharmacokinetics Ceftolozane-Tazobactam in the Treatment of Experimental *Pseudomonas aeruginosa* Pneumonia in Persistently Neutropenic Rabbits

**TABLE 1** Plasma total drug pharmacokinetic parameters of ceftolozane-tazobactam on day 6 after intravenous administration of ceftolozane-tazobactam at 40, 80 and 160 mg/kg every 8 h to healthy New Zealand White rabbits

<table>
<thead>
<tr>
<th>Drug and dosage (mg/kg)</th>
<th>AUC_{0-8} (µg · h/ml)</th>
<th>C_{max} (µg/ml)</th>
<th>CL (ml/h/kg)</th>
<th>V (ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftolozane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26^{b}</td>
<td>155.87 ± 15.02</td>
<td>137.56 ± 20.02</td>
<td>162.23 ± 17.22</td>
<td>173.64 ± 17.53</td>
</tr>
<tr>
<td>53</td>
<td>375.83 ± 23.84</td>
<td>325.96 ± 27.62</td>
<td>142.13 ± 9.01</td>
<td>161.18 ± 6.29</td>
</tr>
<tr>
<td>106</td>
<td>494.06 ± 14.00</td>
<td>356.74 ± 27.04</td>
<td>212.29 ± 5.51</td>
<td>216.58 ± 10.47</td>
</tr>
<tr>
<td>Tazobactam</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13^{b}</td>
<td>15.50 ± 4.73</td>
<td>20.77 ± 8.16</td>
<td>1,199.18 ± 487.82</td>
<td>248.35 ± 65.07</td>
</tr>
<tr>
<td>26</td>
<td>30.09 ± 4.82</td>
<td>47.84 ± 9.28</td>
<td>929.57 ± 127.33</td>
<td>154.14 ± 16.65</td>
</tr>
<tr>
<td>53</td>
<td>25.18 ± 6.81</td>
<td>33.67 ± 9.62</td>
<td>2613.92 ± 747.69</td>
<td>638.25 ± 167.30</td>
</tr>
</tbody>
</table>

^{a}CL, clearance; V, volume of distribution. Values are means ± SEMs.

^{b}Ceftolozane-tazobactam at 40 mg/kg = 26.3 mg/kg ceftolozane and 13.6 mg/kg tazobactam.
Pulmonary Bacterial Burden (log CFU/g) in Lung Tissue in Persistently Neutropenic Rabbits with Experimental *Pseudomonas aeruginosa* Pneumonia: Impact on Strains with Genetically Defined Mechanisms of Resistance

OPRDPL ($P < 0.01$) and ACHE ($P < 0.05$ for CAZ; $P < 0.01$ for C/T) strains in comparison to that of UC

Petraitis V *et al.* AAC.2019
Pulmonary Bacterial Burden (log CFU/ml) in BAL Fluid of Persistently Neutropenic Rabbits with Experimental *Pseudomonas aeruginosa* Pneumonia: Impact on Strains with Genetically Defined Mechanisms of Resistance

*, $P < 0.05$; †, $P < 0.01$ (decreased residual bacterial burden in treatment groups in comparison to that in untreated controls). All values are expressed as mean ± SEM.

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Lung Weights (markers of organism-mediated pulmonary injury) of Persistently Neutropenic Rabbits with Experimental *Pseudomonas aeruginosa* Pneumonia: Impact on Strains with Genetically Defined Mechanisms of Resistance

*, $P < 0.05$; †, $P < 0.01$ (decreased lung weights in treatment groups in comparison to that of untreated controls). All values are expressed as mean ± SEM. The normal lung weight for the rabbits used in this study is approximately 15 g.

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Cumulative Survival Probability of Persistently Neutropenic Rabbits with Experimental *Pseudomonas aeruginosa* Pneumonia: Impact on Strains with Genetically Defined Mechanisms of Resistance

*, P < 0.05; †, P < 0.01; ¶, P < 0.01 (prolonged survival in treatment groups). All values are expressed as percentage of cumulative survival probability.

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Biomarkers in the Persistently Neutropenic Rabbit Model with Experimental *Pseudomonas aeruginosa* Pneumonia

Protein Expression Profiles Distinguish Between Experimental Invasive Pulmonary Aspergillosis and *Pseudomonas aeruginosa* Pneumonia


The time course of the SELDI-TOF relative intensity compared to \( n \) shown for the putative 28 kDa molecule, APOA1, 11.7 kDa molecule TTHY as well as RPA measurement of C-reactive protein...
Pharmacokinetics and Efficacy of Ceftazidime-Avibactam in the Treatment of Experimental Pneumonia Caused by *Klebsiella pneumoniae* Carbapenemase-Producing *Klebsiella pneumoniae* (KPC-Kp) in Persistently Neutropenic Rabbits

Plasma Pharmacokinetics of Ceftazidime-Avibactam in Healthy NZW Rabbits

Plasma pharmacokinetics of ceftazidime/avibactam at 60/15, 90/22.5, and 120/30 mg/kg after administration multiple doses q8h over 5 days to healthy New Zealand White rabbits

Ceftazidime
Dose (mg/kg)

\[ \text{he mean} \pm \text{SEM.} \]

\[ V_{ss} \] volume of distribution at a steady state

Petraitiene R et al. AAC. 2020.
Ceftazidime (CAZ) and avibactam (AVB) on day 5 after multi-dose IV infusions of ceftazidime-avibactam (CZA) at 60, 90, and 120 mg/kg q8h to five NZW rabbits. Dose proportionality following multi-dose infusion of ceftazidime-avibactam to NZW rabbits across dosages of 60 and 120 mg/kg IV.

Petraitiene R et al. AAC. 2020.
Ceftazidime-Avibactam in the Treatment of Experimental Pneumonia Caused by *Klebsiella pneumoniae* Carbapenemase-Producing *Klebsiella pneumoniae* (KPC-Kp) in Persistently Neutropenic Rabbits

**7-day treatment course** with ceftazidime-avibactam or polymyxin B demonstrating decreased of residual bacterial burden, lung weights, pulmonary hemorrhage scores, and BAL bacterial burden in treatment groups in comparison to those of untreated controls. $\S$, $P < 0.01$.  

**14-day treatment course** with ceftazidime-avibactam or polymyxin B demonstrating decreased of residual bacterial burden, lung weights, pulmonary hemorrhage scores, and BAL bacterial burden in treatment groups in comparison to those of untreated controls. $\S$, $P < 0.01$. 
Ceftazidime-Avibactam in the Treatment of Experimental Pneumonia Caused by *Klebsiella pneumoniae* Carbapenemase-Producing *Klebsiella pneumoniae* (KPC-Kp) in Persistently Neutropenic Rabbits

Survival response of KPC-Kp pneumonia demonstrated significantly prolonged survival in rabbits treated with CZA and PMB in comparison to that of UC *, $P \leq 0.05$. 

*Petraitiene R et al. AAC. 2020.*
Survival response of KPC-Kp pneumonia demonstrated significantly prolonged survival in rabbits treated with CZA and PMB in comparison to that of UC *, $P \leq 0.05$.

Significantly prolonged survival was achieved in CZA group in comparison to that of PMB-treated rabbits ¶, $P < 0.05$.

Petraitiene R et al. AAC. 2020.
Summary

• Reviewed the development, challenges, advantages, and limitations of the rabbit models of MDR GNR pneumonia

• Illustrated these concepts with two studies in experimental MDR *Pseudomonas aeruginosa* and KPC pneumonia.

• Use of rabbit models as powerful systems for studying new antimicrobial agents for meeting the challenge of MDR GNRs to our patients and to the country’s public health.
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