Development of a Murine Model of *Pseudomonas aeruginosa* Lung Infection

Presented at, Advancing Animal Models for Antibacterial Drug Development Workshop

U.S. Food and Drug Administration, White Oak MD

March 5, 2020

Lynn Miesel, Ph.D.
Pharmacology Discovery Services, Ltd.
25, Wugong 6th Road, Wugu District,
New Taipei City, Taiwan 24891

Eurofins Panlabs, Inc.
6 Research Park Drive
St. Charles, MO 63304

Lynn Miesel@eurofins.com
www.pharmacologydiscoveryservices.com
NIAID Mission

- Mission: streamline the discovery of therapeutics for \textit{P. aeruginosa}
- \textit{P. aeruginosa} is a leading pathogen that causes VABP and HABP
- Carbapenem resistant \textit{P. aeruginosa} (CRPA) infections have few treatment options
- Related projects
  - Conduct testing services to evaluate therapeutic candidates
  - Developed mouse thigh and lung infection models with MDR isolates
  - Generated PK/PD tutorials with example studies of standard drugs
  - Protocols and example data are available to the drug discovery community
Model features

- Correlate infection mortality with pathogen burden, dissemination and tissue pathology
- Measured endpoints: mortality onset, microbial burden, tissue pathology
- An extended infection period (target ≥ 48 h)
- Compare intranasal (IN) and intratracheal (IT) infection routes
- Conduct with MDR isolates, CDC & FDA AR-BANK

Approach

- Host: persistently neutropenic mice
- Development steps
  - Inoculum optimization
  - Characterize the natural history of infection
  - Benchmark with approved antibiotics
RESEARCH ARTICLE

Development and evaluation of murine lung-specific disease models for Pseudomonas aeruginosa applicable to therapeutic testing

Matthew B. Lawrenz1,2,*, Ashley E. Biller1, Daniel E. Cramer1, Jennifer L. Kraenzle1, Julie B. Sotsky1, Carol D. Vanover1, Deborah R. Yoder-Himes3, Angela Pollard4 and Jonathan M. Warawa1,2,*

FEMS Pathogens and Disease, 73, 2015 ftv025
### P. aeruginosa isolates

**CDC & FDA AR Isolate Bank**

<table>
<thead>
<tr>
<th>AR-BANK#0246</th>
<th>AR-BANK#0266</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CRPA, NDM-1</strong></td>
<td><strong>CRPA</strong></td>
</tr>
</tbody>
</table>

#### Antibiotic susceptibility

<table>
<thead>
<tr>
<th></th>
<th>AR-BANK#0246</th>
<th>AR-BANK#0266</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Notable AMR gene</strong>*</td>
<td>NDM-1</td>
<td>None Detected</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Ceftazidime/avibactam</td>
<td>CAZ-R, AVYCAZ-R</td>
<td>CAZ-R, AVYCAZ-S</td>
</tr>
<tr>
<td>Amikacin</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin, Levofloxacin</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Colistin</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

#### Virulence determinants

- Produce pyocyanin and alginate, motile

*Genomic information from CDC & FDA AR-Bank*
### Inoculum Optimization

In this study, the effect of different inoculum sizes on infection was investigated. The inoculum sizes were titrated to optimize the infection process.

#### Materials and Methods

- **Mice**: Male and Female CD-1 6 weeks
- **Neutropenia**: CP 150 mg/kg D-4, 100 mg/kg D-1
- **Inoculum**: Titration
- **Observations**: body temp 6 h intervals, and body weight daily
- **Humane endpoints**: 4°C body temp Δ, 20% weight loss, moribund, and diarrhea

#### Temperature Transponders

Temperature transponders were used for temperature reads. IPTT300 Bio Medic Data Systems

---

Mouse figure: van Erp EA et al. Viruses 2019, 11(6), 508
Inoculum Optimization
AR-BANK#0246 NDM-1

- Iterative titration studies were conducted for each strain
- Body temperature telemetry was highly effective for humane terminal endpoints
- Persistent neutropenia was added to subsequent studies to prevent immune recovery
- Male mice only were selected for subsequent studies to minimize variables
Natural History of Infection

Mice | Male CD-1 6 weeks
--- | ---
Persistent neutropenia | CP 150 mg/kg D-4, 100 mg/kg D-1, D+1, D+3
Target inoculum | #0246 $1 \times 10^3$; #0266 $3 \times 10^3$
Observations | body temp 6 h, body weight daily
Humane endpoints | 4°C body temp Δ, 20% weight loss, moribund, and diarrhea
Scheduled sacrifice | 4 h, 28 h, or 5D post infection
Measurements | mortality onset (h)
bacterial CFU/g lung and spleen
lung gross pathology and histopathology

Mouse diagram: van Erp EA *et al.* Viruses 2019, 11(6), 508
Natural History of Infection
AR-BANK#0246 NDM-1

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum count</td>
<td>$8.8 \times 10^2$</td>
<td>$1.6 \times 10^3$</td>
</tr>
<tr>
<td>(target $1 \times 10^3$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean survival time (h)</td>
<td>IN $46 \pm 5$</td>
<td>IN $40 \pm 6$</td>
</tr>
<tr>
<td></td>
<td>IT $50 \pm 6$</td>
<td>IT $39 \pm 6$</td>
</tr>
</tbody>
</table>
Natural History of Infection
AR-BANK#0246 NDM-1

Lung CFU

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>IN</th>
<th>IT</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Spleen CFU

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>IN</th>
<th>IT</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Histopathology

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>IN</th>
<th>IT</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Gross Pathology

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>IN</th>
<th>IT</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- mice sacrificed at scheduled time points; ○ mice sacrificed at mortality onset

Log (CFU/g Lung) vs Log (CFU/g Spleen)
Antibiotic Efficacy
AR-BANK#0246 (NDM-1), Colistin (CST)

Mice: Male CD-1 6 weeks
Persistent neutropenia CP 150 mg/kg D-4, 100 mg/kg D-1, D+1, D+3
Target Inoculum 1 × 10³ CFU
CST dosing Start 12 h after infection, 30, 20 and 10 mg/kg BID q12h, one day
Humane endpoints 4°C body temp Δ, 20% weight loss, moribund, diarrhea
Scheduled sacrifice times 12 h, 36 h, or 5 D post infection
Measurements time to mortality onset bacterial counts in lung and spleen lung gross pathology and histopathology

Mouse diagram: van Erp EA et al. Viruses 2019, 11(6), 508
Antibiotic Efficacy
AR-BANK#0246 (NDM-1), Colistin (CST)

Colistin dose selection

- Colistin tolerability in persistently neutropenic mice, 48 h 30 mg/kg q12h
- Colistin efficacy, thigh infection model

Colistin q12h BID vs. *P. aeruginosa* AR#0246
Thigh infection model

Efficacious dose range: 10 - 40 mg/kg q12h BID

HHSN27200005 NIAID PCMID A25
Pharmacology Discovery Services
J. Bulitta, University of Florida team
Antibiotic Efficacy
AR-BANK#0246 (NDM-1), Colistin (CST)

Time prior to dose

IN
Infection

Dose 30 mg/kg q12h

0

1 2

24 h

baseline endpoint termination endpoint

24 h treatment period

<table>
<thead>
<tr>
<th>Time of dose 1 ← 12h → Time of dose 2 ← 12h → Termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h</td>
</tr>
<tr>
<td>6 h</td>
</tr>
<tr>
<td>12 h</td>
</tr>
<tr>
<td>16 h</td>
</tr>
</tbody>
</table>
Antibiotic Efficacy
AR-BANK#0246 (NDM-1), Colistin (CST)

Time prior to dose
Colistin 30 mg/kg q12h BID

The 12 h time to dose was selected:
1. ~10^5 CFU/g at baseline
2. Colistin caused a significant reduction in counts relative to baseline

* p < 0.05 t-test
Antibiotic Efficacy
AR-BANK#0246 (NDM-1), Colistin (CST)

Colistin dosage: BID, q12h, 24 h

IN Inoculation

IT Inoculation

Mean Survival Time

Inoculum count: $1.5 \times 10^3$
Kaplan Meier: Fisher’s exact test
Mean survival time: On-way ANOVA
* $p < 0.05$
Antibiotic Efficacy
AR-BANK#0246 (NDM-1), Colistin (CST)

Colistin dosage: BID, q12h, 24 h

**Lung**

**Spleen**

* $p < 0.05$ t-test

LOD
<table>
<thead>
<tr>
<th></th>
<th>IN Inoculation</th>
<th>IT Inoculation</th>
<th>IN vs IT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (percent)</td>
<td>3.9%</td>
<td>12%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Number of animals</td>
<td>77</td>
<td>41</td>
<td>N/A</td>
</tr>
<tr>
<td>Survival time (mean, SEM)</td>
<td>42 ± 3</td>
<td>54 ± 5</td>
<td>*</td>
</tr>
<tr>
<td>Lung CFU (Log mean, SEM)</td>
<td>7.7 ± 0.2</td>
<td>7.3 ± 0.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Spleen CFU (Log, mean, SEM)</td>
<td>5.5 ± 0.2</td>
<td>5.8 ± 0.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Gross pathology score (SD)</td>
<td>2.5 ± 1.3</td>
<td>3.2 ± 1.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Histopathology score (SD)</td>
<td>1.0 ± 1.1</td>
<td>1.2 ± 1.3</td>
<td>*</td>
</tr>
<tr>
<td>Recommended group size</td>
<td></td>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>

Pooled data from four studies
* $p < 0.05$

Survival comparison: Fisher’s exact test
Pairwise comparisons: t-test
Summary

• Body temperature monitoring facilitates correlation of disease severity with pathogen burden, dissemination, and tissue pathology
• The approach facilitates selection of time points for dosing and sacrifice
• The isolates were highly virulent in neutropenic mice
• Approved antibiotics (colistin and amikacin) were efficacious
• IN and IT infection yielded similar study results
• Limitation, IT infection yielded more variability: survival time and % mortality
Next Steps and Future Directions

- PK/PD to correlate drug exposure with multiple treatment outcomes
- Establish lung infection models with other pathogens
- Establish lung infection models with immune competent mice
Other Resources, NIAID and PDS

- Drug discovery testing services, 75N93019F00131 (A-32)
- Protocols for murine thigh infection models with MDR isolates, HHSN272000003 (A-25)
- PK/PD tutorial PK/PD tutorials with example studies of standard drugs, HHSN272000003 (A-25)
- Protocols and reports are available to the drug discovery community
Acknowledgments

• We thank the CDC & FDA AR Isolate Bank for supplying strains
• This project was funded in whole with Federal funds from the HHS/NIH/NIAID, under Contracts No. HHSN272201700020I / Task Order HHSN27200003 task order A10
## Acknowledgments

<table>
<thead>
<tr>
<th>Name</th>
<th>Organization</th>
<th>Name</th>
<th>Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kun-Yuan Lin</td>
<td>PDST</td>
<td>Chin-Wei Chiang</td>
<td>PDST</td>
</tr>
<tr>
<td>Lucy Chia</td>
<td>PDST</td>
<td>David Lo</td>
<td>PDST</td>
</tr>
<tr>
<td>Pony Lee</td>
<td>PDST</td>
<td>Hsiao-Lung Chen</td>
<td>PDST</td>
</tr>
<tr>
<td>Tim Yeh</td>
<td>PDST</td>
<td>Jiunn-Wang Liao</td>
<td>NCHU</td>
</tr>
<tr>
<td>Nien-Chen Lee</td>
<td>PDST</td>
<td>Ray Slay</td>
<td>NIAID</td>
</tr>
<tr>
<td>Wei-Ting Liao</td>
<td>PDST</td>
<td>Erin Zeituni</td>
<td>NIAID</td>
</tr>
<tr>
<td>Gin-Feng Lai</td>
<td>PDST</td>
<td>Anna Chiang</td>
<td>NIAID</td>
</tr>
<tr>
<td>Jui-Che Chien</td>
<td>PDST</td>
<td>Charles Jackson</td>
<td>NIAID</td>
</tr>
<tr>
<td>Mei-Lee Hsieh</td>
<td>PDST</td>
<td>Ann Eakin</td>
<td>NIAID</td>
</tr>
<tr>
<td>Candance Lee</td>
<td>PDST</td>
<td>Erica Raterman</td>
<td>NIAID</td>
</tr>
<tr>
<td>Timmy Huang</td>
<td>PDST</td>
<td>Jane Knisely</td>
<td>NIAID</td>
</tr>
</tbody>
</table>
Come visit us!