**Results**

**Susceptibility to minocycline and SPR741**

<table>
<thead>
<tr>
<th>Strain of bacteria</th>
<th>Minocycline (µg/mL)</th>
<th>SPR741 (µg/mL)</th>
<th>Combination</th>
<th>Combination (Fold change MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB5075</td>
<td>0.5</td>
<td>&gt;64</td>
<td>0.0125</td>
<td>40-fold</td>
</tr>
<tr>
<td>KP4640</td>
<td>0.5</td>
<td>&gt;64</td>
<td>0.025</td>
<td>16-fold</td>
</tr>
</tbody>
</table>

**Figure 2: MIC and Time-Kill**

Minimal Inhibitory Concentration is determined doing a dilution series of minocycline and SPR741 in cation-adjusted Mueller Hinton broth (CAMHB) in 96-well plates according to CLSI at range from 0.0625 – 64 µg/mL.

Time-kill evaluated SPR741 and minocycline alone and in combination at 1/4X and 1X the MIC. The combination at 1X yielded synergistic, bactericidal killing.

**Figure 4: Wound model of infection**

BALB/c mice (n=10) are pretreated with cyclophosphamide and infected topically with 5 x 10^6 CFU of bioluminescent AB5075. Mice are treated i.p. with minocycline, SPR741, or the combination twice a day for three days. On Day 6, animals are imaged using the IVIS system to measure luciferase activity. Survival of each group – untreated control, SPR741 (60 mg/kg BID), minocycline (7.0 mg/kg), combination are shown. (B) Legend for radiance (quantity of bioluminescence units).

**Figure 5: K. pneumoniae Pulmonary Model of Infection**

BALB/c mice (n=20) are pretreated with cyclophosphamide and infected intranasally with 2 x 10^6 CFU KP4640. Mice are treated with minocycline, SPR741, or the combination twice a day for three days. Survival is monitored until Day 7. Results are significant using the Mantel-Cox test. Untreated and minocycline controls fail to reach statistical significance. SPR741 potentiates infection to not enough to promote survival in this animal model.

**Ongoing Investigations - Future Directions**

1. **Evaluate bacterial burden and wound closure in the wound model of infection.**
2. **Identify E. coli and K. pneumoniae minocycline susceptible strains for animal testing.**
3. **Evaluate SPR206, another polymyxin-like compound with other antibiotic combinations.**
4. **Evaluate topical application along with systemic delivery.**

**Conclusions**

- **SPR741 potentiation molecule lowers the MIC of minocycline against A. baumannii and K. pneumoniae. K. pneumoniae** was once resistant, now becomes susceptible.
- **The combination results in bactericidal activity as visualized via time-kill assay.**
- **In vivo, in the lung model of infection the combination of SPR741 and minocycline results in a significant 2-3 log reduction in A. baumannii burden promoting survival.**
- **In vivo, in the wound model of infection the combination also shows a reduction in A. baumannii burden, which also improved the wound inflammation and tissue damage.**

**References**


**Disclaimers**

- Material has been reviewed by the Walter Reed Army Institute of Research. There is no opinion or representation concerning the accuracy or correctness of these materials. Items on this slide are not to be construed as official, or as reflecting the views of the Department of Defense or any other Federal Agency.

- Discretion was exercised with respect to personally identifiable information. All other details have been removed from the original data sets for presentation purposes.

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