Efficacy of NXL101, a Novel Topoisomerase Inhibitor, against Multi-Resistant Staphylococcus aureus in Murine Septicaemia and Thigh Muscle Infections.

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Methods: Septicaemia: Groups of mice (n=10) were intraperitoneally infected with one of 8 S. aureus strains; 2 susceptible and 6 multi-resistant (MRSA) phenotypes (MIC range <0.06-32 µg/ml). NXL101 MIC range 0.03-12 µg/ml). Parenteral treatments were given immediately and 4 hours post-infection and ED50s were calculated by the probit method. Thigh muscle: Groups of neutropenic mice (n=5), were inoculated intramuscularly with one of 6 S. aureus strains (1 MSSA, 5 MRSA). MOX MIC range <0.06-32 µg/ml. NXL101 MIC range 0.03-12 µg/ml). Subcutaneous treatments at 50mg/kg were given 1 and 7 hours post-infection. Bacterial burden was evaluated in mice sacrificed 24 hours post infection.

INTRODUCTION
NXL101 is a clinical candidate from an entirely novel chemical class of antibacterial agents and its in vitro activity has previously been shown against difficult to treat Gram-positive pathogens (including multi-resistant strains) involved in nosocomial and community acquired infections. NXL101 does not show cross-resistance to existing classes of anti-infectives including quinolones (1,2). Demonstration of efficacy in vivo has been facilitated by improving the solubility of NXL101 by formulation in cyclodextrin (HCO70). NXL101 is a solubile and stable in a 30% Keltose solution at 50mg/ml.

STRUCTURE OF NXL101

METHODS
Preparation of S. aureus strains
The strains tested were isolated from isolates from Novexel collection. MICs were measured by using a two-fold agar dilution method. Mueller-Hinton agar medium (pH 7.4; Biorad, France) was used throughout the study. A standard inoculum of 10<sup>6</sup>CFU/ml (Colonby Forming Units) was used throughout. All plates were incubated at 37°C for 20 hours.

Preparation of test compounds
NXL101 was solubilised in 30% Keltose HPB® to a concentration of 50mg/ml. Biorad, France) was used throughout the study. A 50 mg/ml stock solution was diluted for dosing in physiological saline. Other compounds were dissolved and diluted in physiological saline.

Animals
Male Swiss mice (CD1) 20-23 g Charles River-France were used and kept in controlled conditions of temperature (21 ± 2°C) relative humidity and a lighting time of 12 h cycles. Mice had free access to food and filtered water. Experimental protocols were approved by the local ethics committee. The research complied with the national legislation and with Novexel Policy with regard to care and use of animals and related codes of practice.

Murine septicemia model
Each strain was diluted in 5% hog gastric mucin (Sigma) and injected by intraperitoneal route. Antibiotic treatments were given at 0 and 4 hours post infection.-Antibiotic treatments were given at 0 and 4 hours post infection-subcutaneously. For NXL101, some groups of mice were also treated intra-venously.

Murine thigh infection model
Tonsillar fluid from patients with culture negative otitis media was used to infect mice sub-cutaneously as described previously (1). Antibiotic treatments were administered 1 and 7 hours post infection. The anti-staphylococcal activity of NXL101 was studied in 2 murine infection models; the murine septicemia model and the murine thigh muscle infection model.

RESULTS
Table 1 The efficacy of NXL101 against S. aureus strains in a mouse septicemia model compared to vancomycin, linezolid and moxifloxacin

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype</th>
<th>Treatment</th>
<th>ED50 (µg/ml)</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NXL101</td>
<td>metR, FQR</td>
<td>NXL101 SC</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>NXL101</td>
<td>metR, FQR</td>
<td>Vancomycin</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>NXL101</td>
<td>metR, FQR</td>
<td>Linezolid</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>NXL101</td>
<td>metR, FQR</td>
<td>Moxifloxacin</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

Thigh muscle infection
- NXL101, LIN and VAN treated animals had significantly less bacterial burden at 24 hours compared to untreated controls for all the strains tested (p < 0.01).
- NXL101 was able to prevent proliferation of N. cinerea and S. aureus in thigh. The tissue was infected with a single clone of S. aureus and treated with NXL101, with or without MRSA. NXL101 was equally effective when dosed SC or IV showing that NXL101 was equally bioavailable in the cyclodextrin formulation by either route.

CONCLUSION
• The anti-staphylococcal activity of NXL101 seen in vitro translated into good efficacy in vivo.
• NXL101 was uniformly efficacious against metS<sup>+</sup> and metS<sup>-</sup> and FO<sup>+</sup> or FO<sup>-</sup> strains.
• NXL101 was equally effective when dosed SC or IV showing that NXL101 was equally bioavailable in the cyclodextrin formulation by either route.

REFERENCES