LTX-109 Demonstrates Rapid Membranolytic Activity against *Escherichia coli* and *Staphylococcus aureus*

B. MORTENSEN, A. FUGELLI, H.M. BLENCKE, K. STENSVÅG, J.S. SVENDSEN, W.M. OLSEN
1Lytx Biopharma, Norway, 2Norwegian College of Fishery Science, Univ. of Tromsø, Norway

**Background**

LTX-109 is a novel antimicrobial drug in clinical development for skin infections and nasal decolonisation of MRSA. The drug mimics the effects of natural antimicrobial peptides in a synthetic small molecule. LTX-109 has demonstrated a broad activity against several Gram+ and Gram- bacteria in vitro, as well as activity against a range of yeast and fungal species. The compound is equally effective against antibiotic-resistant species such as methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant Enterococci (VRE) and multi-resistant Pseudomonas isolates. The ultra-rapid membrane-lysing mode of action may result in a lower propensity to resistant development and a rapid bactericidal mechanism of action, as shown in in vitro studies. To date LTX-109 demonstrates no in vitro cross-resistance with other classes of antibiotics.

The present study investigated the mode of action of LTX-109 by means of bacterial real-time assays.

**Methods**

A real-time viability assay based on the expression of bacterial luciferase in *E. coli* and *S. aureus* was used to assess the metabolic state of sensor bacteria. Light produced by *E. coli* HB101 constitutively expressing the luxCDABE operon indicates viable bacterial cells. Reduced relative light emission indicates bactericidal activity.

A second real-time assay based on *E. coli* cells expressing insect luciferase was used to analyse bacterial cell membrane integrity. Relative light production by *E. coli* HB101 constitutively expressing an insect luciferase requires externally added D-luciferin as its substrate. Light induction results from the increased passage of D-luciferin into the bacteria in response to decreased membrane integrity induced by the treatment with AMPs. Concentrations of LTX-109 from sub-MIC to 5xMIC were used for both assays.

**Results**

LTX-109 demonstrated rapid membranolytic activity against *E. coli* (see figure below) and *S. aureus* (not shown) in the real-time Luciferase cell viability assay. In *E. coli* this quick response was comparable to results obtained with cecropin P1, which is a well-known membrane active antimicrobial peptide. In the membrane-integrity assay, at MIC and higher concentrations LTX-109 induced a peak of light emission comparable to cecropin P1, whereas at sub-MIC concentrations of LTX-109 no light peak was observed.

**Conclusions**

- LTX-109 appears to kill bacteria by interfering with plasma membrane integrity.
- Since this activity is measurable at concentrations equal to the MIC, we propose that the main mode of action is damaging the bacterial plasma membrane.
- The drug has been tested in Phase I and two Phase IIa trials with good tolerence, minimal systemic bioavailability
- LTX-109 has demonstrated Proof-of-Concept in decolonisation of nasal MRSA / MSSA
- Further Phase II studies are planned to demonstrate efficacy in larger patient populations.