

ARTILYSINS: ANTIBACTERIAL ENZYMES THAT ATTACK BACTERIAL SURFACE STRUCTURES

Stefan Miller, CSO, Lisando GmbH

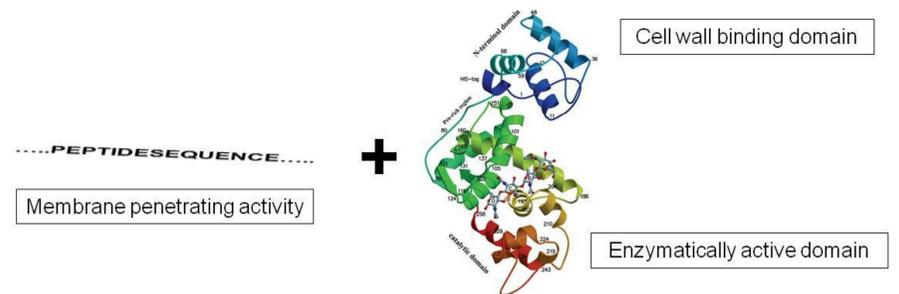
Contact: Dr. Stefan Miller, CSO; Lisando GmbH; Josef-Engert-Straße 13; 93053 Regensburg, Germany; Mail: stefan.miller@lisando.com

Abstract:

Increasing antibiotic resistance of bacteria provides a clear need for novel ways to combat bacterial pathogens. Artilysins are novel designed recombinant polypeptides that are modified specifically to provide the activities needed to kill bacterial pathogens. Artilysins combine an efficient enzyme with membrane penetrating activities. Upon contact from the outside, Artilysins efficiently disrupt surface structures of both gram negative and gram positive target bacteria. MIC data, as well as infection experiments show that using an enzymatic mechanism Artilysins are killing strains of *Pseudomonas aeruginosa* and MRSA independent whether or not these strains are antibiotic resistant. Furthermore Artilysins are also active in reducing biofilm formation of both bacterial species.

In contrast to most classical antibiotics, Artilysins will not be metabolized and are attacking highly conserved structures on the bacterial surface. Thus bacteria will hardly be able to adapt to this new mode of action provided by Artilysins and, thus, the risk of the development of resistances by bacteria against Artilysins is significantly low. Thus Artilysins are an efficient tool to combat pathogenic bacteria.

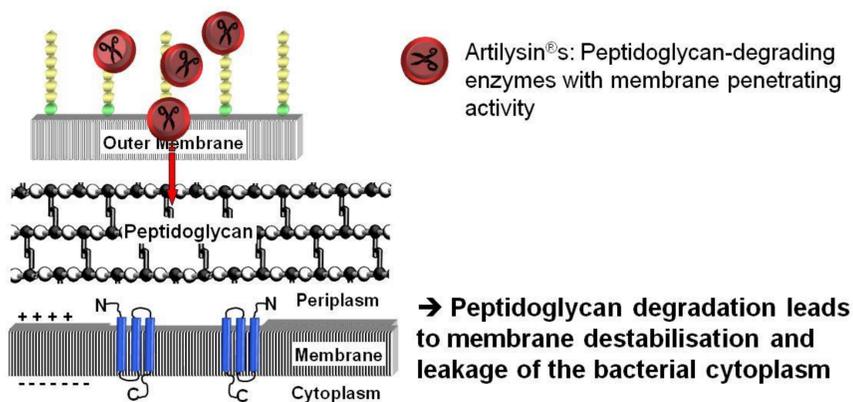
Artilysin® technology – key facts



Artilysin®

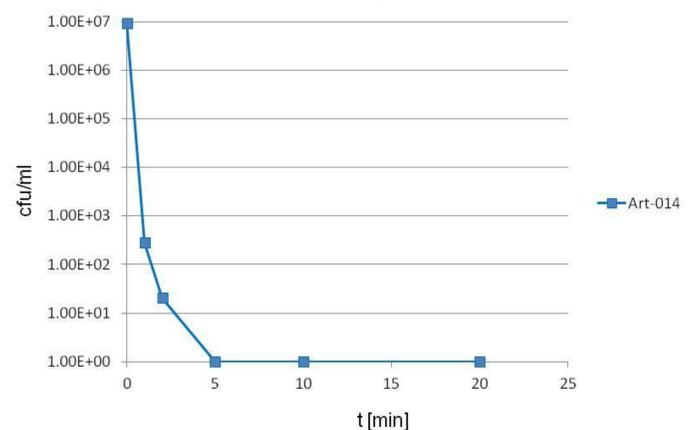
- Novel designed fusion proteins
- Combination of efficient enzymes with membrane penetrating activities
- Toolbox for all bacteria of interest
- Fusion proteins – expressed recombinantly and purified to homogeneity
- Peptide and enzyme libraries available

Artilysin® - functional principle



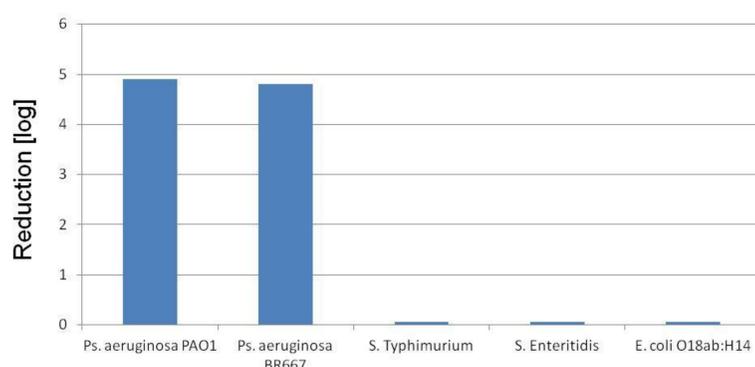
Artilysin®s are attacking conserved bacterial surface structures

Artilysin® - fast killing of bacteria



7-log reduction of *Pseudomonas aeruginosa* PAO1 within few minutes

Artilysin® - targeted killing of bacteria



Artilysin Art-014, designed to kill *Pseudomonas aeruginosa*:

→ Specific antimicrobial activity against *Pseudomonas aeruginosa*, including strain Br667, a clinical isolate, broadly antibiotic resistant (10/11)

Minimal Inhibitory Concentration

MIC of Art-085 (Artilysin® targeting *Pseudomonas aeruginosa*):
Pseudomonas aeruginosa: 60 strains, including 50 clinical isolates

Examples:

PAO1p	3-4 µg/ml
2572	3-4 µg/ml
BR680	10 µg/ml
PAK	7-9 µg/ml
UG449	4-6 µg/ml

MIC₅₀ = 10 µg/ml (w/o optimisation)
(Testing performed according to CLSI reference M31-A3)

For comparison :

Lysozyme: MIC >500µg/ml for *Pseudomonas fluorescens*
(Branen & Davidson, 2004; Int. J. Food Microbiology 90, 63-74)

Otitis externa – case study



- Dog was treated with Neomycin for 6 weeks without effect.
- Treatment with Art-085: within 1 day massively reduced inflammation symptoms.
- within less than 5 days *Ps. aeruginosa* was eliminated using Art-085

Artilysin® - highlights

- Targeted antibacterials with a new mode of action
- Rapid and efficient enzymatic killing of:
 - *Pseudomonas aeruginosa* and other gram negative bacteria
 - *Staphylococcus aureus* and other gram positive bacteria
- Artilysin® Art-085 is active *in vivo*, in animal models
- Artilysin® Bat-072 is active *in vivo*, in decubitus application
- Artilysin®s are active in reducing biofilm formation

The Artilysin® platform addresses the growing problem of multi-resistant gram negative and gram positive bacteria.