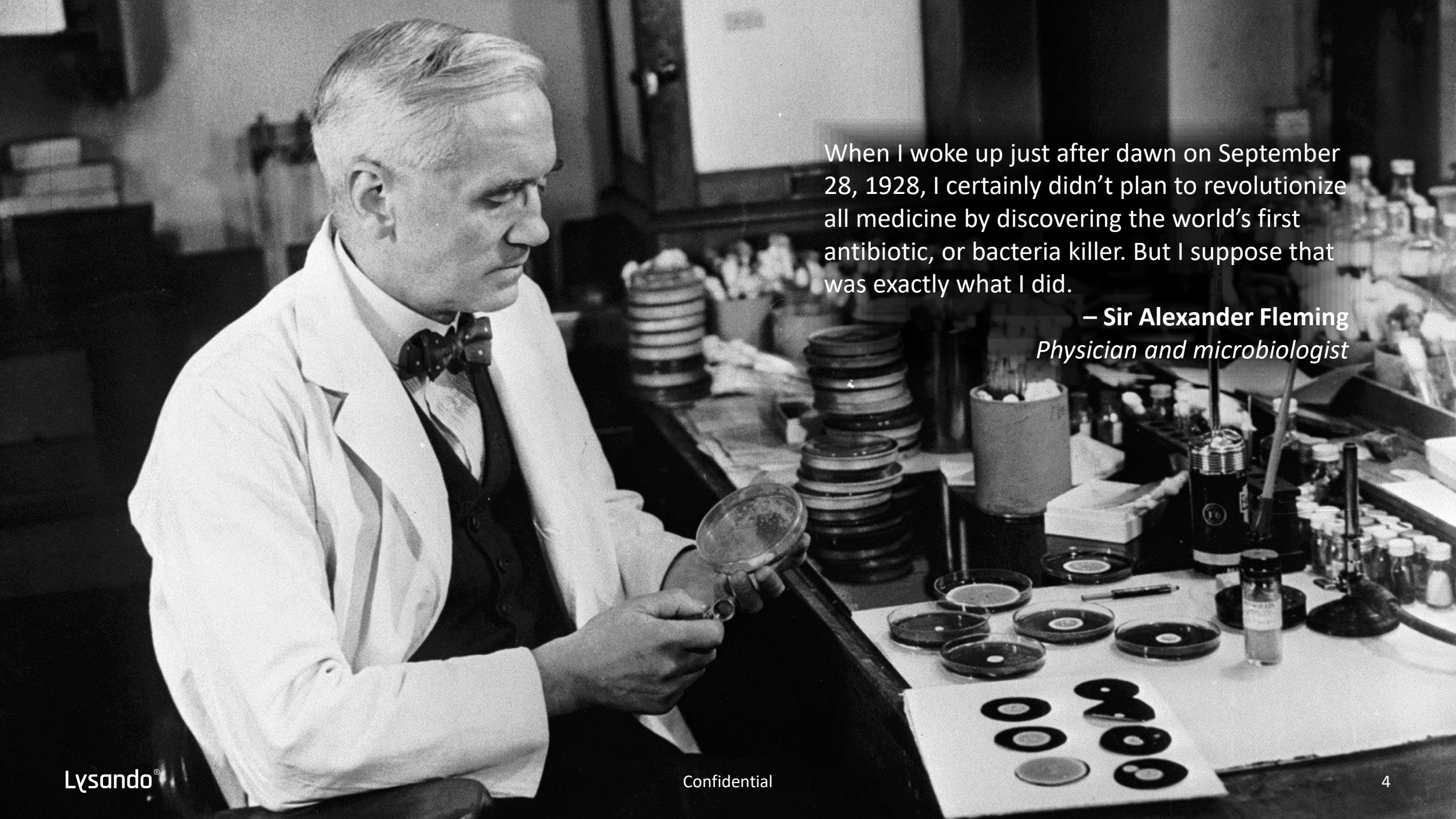

Lysando®
From Invention to Cure
bayresq.net
Neue Strategien gegen
multiresistente Krankheitserreger
mittels digitaler Vernetzung
Fabian Geldmacher – February 23, 2024

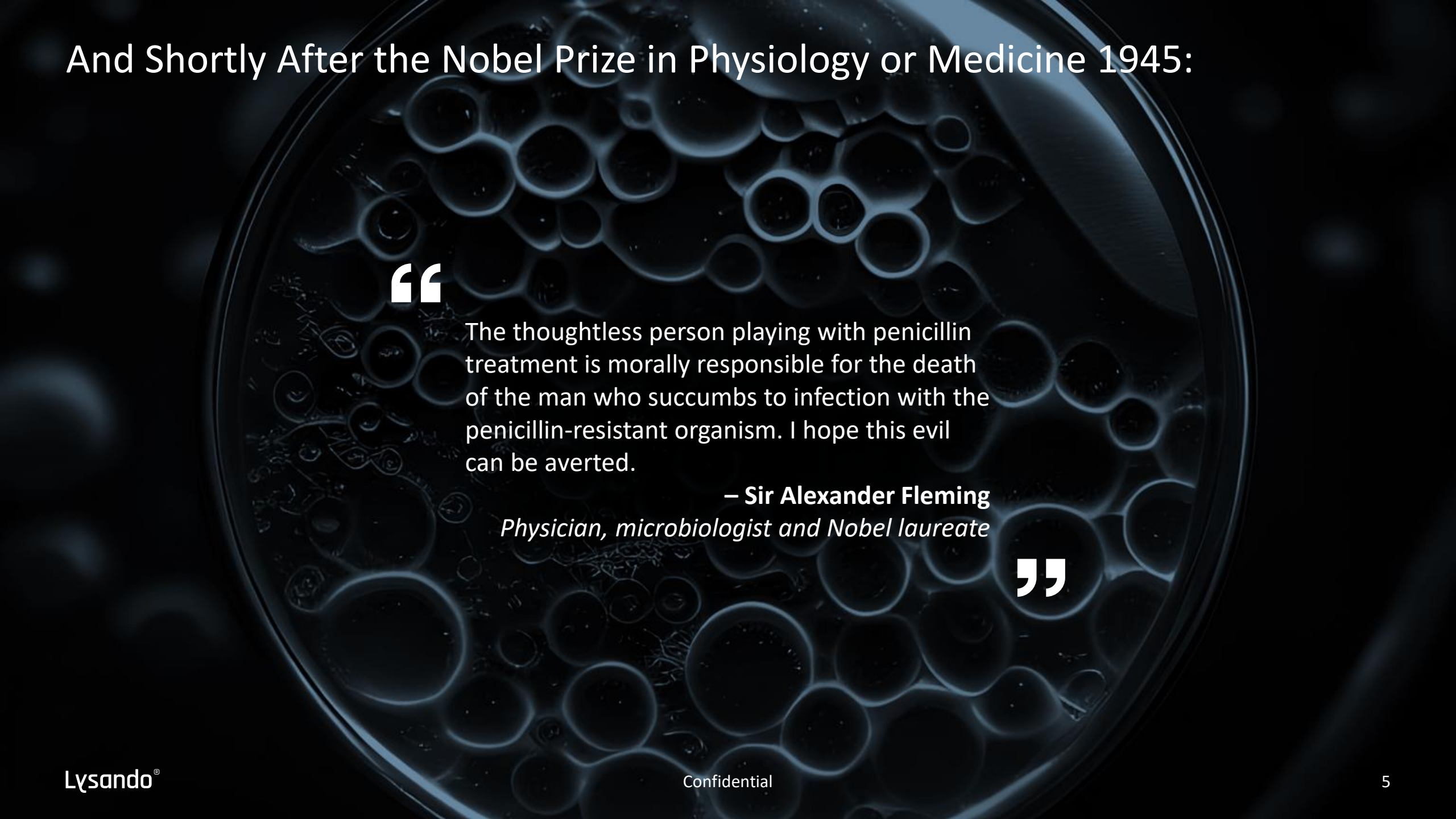

Lysando®

Artilysin®

Lysando®


When I woke up just after dawn on September 28, 1928, I certainly didn't plan to revolutionize all medicine by discovering the world's first antibiotic, or bacteria killer. But I suppose that was exactly what I did.
– Sir Alexander Fleming
Physician and microbiologist

And Shortly After the Nobel Prize in Physiology or Medicine 1945:



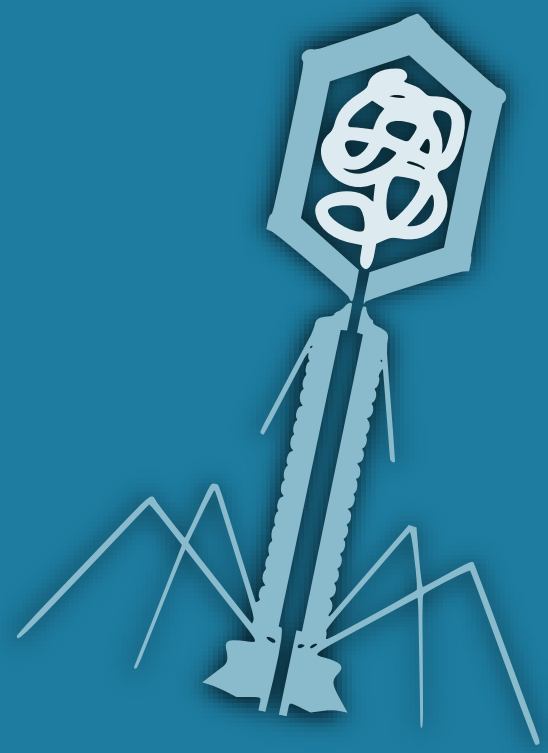
> The thoughtless person playing with penicillin treatment is morally responsible for the death of the man who succumbs to infection with the penicillin-resistant organism. I hope this evil can be averted.
>
> **– Sir Alexander Fleming**
> *Physician, microbiologist and Nobel laureate*

Bacteriophages

SIMPLIFIED

Criteria

Potency against Gram-positive bacteria	✓
Potency against Gram-negative bacteria	✓
Precision targeting of distinct bacteria	✓
Activity against persister bacteria	✗
Resistance avoidance	✗
No gene transfer risk	✗
Low cytotoxicity/ no side effects	✗
“One size fits all”	✗

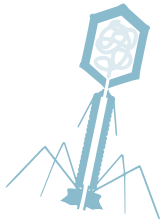


Bacteriophages are a group of usually complex viruses who specialize in infecting and re-programming bacteria.

They replicate intracellularly and utilize endolysins to break down the bacterial host cell wall, ultimately resulting in the release of new phage progeny.

Endolysins

SIMPLIFIED

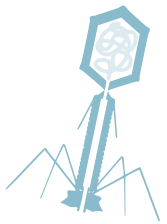
Criteria	Bacteriophages	
		
Potency against Gram-positive bacteria	✓	✓
Potency against Gram-negative bacteria	✓	(✗)
Precision targeting of distinct bacteria	✓	✓
Activity against persister bacteria	✗	✓
Resistance avoidance	✗	(✗)
No gene transfer risk	✗	✓
Low cytotoxicity/ no side effects	✗	✓
“One size fits all”	✗	✓

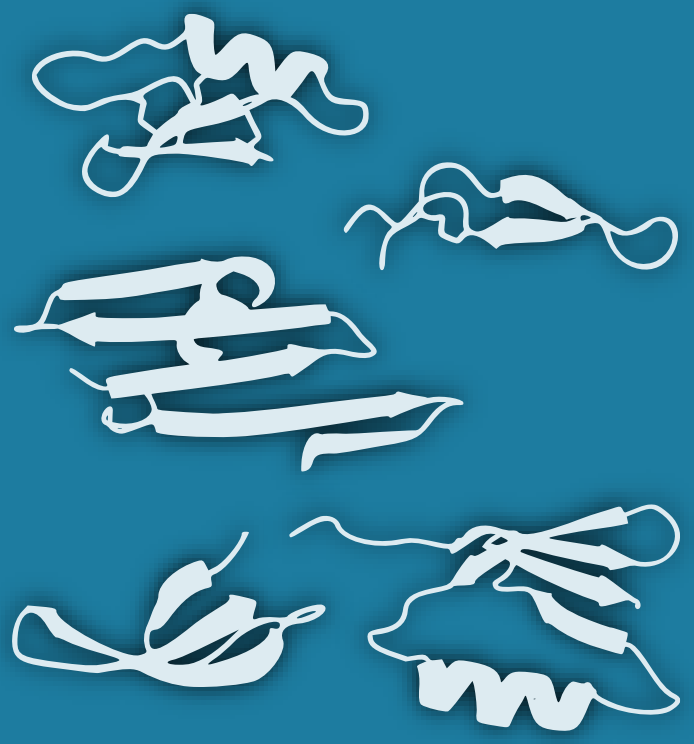
Endolysins are highly specialized enzymes. As the cell wall-cleaving components of bacteriophages they play a pivotal role in cell lysis by specifically cleaving the peptidoglycan layer of bacterial cells.

This rupture of the cell wall leads to the destruction of the bacterial host.

Antimicrobial Peptides

SIMPLIFIED

Criteria	Bacteriophages	Endolysins	
			
Potency against Gram-positive bacteria	✓	✓	✓
Potency against Gram-negative bacteria	✓	(✗)	✓
Precision targeting of distinct bacteria	✓	✓	✗
Activity against persister bacteria	✗	✓	✓
Resistance avoidance	✗	(✗)	✗
No gene transfer risk	✗	✓	✓
Low cytotoxicity/ no side effects	✗	✓	✗
“One size fits all”	✗	✓	✓

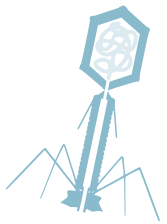
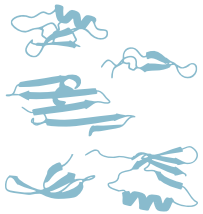


AMPs are a diverse group of naturally occurring molecules known for their broad-spectrum antimicrobial activity against bacteria, viruses, fungi, and parasites.

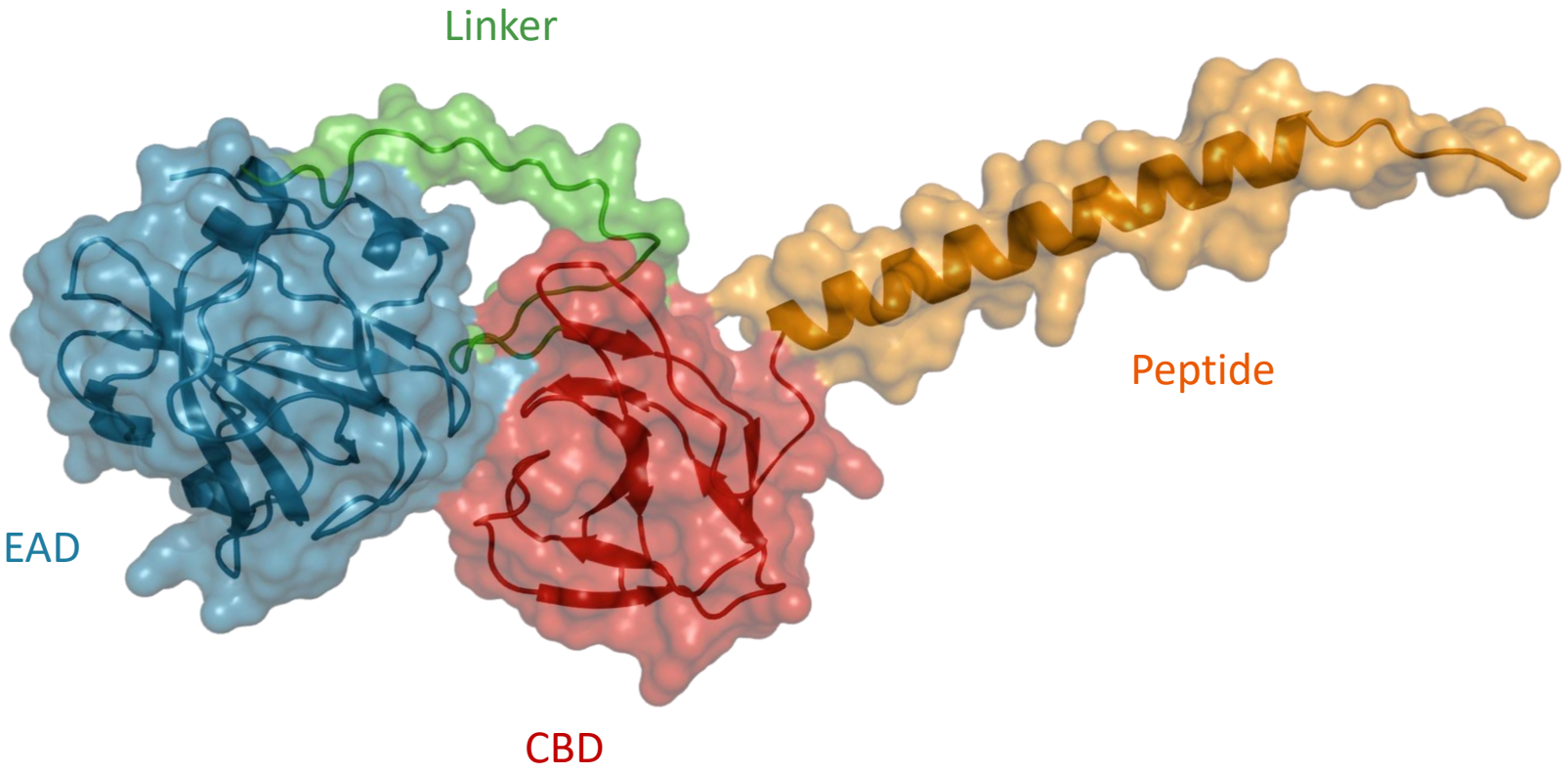
Their unique mechanism of action involves the disruption of microbial membranes.

So, What Is Artilysin®?

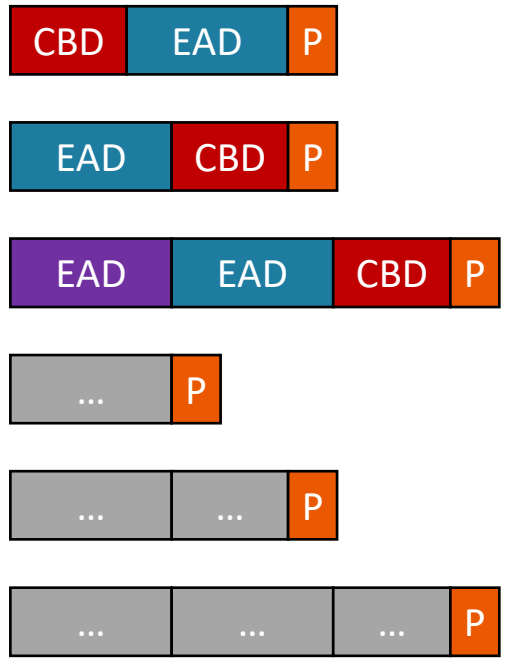
SIMPLIFIED

Criteria	Bacteriophages	Endolysins	Antimicrobial Peptides	Artilysin®
				
Potency against Gram-positive bacteria	✓	✓	✓	
Potency against Gram-negative bacteria	✓	(✗)	✓	
Precision targeting of distinct bacteria	✓	✓	✗	
Activity against persister bacteria	✗	✓	✓	
Resistance avoidance	✗	(✗)	✗	
No gene transfer risk	✗	✓	✓	
Low cytotoxicity/ no side effects	✗	✓	✗	
“One size fits all”	✗	✓	✓	

So, What Is Artilysin®?



Many, many variants are possible



Mode of Action

Endolysin component

- Targets highly conserved peptidoglycan, a “hard-to-change” bacterial structure
- Derived from bacteriophages

Antimicrobial peptide

- Confers broad-spectrum antimicrobial activity
- Engages in a dual-mode of action alongside endolysin moiety

Dual-mode of action

Adhesion to the bacterial cell wall

- First, Artilysin® interacts with the bacterial cell wall through electrostatic interactions
- Positive net charge facilitates the attachment to negatively charged bacterial surfaces, disrupting lipopolysaccharide (LPS) stability

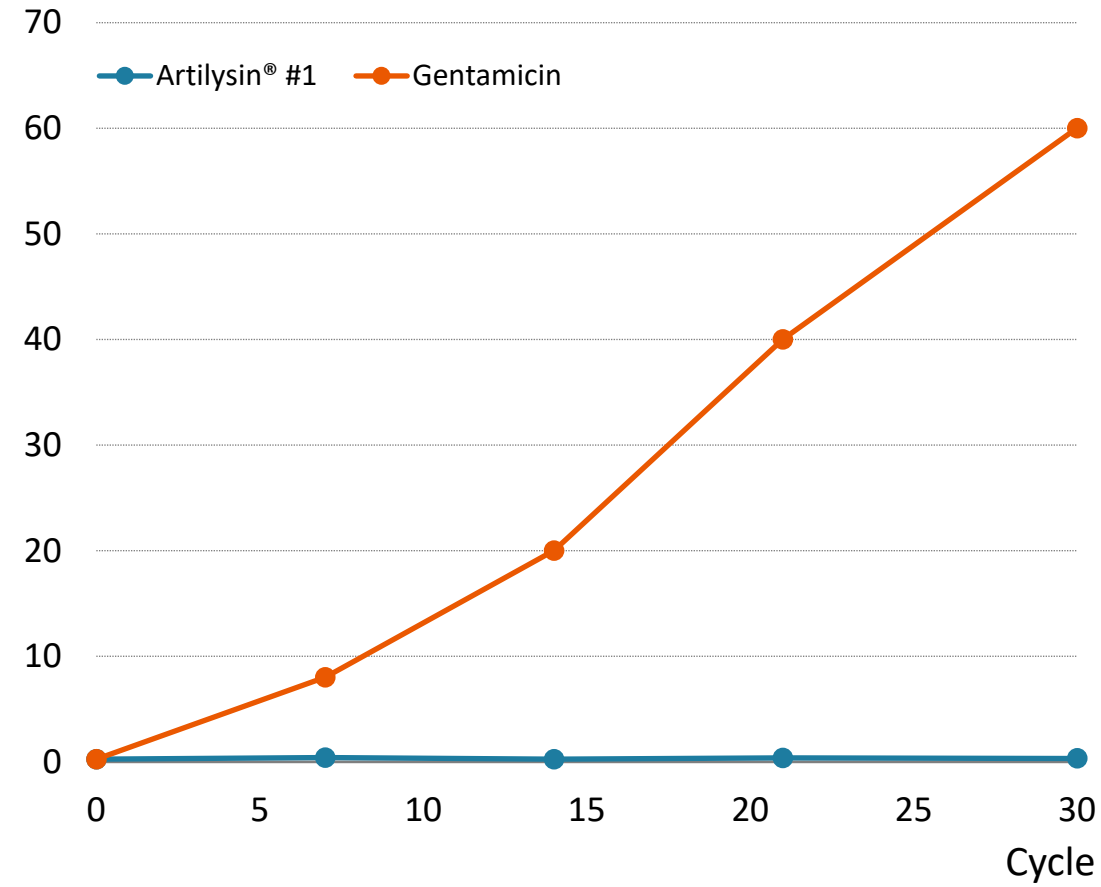
Enzymatic activity and cell lysis

- As a next step, Artilysin® facilitates the passage through the outer membrane
- The active hydrolase site then destabilizes the peptidoglycan layer, resulting in cell rupture through osmotic pressure

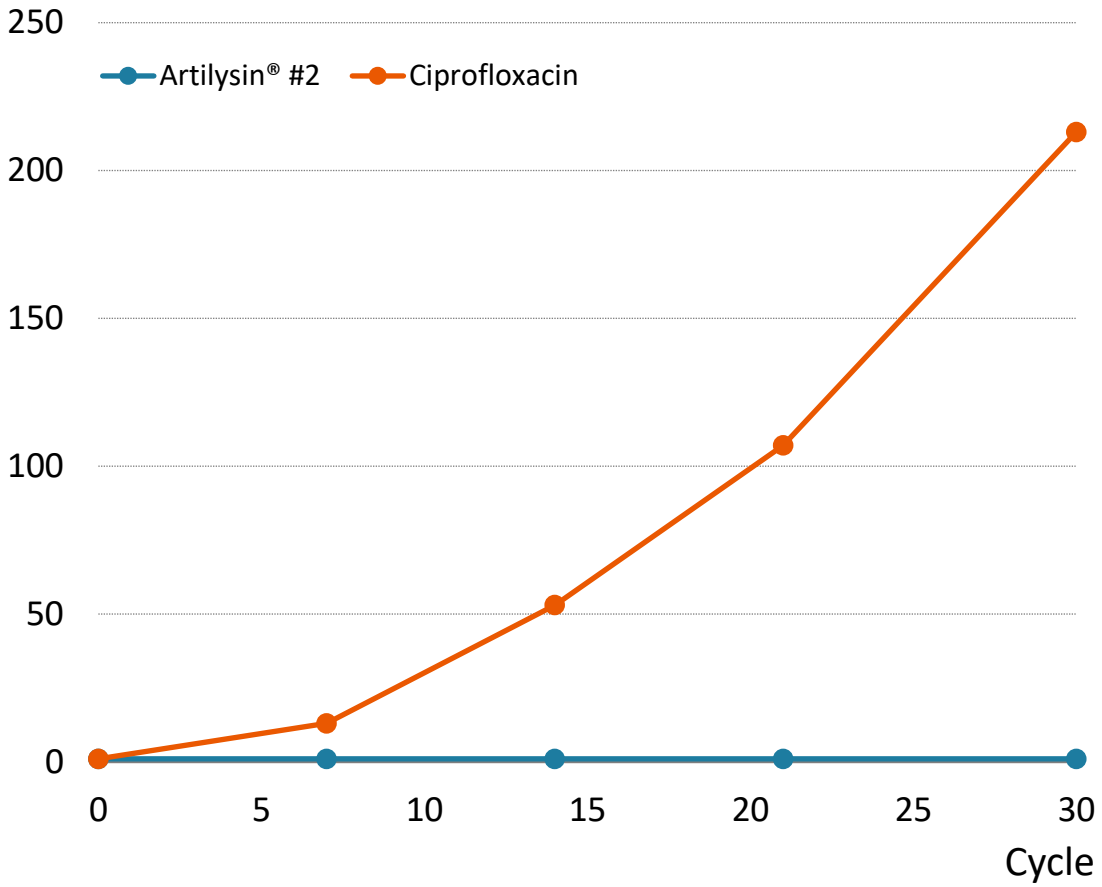
Artilysin® operates **without the need for intracellular targets or receptors** and is **independent of active bacterial metabolism**

No Resistance Development

Resistance induction in *Staphylococcus aureus* Sp 10,
MIC/MIC$_0$



Resistance induction in *Pseudomonas aeruginosa* PAO1,
MIC/MIC$_0$

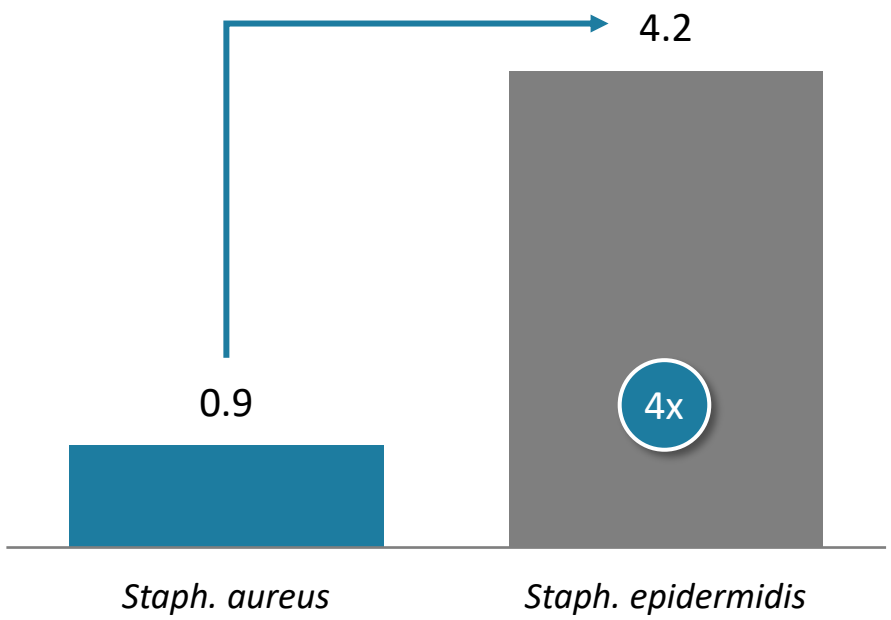


MIC = Minimum Inhibitory Concentration

Differentiation Between Closely Related Species Possible

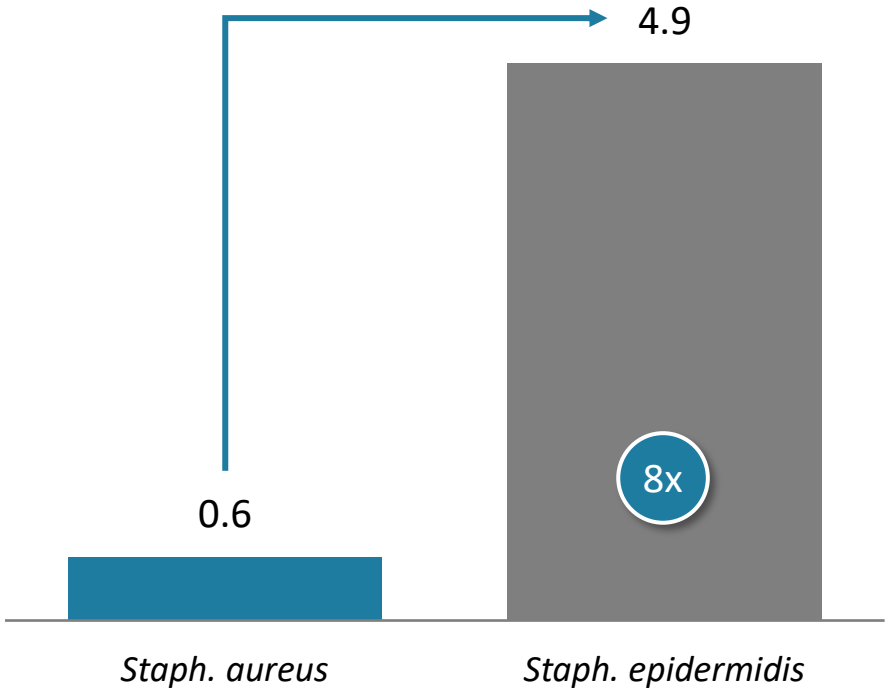
Artilysin® #3

MIC_{90}, in µM



Artilysin® #4

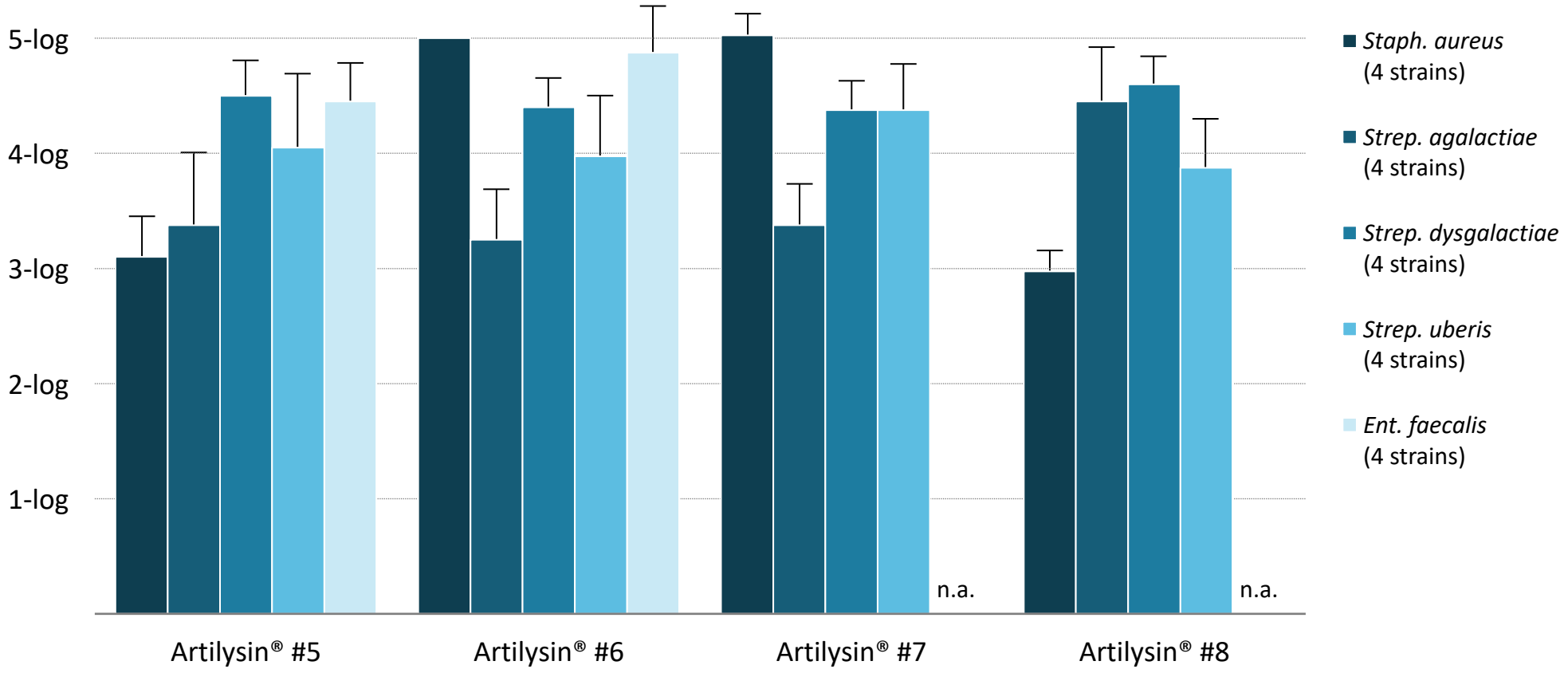
MIC_{90}, in µM



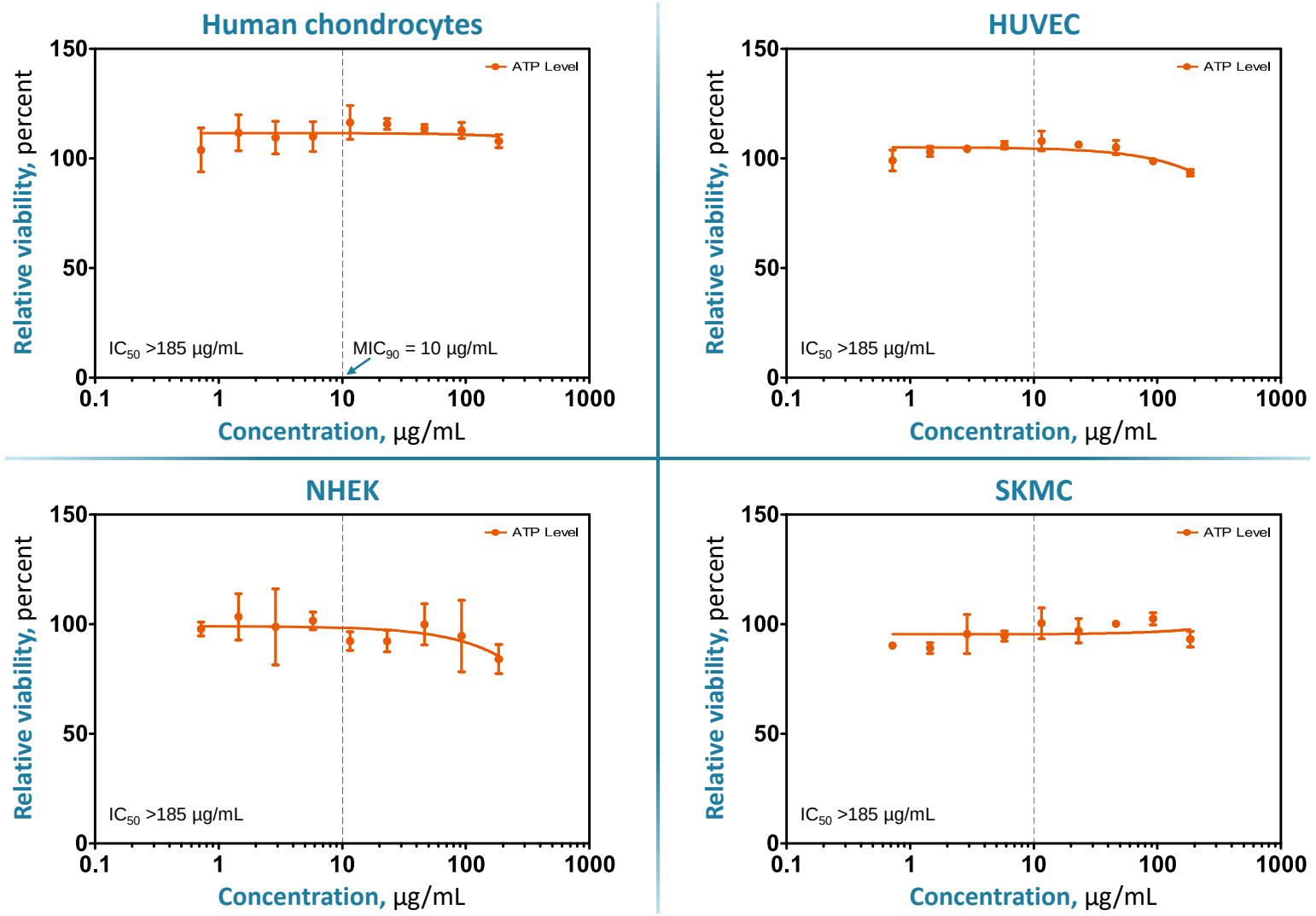
MIC = Minimum Inhibitory Concentration

Activity Against a Broader Spectrum of Bacterial Species

Bacterial activity assay, average log reduction (CFU)



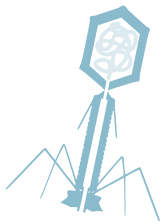
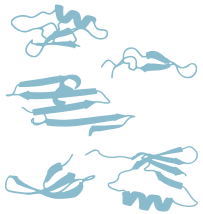
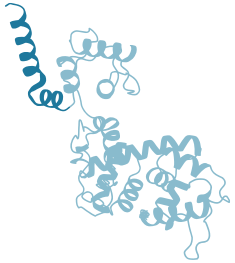
Highly Favorable Compatibility Data and Safety Profile



HUVEC = Human Umbilical Vein Endothelial Cells; NHEK = Normal Human Epidermal Keratinocytes; SKMC = Skeletal Muscle Cells

Summary: Advantages of Artilysin®

SIMPLIFIED

Criteria	Bacteriophages	Endolysins	Antimicrobial Peptides	Artilysin®
				
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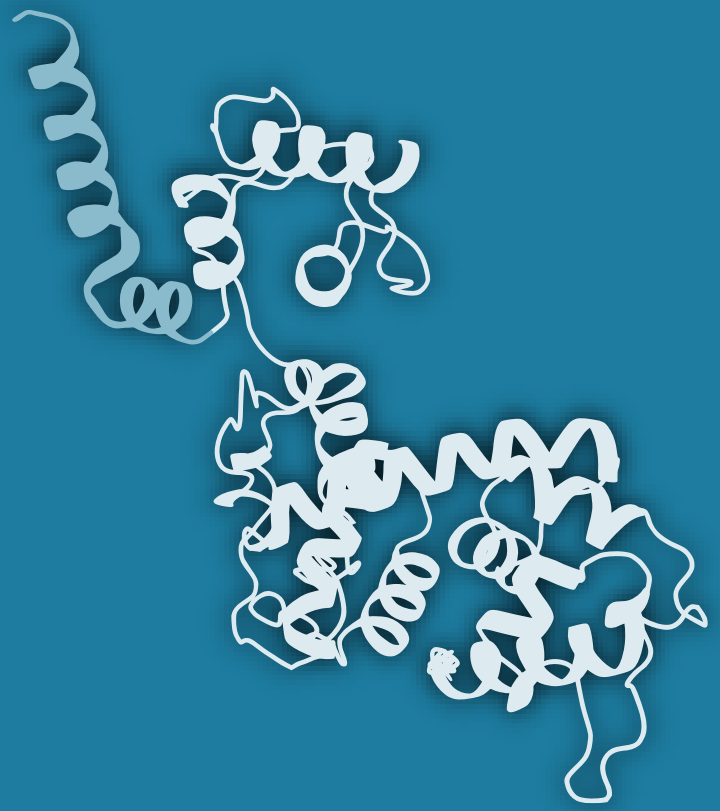
No gene transfer risk	✗	✓	✓	✓
Low cytotoxicity/ no side effects	✗	✓	✗	✓
“One size fits all”	✗	✓	✓	✓



Artilysins are novel designed recombinant polypeptides that are modified specifically to provide the activities needed to kill bacterial pathogens.

Artilysins combine an endolysin activity with membrane penetrating activities.

The Challenge

“Shortcomings of wildtype endolysins”

In nature, wildtype endolysins are typically characterized by a short half-life / low stability – why is that?

Rapid action	Endolysins act rapidly to aid phage escape, becoming redundant upon successful lysis
Transient need	Their short-lived presence prevents harm to neighboring cells post-infection

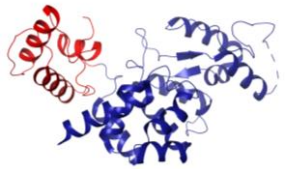
“What we would like to see”

1. Long shelf life
2. Storability at room temperature
3. Room for improvement

We Leverage Artificial Intelligence

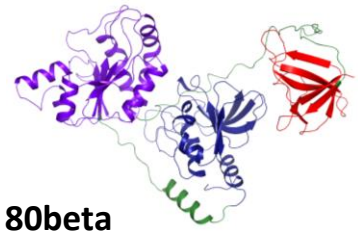
AlphaFold
Rosetta

Level 1: Optimization



AP3-phage EL

CBD | EAD



80beta

EAD | EAD | CBD

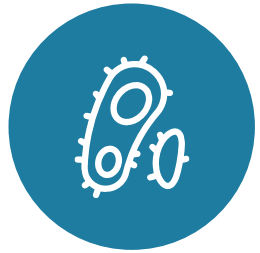
- Wildtype endolysins feature independent domains connected by linkers without/with limited domain-domain interactions
- Consequently, our design strategy is focused on individual domains to construct a modular domain system (“LEGO blocks”) for Artilysin® design
- Objectives:

Stability

Improvement of protein stability

Producibility

Optimization of expression construct and yields



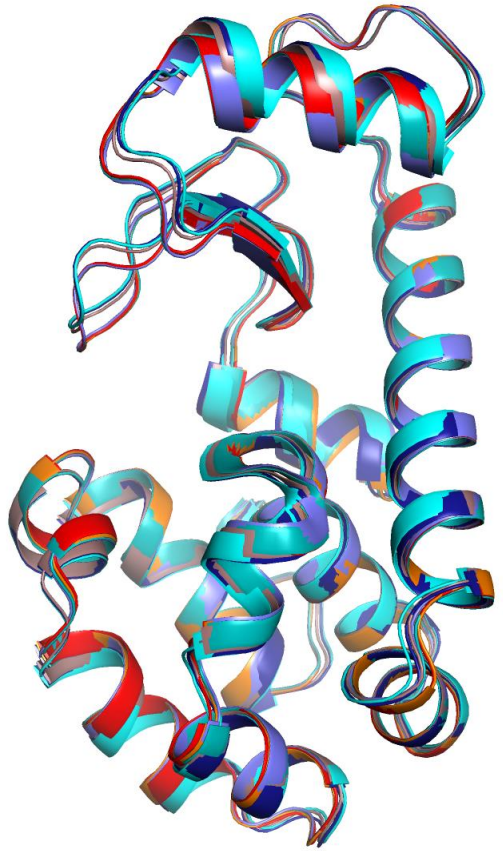
Activity / affinity

Modulation of protein activity and affinity

Level 2: Prediction of novel combinations for further assessment

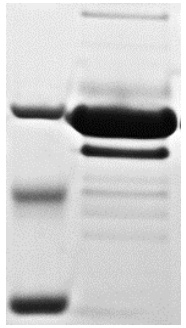
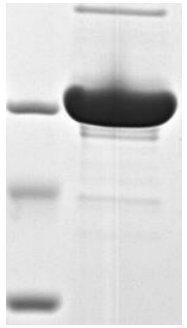
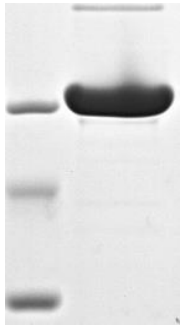
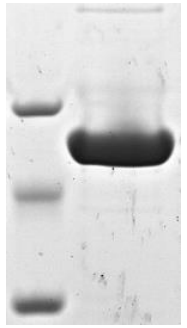
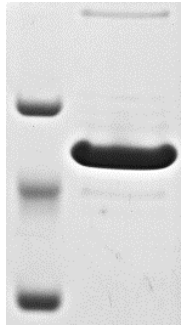
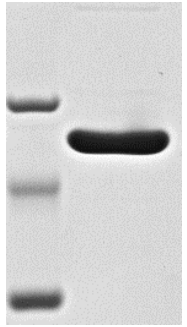
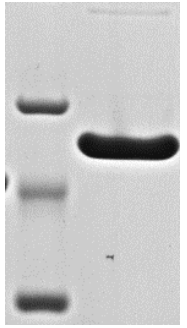
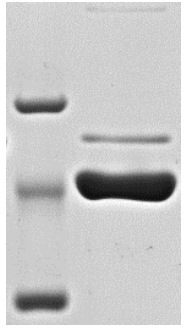
- Building a proprietary database on antimicrobial activity of both individual modules and complete Artilysins towards individual species
- The goal is to predict the activity and specificity of novel Artilysins in advance

We Leverage Artificial Intelligence – Example Use Case



Structural superposition of parent Artilysin® and eight design variants

Optimization of producibility

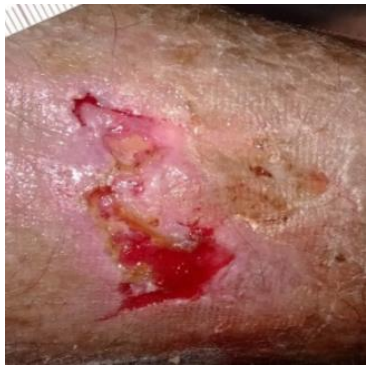
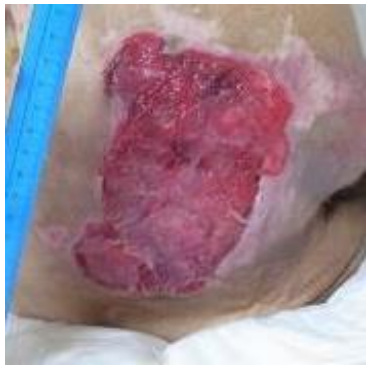
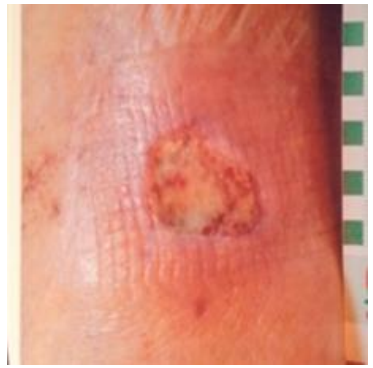
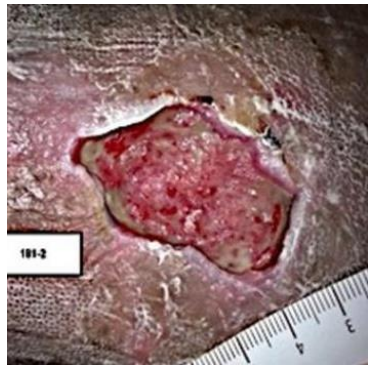
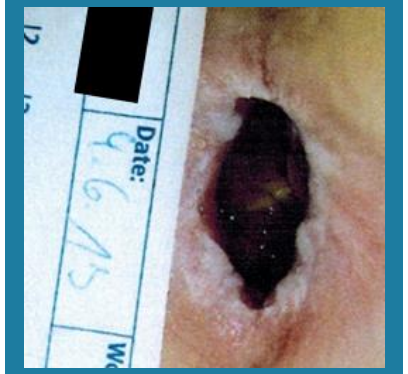
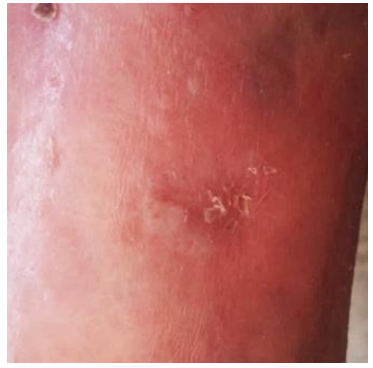
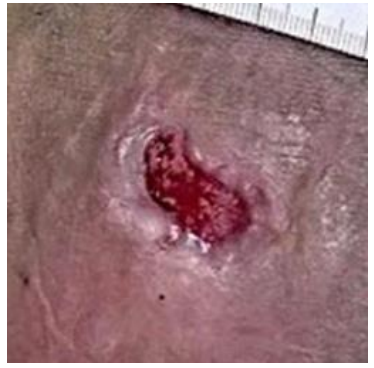
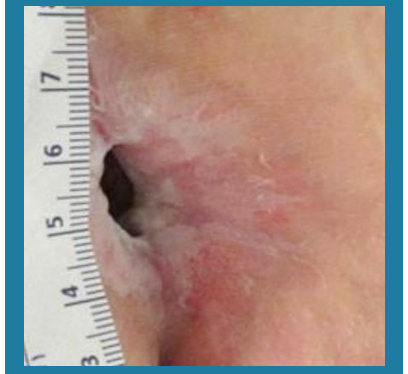
	Parent	Variant 5	Variant 6	Variant 8
				
Yield post IMAC:	**21.5 mg/L**	**22.7 mg/L** *(+ 5.6%)*	**39.1 mg/L** *(+ 81.9%)*	**48.3 mg/L** *(+ 124.7%)*
				
Yield post IEX:	**11.0 mg/L**	**15.1 mg/L** *(+ 37.3%)*	**24.5 mg/L** *(+ 122.7%)*	**32.2 mg/L** *(+ 192.7%)*

IMAC = Immobilized Metal Affinity Chromatography; IEX = Ion Exchange Chromatography

Let‘s See How It Works

Wound healing was initiated in every patient

– detailed in the following –

	Example case #1	Example case #2	Example case #3	Example case #4	Example case #5
Age of wound	4 weeks	21 weeks	24 weeks	25 weeks	36 weeks
Bacteria found in wound	*S. aureus* and *E. coli*	*S. aureus* and *S. agalactiae*	*C. freundii* and *P. mirabilis*	*E. corrodens* and *E. coli*	*K. pneumoniae*, *P. mirabilis* and *S. agalactiae*
Before treatment					
After treatment		 After 2.5 years			
Treatment/follow-up	3 months	9 weeks	3 months	3 months	3 months

Let‘s See How It Works

Patient data

- **Gender:** male
- **Age:** 66
- **Complicating factors:** patient in a coma

Case characteristics

- **Indication:** chronic infected wound
- **Wound type:** decubitus, stage IV
- **Location:** sacrococcygeal region
- **Size of wound:** 10 cm in depth, extending down to the bone, with two “pockets”
- **Duration of wound persistence:** approx. 36 weeks

Microbiology

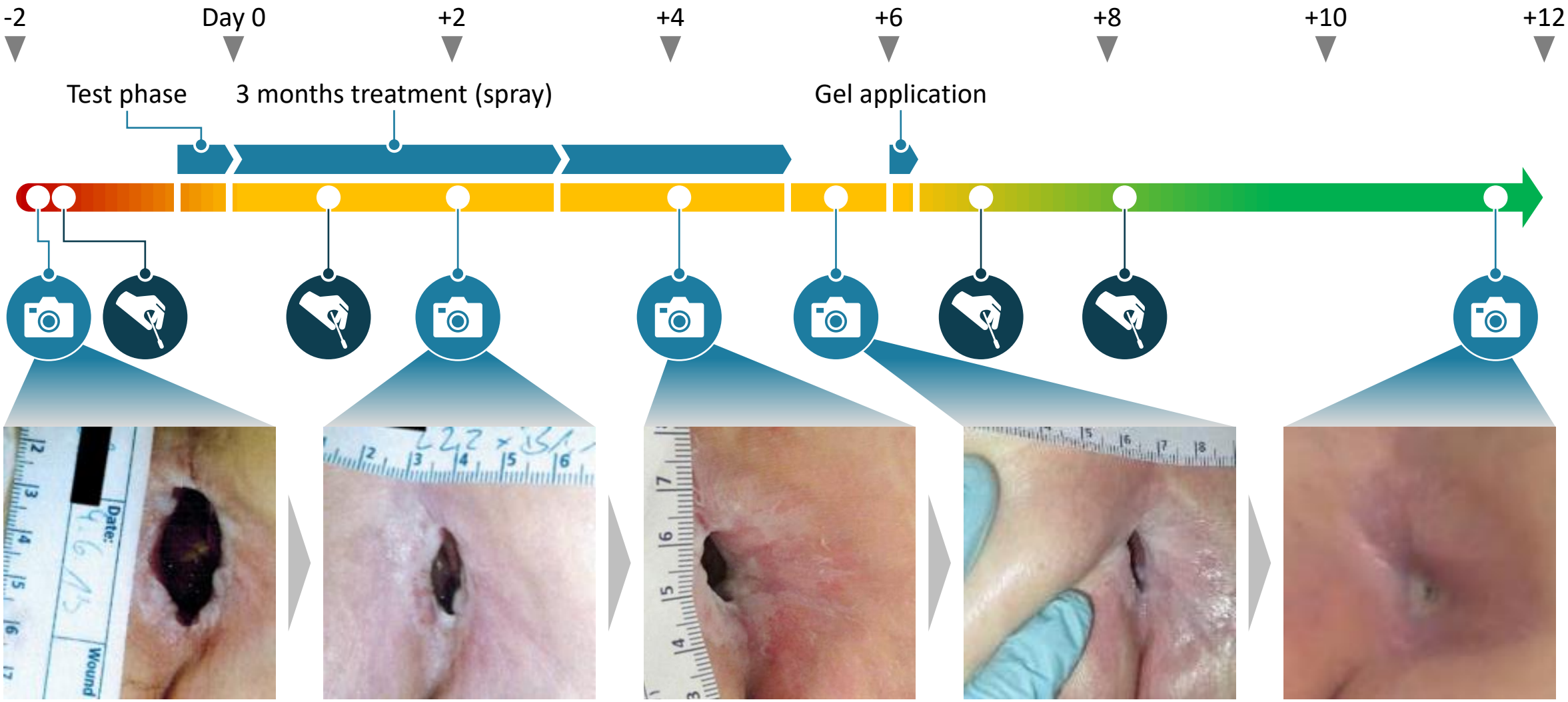
Wound colonized with

- *Klebsiella pneumoniae* (ESBL-positive, non-MDR)
- *Proteus mirabilis* (non-MDR)
- Streptococcus agalactiae

Primary treatment objective

Initiation and promotion of wound healing

Let’s See How It Works



Time For…

Lysando®

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