The Guanidino-Lipoglycopeptides: Novel Glycopeptide Antibiotics with *Best-in-Class* Potential

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LEIDEN
DRUG DEVELOPMENT
CONFERENCE

Gram-positive pathogens remain a serious threat

- MRSA and VRE account for half of all AMR-associated deaths in USA and Europe

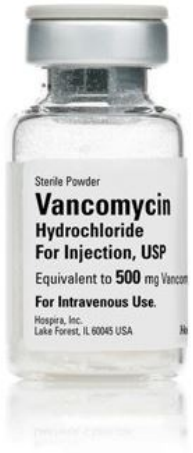
- 2018 global MRSA market worth US$ 922.2M (by 2026 estimated to grow to US$ 1,3B)
- Global VRE market estimated between US$ 280 and 440M

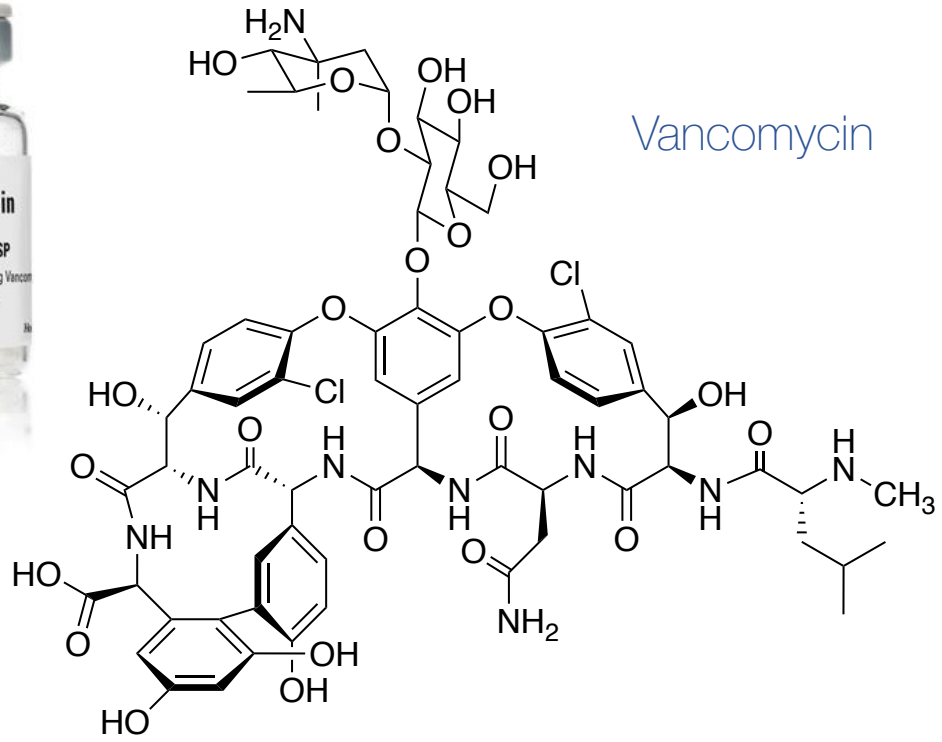
CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019.

*C. J. L. Murray, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis, Lancet **2022**, 399, 629-655.*

Glycopeptide antibiotics

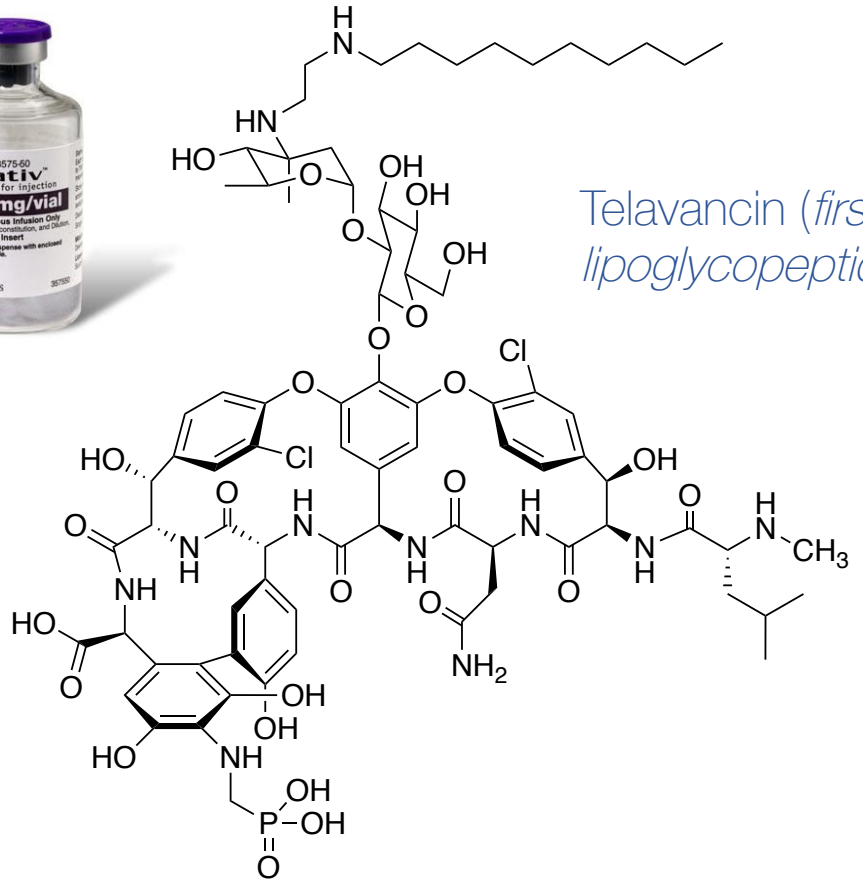
- Since the 1970's vancomycin used in treatment of serious infections, including acute bacterial skin and skin structure infections (ABSSSI), due to staphylococci and enterococci
- Resistance now a serious problem





Lipoglycopeptide antibiotics

- Vancomycin resistance led to development of the lipoglycopeptides (e.g. teicoplanin, telavancin, dalbavancin, and oritavancin)
- Increased activity but also renal toxicity and unusual PK ($t_{1/2}$ up to 300hr)



Telavancin (*first semisynthetic lipoglycopeptide brought to market*)

FDA

BLACK BOX WARNING

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There is a growing unmet need for novel antibiotic compounds with activity against resistant pathogens and improved safety profiles.

Introducing the guanidino lipoglycopeptides

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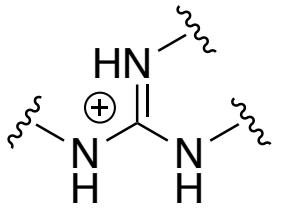
- The guanidine group carries a strong positive charge



Guanidine

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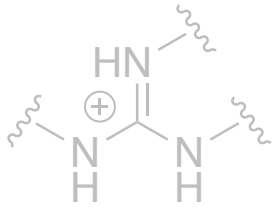
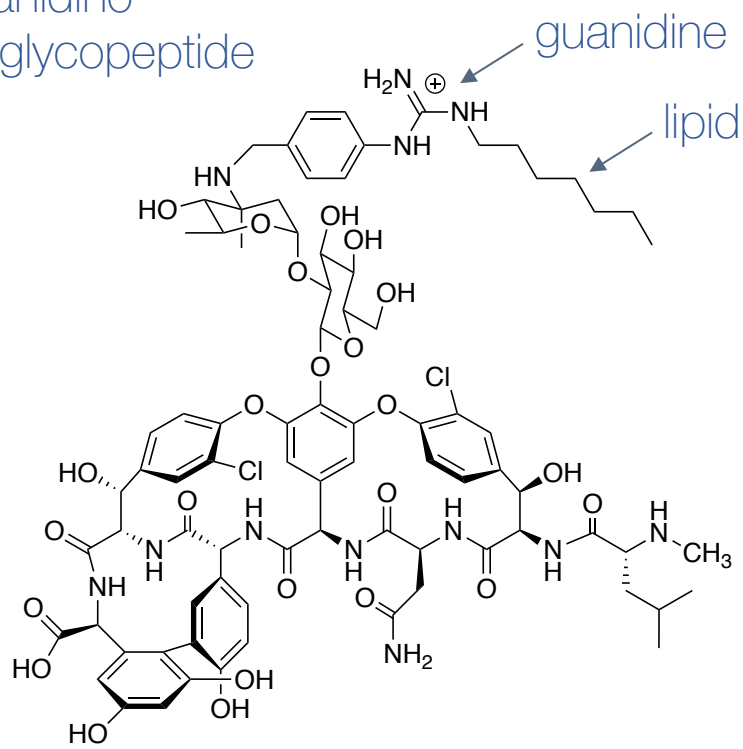


Guanidine

- In 2019 the Martin group (Leiden University) discovered the guanidino lipoglycopeptides:

Guanidino lipoglycopeptide

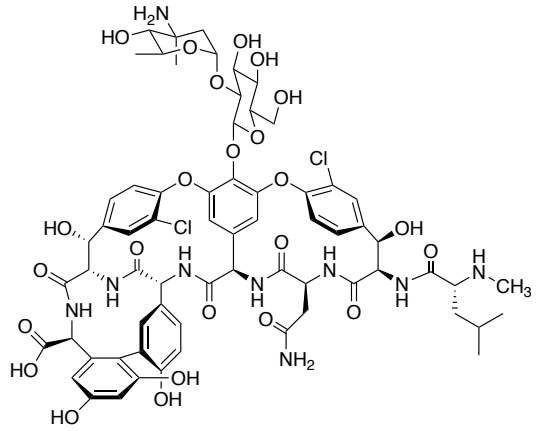


- Readily synthesized from vancomycin (scalable and cost effective)
- Extremely potent antibiotics

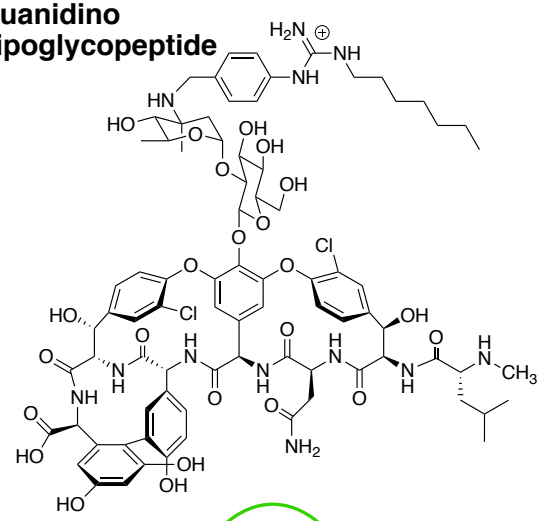
In vitro activity

- The guanidino lipoglycopeptides exhibit potent antibacterial activity (very low MIC values) superior to vancomycin:

Vancomycin



Guanidino Lipoglycopeptide



Strain		
MSSA	1	≤0.0078
MRSA	1	0.0156
VISA	8	0.0313
VRSA	>128	1
VSE	0.5	≤0.0078
VRE (vanB)	128	≤0.0078
VRE (vanA)	>128	2
SP	0.5	≤0.0078

MSSA ATCC29213; MRSA USA300; VISA LIM-2, NR-45881; VRSA HIP13419, NR-46413; VSE 980; VRE 7314 (vanB); VRE 155 (vanA); *S. pneumoniae* 153

Potent antibacterial effect

Cell-based assays

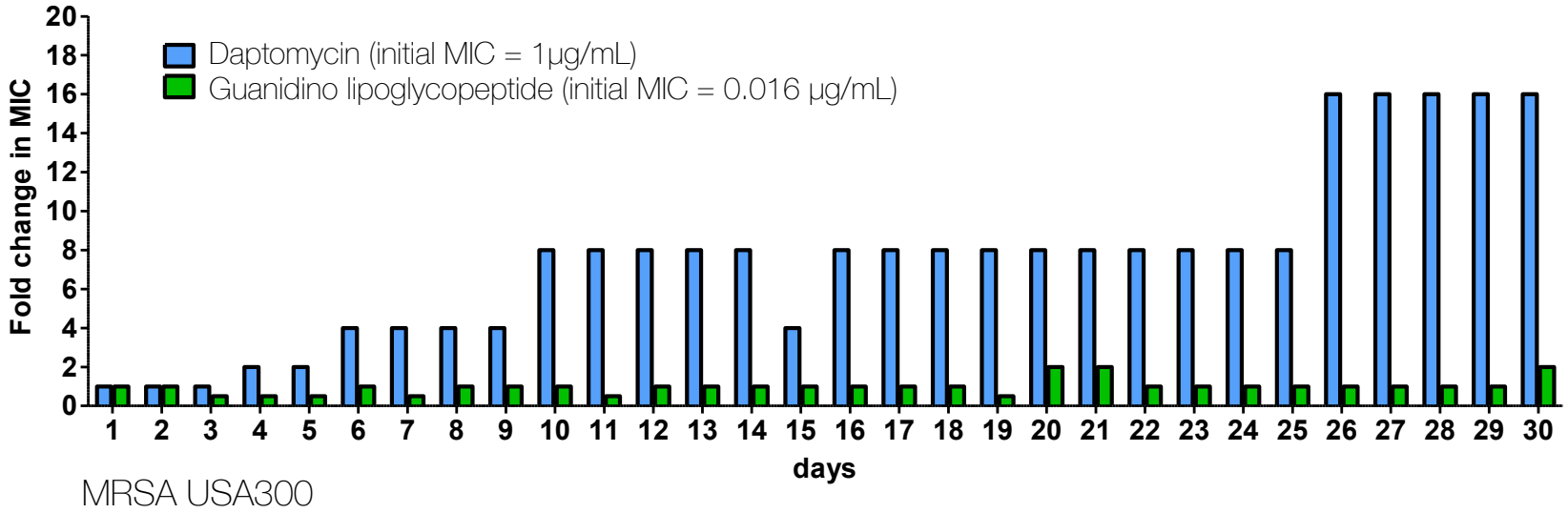
- Guanidino lipoglycopeptide found to be non-haemolytic up to 1000x MIC. Clinically-used oritavancin exhibits significant hemolysis (>40% at 1000x MIC)
- Guanidino lipoglycopeptide not toxic to kidney cells at 100 μM. Clinically-used oritavancin and telavancin both demonstrate toxicity:

Compound	**Cytotoxicity CC_{50} (μM)**
Vancomcyin	>100
Guanidino lipoglycopeptide	>100 ✓
Telavancin	24
Oritavancin	3.5

MTT assay with HEK293 cells, 24-hour incubation.

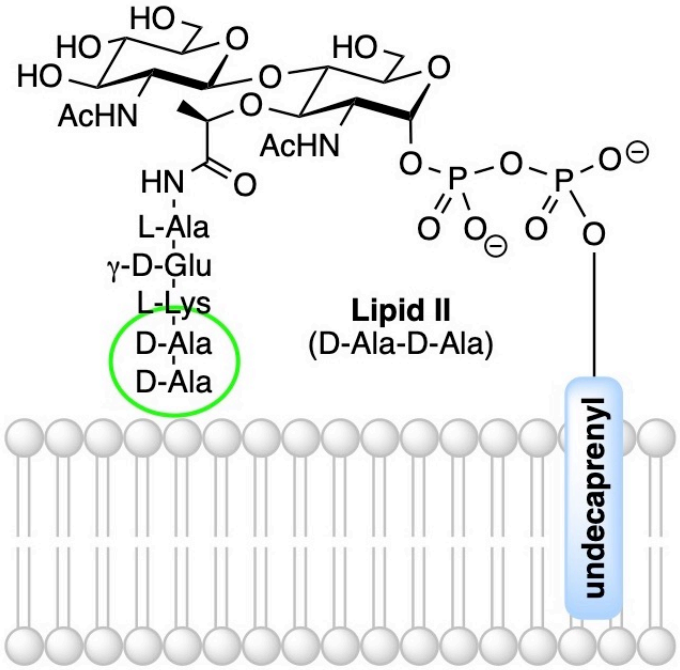
Resistance studies

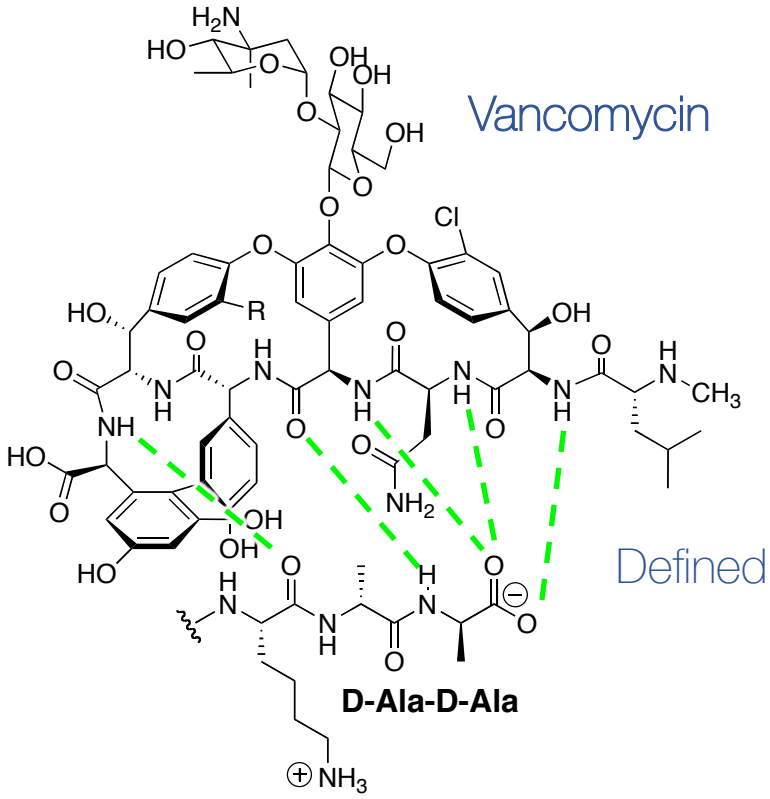
- Serial passage assays indicate guanidino lipoglycopeptides have low propensity to induce resistance compared with the clinically-used daptomycin:



Lipid II and vancomycin resistance

- Vancomycin binds the D-Ala-D-Ala motif of Lipid II with high affinity:





Defined hydrogen bond network

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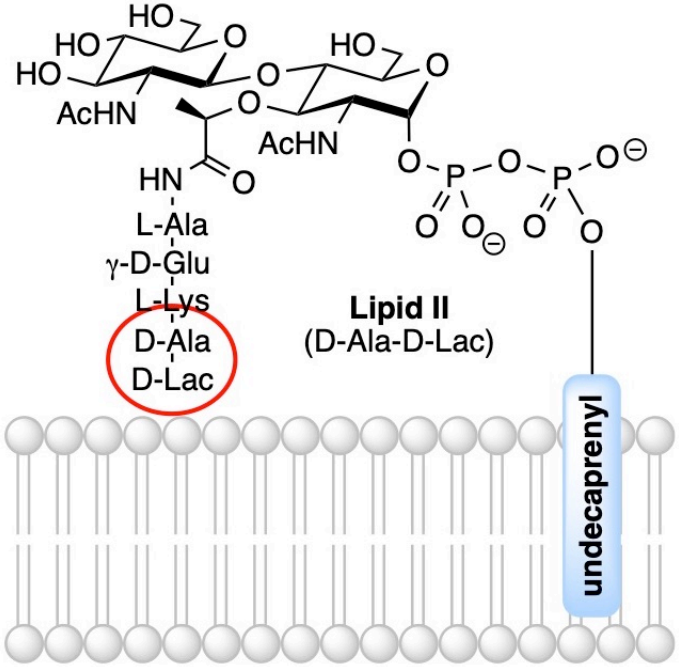
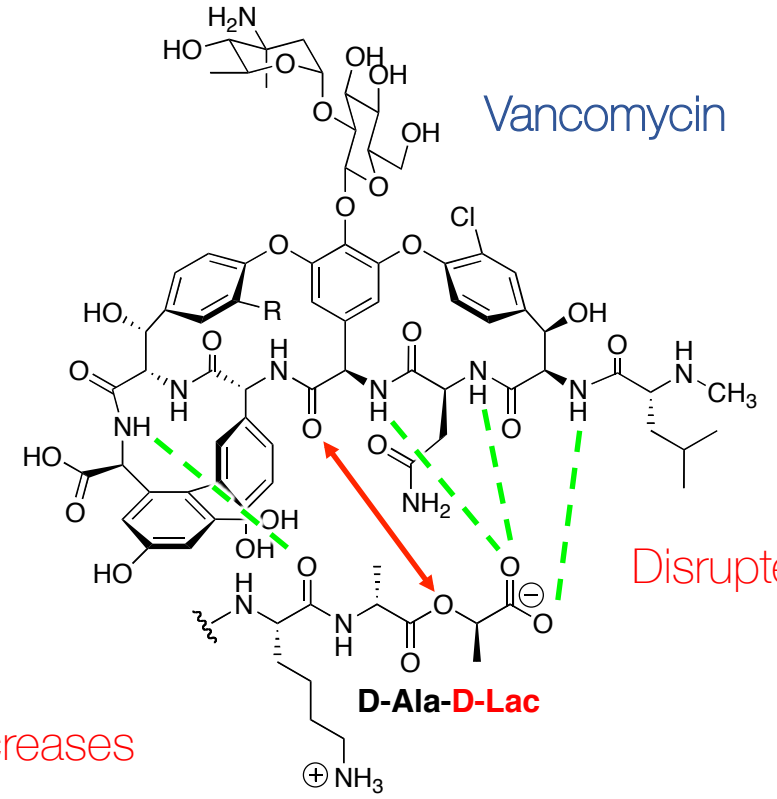




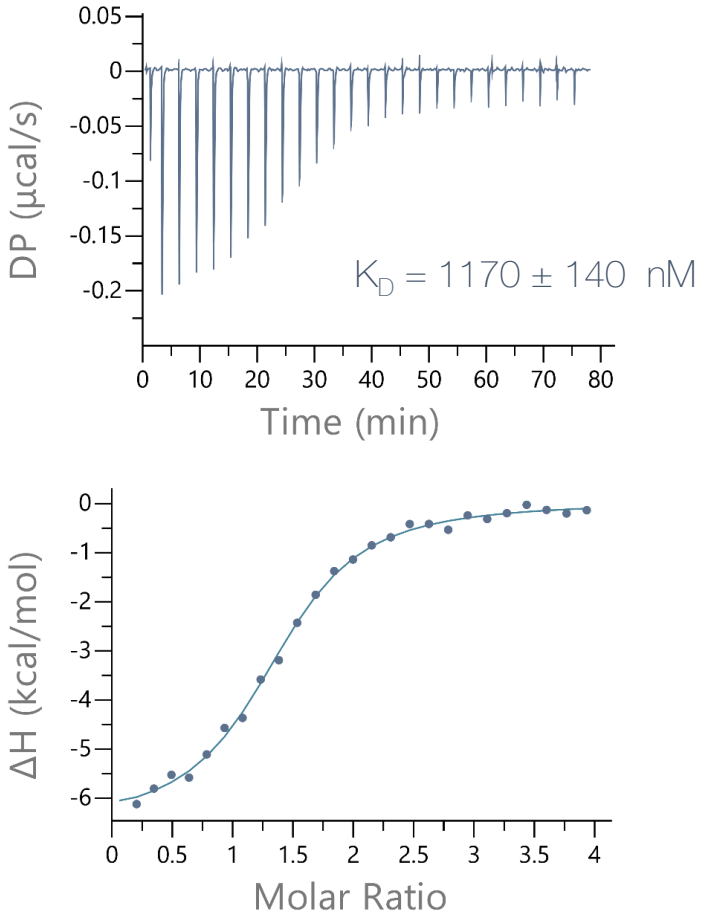
Disrupted hydrogen bond network

- D-Alanine to D-Lactate mutation decreases binding affinity 1000x
- One less H-bond = vancomycin resistance

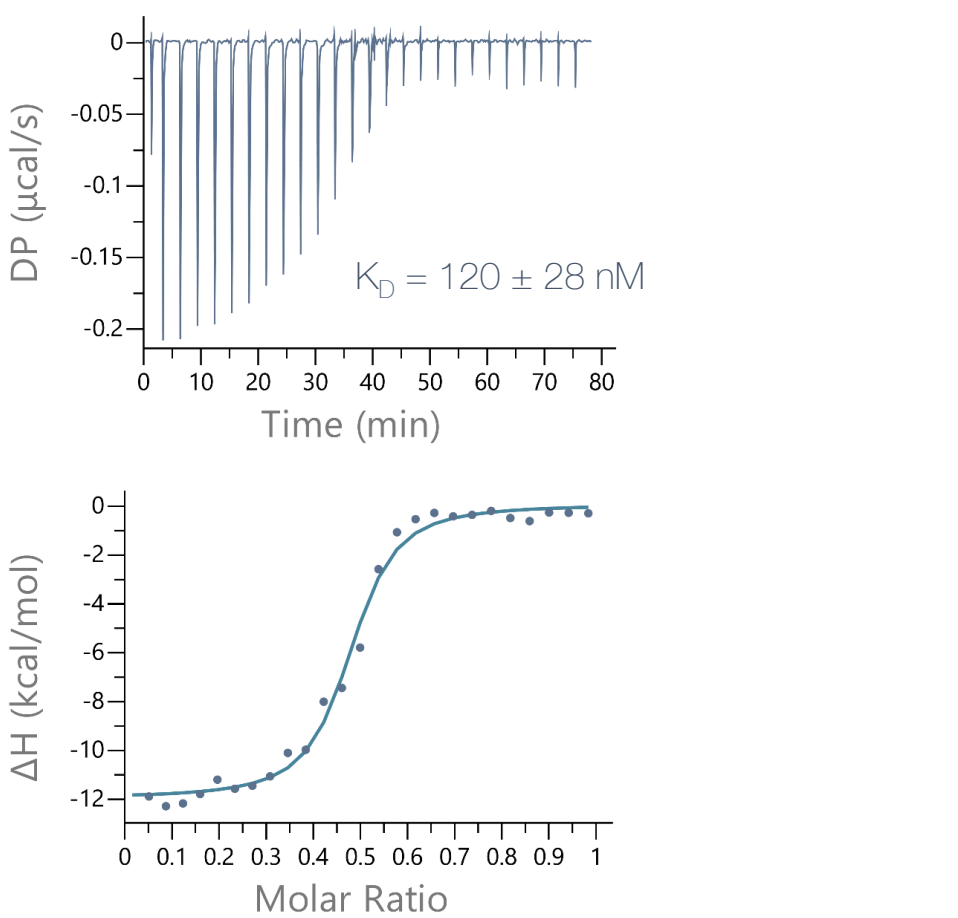
Mechanistic studies: Lipid II binding with ITC

- Guanidino lipoglycopeptides show superior binding to both wild-type and D-Ala-D-Lac forms of lipid Lipid II:

Vancomycin + WT Lipid II



Guanidino analogue + WT Lipid II



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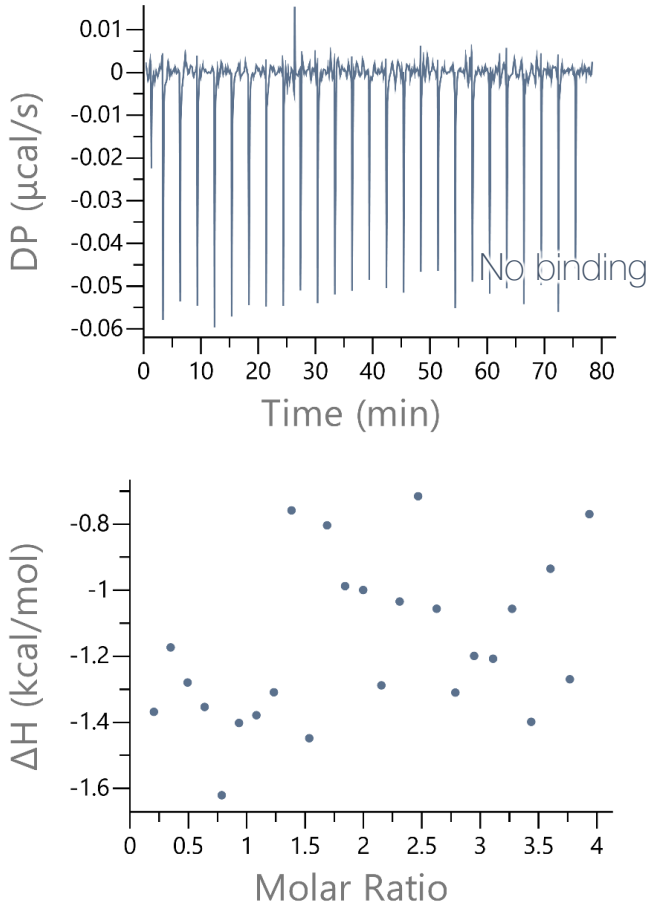

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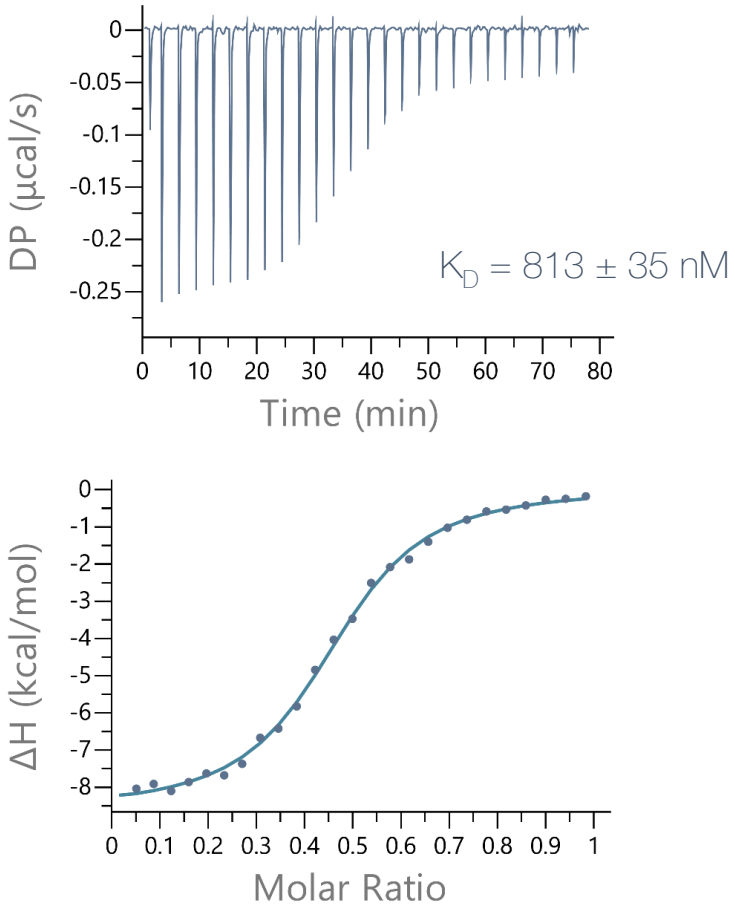

In vivo studies: Tolerability, PK & efficacy

- Guanidino lipoglycopeptide well tolerated in mice as high as 100 mg/kg
- Favorable pharmacokinetics ($t_{1/2}$ = 1.22 hours) with blood concentrations maintained above MIC >8 hours when dosed at 3 mg/kg

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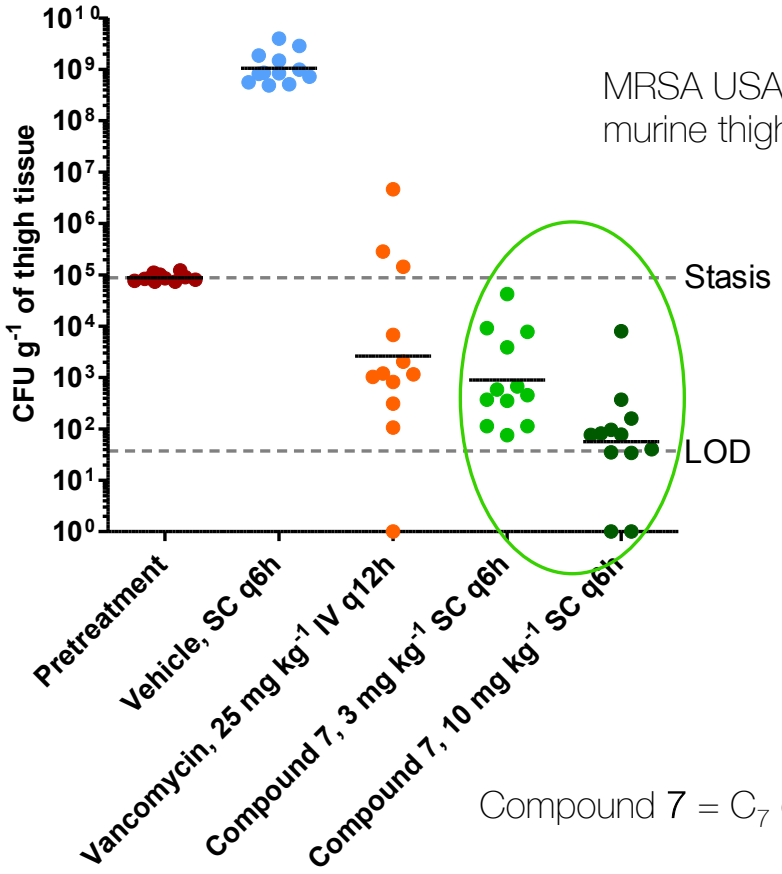


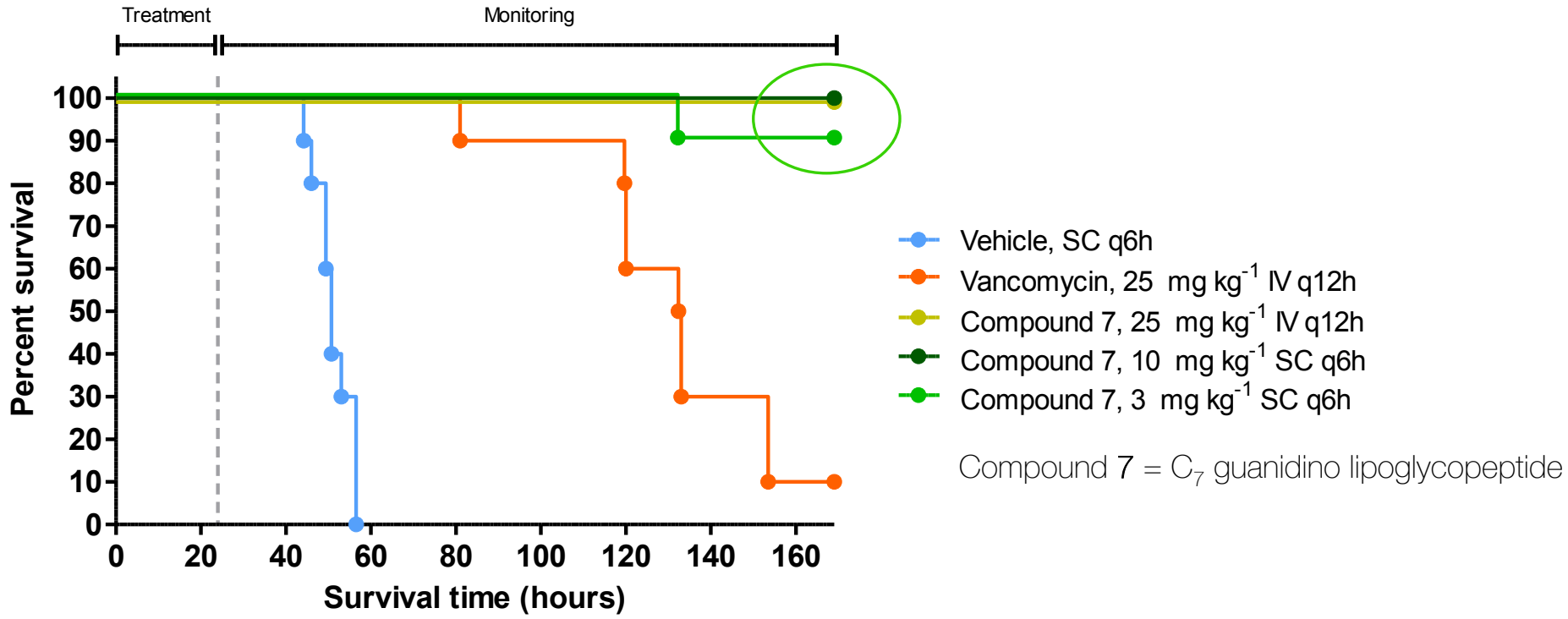
MRSA USA300 neutropenic murine thigh infection model

Guanidino lipoglycopeptides significantly more effective than vancomycin in vivo

Compound 7 = C_7 guanidino lipoglycopeptide

In vivo studies: Sepsis survival study

- 7-day survival study with immunocompetent mice infected with *S. aureus* demonstrates superiority of guanidino lipoglycopeptide vs vancomycin:



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Next steps

- Guanidino lipoglycopeptides outperform vancomycin and the other clinically used glycopeptides = *best in class potential*

Enhanced Activity + Lower Toxicity = Greater Therapeutic Window

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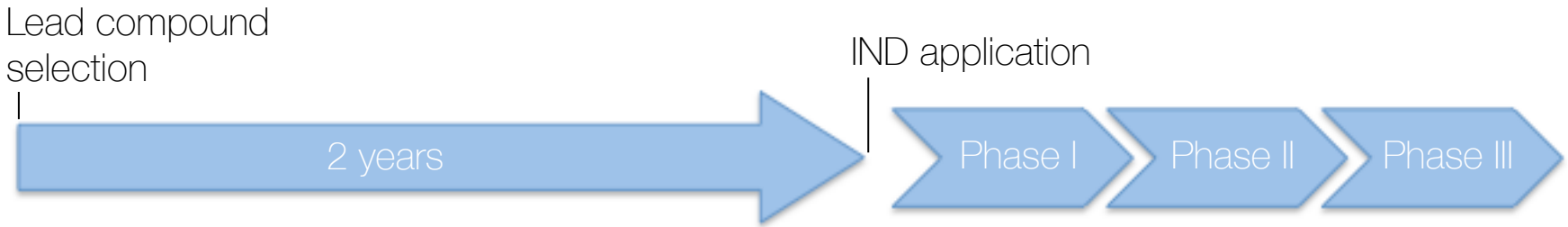
- Preclinical work required prior to IND application:



IND Enabling studies (€4-5M)

- Chemistry, manufacturing & controls (CMC)
- PK/PD
- Metabolism
- Toxicology

NACTAR

Stay tuned!

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- Roos Masereeuw (Utrecht)
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