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Polymyxins: a new hope in combating Gram-negative superbugs?

Polymyxins have emerged as an important last-line of defense against Gram-negative ‘superbugs’. Unfortunately, the effective use of polymyxins in the clinic has been hampered by their nephrotoxic side effects. Over the last 10 years various industry and academic groups across the globe have been trying to develop new polymyxins that are safer and more efficacious than the currently approved polymyxin B and colistin. However these drug discovery programs are yet to deliver a new and improved polymyxin drug into the clinic. In this piece we provide an overview of the current state of these polymyxin drug discovery programs from a medicinal chemistry perspective as well as some thoughts on how future drug discovery efforts may ultimately find success.

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Polymyxins versus ‘superbugs’

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Bad bugs no drugs

Antibiotic resistance has evolved into a global health crisis [1]. The dry antibiotic pipeline together with the increasing incidence of bac-

terial resistance in the clinic has been dubbed ‘the perfect storm’ [2,3]. The Centres for Disease Control and Prevention have alerted regulatory agencies, politicians and the pharmaceutical industry of the urgent need for the discovery of novel antibiotics [4]. Numerous hospitals globally have experienced outbreaks of infections caused by Gram-negative *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* that are resistant to all current antibiotics, including the last-line polymyxin B and colistin (polymyxin E) [5–8]. Unfortunately, for Gram-negatives no novel antibiotic classes have been introduced into the clinic over the last few decades. This potentially dire situation has forced clinicians and researchers to venture back into the past and resource ‘old’ antibiotics that were previously shelved. The primary example of this is the polymyxin class of antibiotics, which is the focus of this perspective. Polymyxins were first discovered in 1947 and introduced into the clinic in the late 1950s. However, early clinical experience showed

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that parenteral administration of the polymyxins led to cases of nephrotoxicity and neurotoxicity, and their clinical use decreased by the 1970s [9–12]. Over the last two decades, there has been a greatly renewed interest in polymyxins because of the increasing prevalence of multidrug-resistant (MDR) *P. aeruginosa*, *A. baumannii* and *K. pneumoniae*, which are resistant to all other available antibiotics [9–12]. The excellent antibacterial activity of the polymyxins comes with a catch 22: nephrotoxicity. Simply increasing daily doses of polymyxins is not an option because nephrotoxicity can occur in up to 60% of patients and is the major dose-limiting factor [13]. Even more worryingly, are the recent reports in *Lancet Infectious Diseases* of the emergence of plasmid-borne polymyxin resistance in these species [14,15], which implies resistance to these last-line antibiotics can now rapidly spread and no antibiotics are available for the treatment of life-threatening Gram-negative infections. As such there is a need to develop new polymyxin lipopeptides with improved safety and efficacy profiles over the existing drugs, polymyxin B and colistin.

Polymyxins, early medicinal chemistry efforts

Polymyxins are natural product lipopeptide secondary metabolites produced by the Gram-positive soil microbe *Paenibacillus polymyxa* [16]. In terms of their chemical structures, the polymyxins comprise a cyclic heptapeptide core linked to a linear tripeptide segment with an *N*-terminal fatty acyl tail (Figure 1) [17]. They are polycations at pH 7.4 owing to the 5 L- α,γ -diaminobutyric acid (Dab) residues. Polymyxin B and colistin, which are the only polymyxins used in the clinic, are actually mixtures of multiple structurally similar components, a consequence of the fact that they are obtained via bacterial fermentation (Figure 1). Since the 1970s, attempts have been made to modify the structures of the polymyxins to explore their structure–activity relationship (SAR) or improve their safety [17]. However, the majority of these early efforts were limited to semi-synthetic modifications to the peripheries of the polymyxin core structure, namely the acylation/alkylation of the side-chain amino groups of the Dab residue, or substitution of the *N*-terminal fatty acyl chain [17]. The limited scope of modifications was due in part to the lack of access to robust synthetic platforms that allow for the total synthesis of polymyxin lipopeptides, as well as limited understanding of polymyxin pharmacology. This body of work has been previously summarized in detail by our group and provided insights into their mode of action [17]. The initial cellular target of the polymyxins is the lipid A component of lipopolysac-

charide (LPS) in the bacterial outer membrane. This initial interaction involves an electrostatic interaction of the cationic Dab residues of the polymyxin molecule with the negatively charged phosphate groups of lipid A (Figure 2) [18]. The fatty acyl tail of polymyxins is also crucial for the bacterial killing activity [19], indicating that the hydrophobic interaction between the fatty acyl chain of polymyxins and lipid A plays a very important role in this first step in the action of polymyxins. Not surprisingly, many of the bacterial mechanisms of resistance to polymyxins are based on modifications to lipid A which reduce or abolish this initial electrostatic interaction. Modification of the phosphates of lipid A with positively charged moieties such as 4-amino-4-deoxy-L-arabinose (L-Ara4N) or phosphoethanolamine, that reduce the net negative charge of lipid A, thereby increasing resistance to polymyxins [20–27]. In terms of their nephrotoxicity it was found that complete removal of the *N*-terminal fatty acyl group afforded compounds with reduced potential for nephrotoxicity, however as discussed above this came at the cost of significantly reduced antibacterial activity compared with polymyxin B and colistin.

Recent polymyxin drug discovery programs

Over the last 10 years, various academic groups and pharmaceutical/biotech companies have undertaken extensive medicinal chemistry programs focused on discovering novel polymyxin lipopeptides with improved efficacy and safety profiles than the currently approved polymyxin B and colistin. These programs have been driven by the increasing use of the polymyxins in the clinic to combat MDR Gram-negative ‘superbugs’ as well as the advancement in polymyxin pharmacology over the last decade [13]. The ‘holy grail’ of these polymyxin drug development programs is to develop low nephrotoxic polymyxin lipopeptides. In its entirety this is not an infinitesimal task, given that little is known about the mechanism of nephrotoxicity, the molecular targets involved, and the structure–nephrotoxicity relationship of polymyxins. As a result, all of the polymyxin drug discovery programs to date have relied heavily on phenotypic screening, to guide drug design. Many of the recent polymyxin drug discovery programs undertaken by pharmaceutical and biotech companies have also continued to focus on semi-synthetic modifications of the core structure of polymyxin B and colistin [28–31]. This is driven mostly by the economics of large-scale peptide active pharmaceutical ingredient (API) manufacturing. While polymyxin B and colistin have complex cyclic peptide structures containing nonproteinogenic amino acid residues, they are a cheap source of starting material that could be

Figure 1. Structures of the major lipopeptide components of polymyxin B and colistin.

Figure 2. Model of polymyxin B, complexed with *Klebsiella pneumoniae* lipid A.

potentially converted to a new drug relatively easily in two to three steps. This is a very attractive approach to pharmaceutical and biotechnology companies involved in developing a new drug.

To date, the most advanced of these polymyxin drug discovery programs was undertaken by Cubist Pharmaceuticals, the company that developed and marketed the lipopeptide antibiotic daptomycin for treatment of Gram-positive infections. Their program was based on intellectual property originally developed by BioSource Pharmaceuticals [29]. The strategy involved removing the *N*-terminal fatty acyl groups of polymyxin B or colistin mixtures using novel semi-synthetic methodology to provide a single ‘polymyxin core’ of which the *N*-terminus was derivatized with novel halo-aryl groups. The idea behind these modifications was to reduce nephrotoxicity by decreasing the hydrophobicity of the *N*-terminal fatty acyl group (which has been identified as a nephrotoxicity ‘hot-spot’ in the polymyxin molecule), but leaving enough hydrophobicity to maintain antimicrobial activity. The lead compound to emerge from the Cubist program was the *N*-terminal 2-chlorophenylurea derivative, **CB-182,804** (Figure 3) [29,32]. In preclinical studies **CB-182,804** had a comparable *in vitro* antimicrobial profile to polymyxin B against target Gram-negative clinical isolates and showed efficacy and reduced nephrotoxicity in a monkey model. It was taken into a Phase I clinical trial in 2009, the only polymyxin drug discovery program to do so far to date, but has not progressed any further. Cubist has since discontinued this program and in 2014 the company was purchased by Merck Pharmaceuticals. One can only assume that toxicity issues were encountered

in the Phase I study for the program not to progress further.

Pfizer has also conducted a novel polymyxin discovery research program, focused on modification of the *N*-terminal fatty acyl group along with the modification of the Dab residue at position 3 [28,30]. It was initially discovered that substitution of the Dab residue at position 3 of polymyxin B with a diaminopropionic acid residue to give lipopeptide **5a** (Figure 3) resulted in a twofold improvement in MIC values compared with polymyxin B against *P. aeruginosa* and *A. baumannii*. Furthermore, screening of lipopeptide **5a** for *in vitro* nephrotoxicity utilizing human renal proximal epithelial cells showed a twofold decrease in cytotoxicity relative to polymyxin B. Further modifications to modulate the hydrophobicity of the *N*-terminal fatty acyl group of lipopeptide **5a** with novel substituted biphenyl and hetero-aromatic groups afforded their lead compound **5x**, which contains the *N*-terminal hetero-aromatic group, *N*-phenyl pyridone (Figure 3). These compounds were initially accessed through total synthesis but could also be accessed by semi-synthetic modification of polymyxin B [30]. Lead compound **5x** had a comparable *in vitro* antimicrobial profile to polymyxin B against polymyxin-susceptible strains as well a twofold to fourfold improved potency *in vitro* against polymyxin-resistant subpopulations of *P. aeruginosa* and *A. baumannii*. Most importantly, screening lipopeptide **5x** for *in vitro* nephrotoxicity utilizing human renal proximal epithelial cells revealed a greater than fivefold decrease in cytotoxicity relative to polymyxin B. While **5x** had a good nephrotoxicity profile in rats compared with polymyxin B, this did not translate to the dog. The variation observed with the animal nephrotoxicity data of lipopeptide **5x** was considered significant barriers for the further preclinical development [30]. To the best of our knowledge, Pfizer has since terminated its polymyxin discovery program.

Cantab anti-infectives, a UK-based biotech company with an active polymyxin drug discovery program, has also focused on modification of the *N*-terminus of polymyxin B [33,34]. Their drug design strategy has been to replace the *N*-terminal fatty acyl group and Dab residue at position 1 with novel cyclic and acyclic aminoacyl groups such as shown with the lipopeptide **D69** [34] (Figure 3). These compounds are derived from the polymyxin B nonapeptide (a product resulting from enzymatic cleavage of polymyxin B) and have shown improved *in vitro* antimicrobial activity and cytotoxicity against HK-2 cells compared with polymyxin B. In a 7-day rat nephrotoxicity study, they have shown a twofold to threefold reduction in the levels of *N*-acetyl- β -D-glucosamine

(NAG), albumin and cystatin (key renal biomarkers of kidney injury) relative to colistin [33]. This program is ongoing and their compounds are undergoing further preclinical evaluation. In a strategically different approach, the Finland-based biotech company Northern Antibiotics has developed a series of polymyxin analogs which possess only three positive charges compared with the five carried by polymyxin B and colistin [35–42]. The five positively charged Dab residues are thought to be a structural feature of the polymyxins that contributes to their nephrotoxicity. The most promising lead **NAB739** shares an identical cyclic core to that of polymyxin B, and a modified linear segment where the Dab residue at position 1 has been removed and the Dab residue at position 3 replaced with D-Serine (Figure 3) [37]. The substitution of the two Dab residues within the linear segment means the compound only carries three positive charges at physiological pH. **NAB739** displayed good *in vitro* and *in vivo* activity against *Escherichia coli* but not against *A. baumannii* or *P. aeruginosa*. Furthermore, **NAB739** was not active against polymyxin-resistant *E. coli* and *K. pneumoniae* [37–40]. Based on *in vitro* studies, the toxicity of **NAB739** appears to be lower than polymyxin B and colistin [37,41]. In addition to **NAB739**, two additional Northern Antibiotics compounds are noteworthy, **NAB7061** and **NAB741** which do not possess potent direct antibacterial activity; however, they retained the ability to permeabilize the Gram-negative outer membrane [37,39]. This permeabilizing activity facilitates the intracellular access to target sites of hydrophobic antibiotics or large hydrophilic antibiotics which normally permeate poorly through or are excluded by the Gram-negative outer membrane. Accordingly, Northern Antibiotics purports that **NAB739**, **NAB741** and **NAB7061** may be useful as sensitizing agents for combination therapy. To this end these compounds are currently undergoing preclinical development as antibiotic potentiators by Spero Therapeutics.

The Cooper group at the University of Queensland (UQ) has developed new synthetic methodology for the preparation of polymyxin lipopeptides where the key cyclization step is also carried out on the solid phase. This makes this methodology amenable to high-throughput parallel synthesis of polymyxin lipopeptide libraries, which may greatly facilitate the discovery of new polymyxin lipopeptides [43]. To date the group has reported the synthesis and *in vitro* screening of 32 polymyxin lipopeptides, a mixture of new polymyxin lipopeptides and previously published analogs to validate their synthetic methodology. With their new polymyxin lipopeptides, SAR

data revealed that the inversion of the L-Dab¹ to D-Dab¹ was poorly tolerated with respect to the antibacterial activity whereas substitution of Dab³ with Gly or Ser had little or no impact on antibacterial activity. Furthermore, substitution of the *N*-terminal fatty acyl group with biphenyl or biphenyl ether groups improved activity against polymyxin-resistant strains. *In vitro* toxicity assessments revealed that UQ compound **38** (Figure 3) was the least cytotoxic in HepG2 and HEK293 cell lines with a Cytotoxic Concentration₅₀ >300 μM, and similarly showed low LDH and GGT release from primary human kidney cells compared with polymyxin B and colistin. This program is ongoing and they plan to investigate further combinations of the promising modifications identified.

Our team at Monash University has looked beyond the peripheries of the polymyxin scaffold and extensively explored the SAR and structure–toxicity relationships (STR) of modifications to positions 6 and 7, which are hydrophobic residues (Phe and Leu) in polymyxin B and colistin [44]. Previous polymyxin drug discovery programs have paid very little attention to section of the polymyxin scaffold, hence not a lot was known about the role of the position 6 and 7 residues in terms of their contribution to activity and nephrotoxicity. These residues are not amenable to semisynthetic modification like the Dab residues; therefore, they need to be completely substituted out, in order to be modified. This was achieved using total synthesis methodology developed in our laboratory [44]. We have also employed an SAR-based mechanistic model (developed in-house) to help guide the design of our novel polymyxin lipopeptides, the first program to do so [44]. From his work we have discovered that incorporation of amino residues with long hydrophobic alkyl or biphenyl side chains, such as octyl-glycine and biphenylalanine at position 6 or 7, results in increased activity against polymyxin-resistant isolates [44]. With compounds such as **FADDI-002** (Figure 3) a 32-fold improvement in potency was obtained against polymyxin-resistant clinical isolates of *P. aeruginosa*. Serendipitously, these modifications to positions 6 and 7 of the polymyxin core also afforded compounds with activity against MDR Gram-positive *Staphylococcus aureus* and *Enterococcus faecium* which are intrinsically resistant to polymyxins. It is proposed that these modifications help overcome polymyxin resistance (due to L-Ara4N modification of the lipid A phosphates) by accentuating the hydrophobic interactions of the position 6 and 7 residues and the fatty acyl chains lipid A. Further exploration of modifications to position 6 and 7 has found that the hydrophobicity of the residues at these

two positions also plays a role in the nephrotoxicity of the polymyxins. Compounds such as **FADDI-100** which have decreased hydrophobicity at position 6 and 7 relative to polymyxin B and colistin, showed no histological damage in a mouse nephrotoxicity model, whereas polymyxin B and colistin showed significant damage [45]. Furthermore, these lipopeptides demonstrated comparable or improved *in vitro* and *in vivo* efficacy to polymyxin B and colistin. To date, exploring ‘outside the box’ and looking internally at modifications to positions 6 and 7 of the polymyxin

core has generated very interesting and potentially promising compounds. This work is ongoing and these novel polymyxin lipopeptides are undergoing further preclinical evaluation.

Conclusion & future perspective

In wake of our increasing understanding of polymyxin pharmacology, mode of action and toxicity mechanisms, recent medicinal chemistry efforts have yielded a number of promising novel polymyxin lipopeptides, with improved activity against polymyxin-

Figure 3. Examples of novel polymyxin lipopeptides from recent polymyxin drug discovery programs (cont. from facing page). Modifications to the polymyxin scaffold are highlighted in red.

resistant strains and nephrotoxicity profiles compared with polymyxin B and colistin. However, none of these new polymyxins are yet to make it into the clinic or look like a chance of doing so anytime in the near future. In terms of structure–toxicity relationships, these medicinal chemistry programs have highlighted that the *N*-terminal fatty acyl chain and the five positive charges represent nephrotoxicity ‘hot-spots’ around which medicinal chemistry efforts have been based in order to heal the Achilles’ heel of polymyxins, dose-limiting nephrotoxicity. Nevertheless, trying to re-engineer the polymyxins by focusing on only the *N*-terminal fatty acyl group or the Dab side-chains alone has yet to deliver a compound beyond Phase I. More fruitful results might be obtained by investing in drug design strategies that look beyond the peripheries of the polymyxin scaffold and examine all the amino acid residues within the polymyxin core structure. Several of the polymyxin drug discovery programs discussed here have started to venture into the core of the polymyxin, which has given rise to interesting novel polymyxins. The findings from all medicinal chemistry efforts to date suggest that the structural features of polymyxins that confer antibacterial activity are often inextricably linked to their nephrotoxicity. Moving forward, there is a need to further develop our understanding of the molecular mechanisms underlying the renal uptake disposition and toxicity of polymyxins, in order to find ways to structurally uncouple their antimicrobial activity from their nephrotoxicity. Adopting a target based or structure-based drug design approach rather

than relying solely on phenotypical screening to guide drug design, may be required in order to overcome nephrotoxicity. Furthermore, when it comes to screening for nephrotoxicity there appears to be a disconnect between what is observed *in vitro* with cell-based assays and what is observed *in vivo*. Pfizer highlighted this with their polymyxin drug development work [30]. The Cooper group at the University of Queensland has also discussed the inadequacies of current cell based assays to assess human nephrotoxicity [43,46]. Recently our team at Monash University has published work which showed a significant difference between the *in vitro* and *in vivo* nephrotoxicity observed for the major lipopeptides found in polymyxin B and colistin [47]. Reliance on cell cytotoxicity assays for screening compounds may lead to erroneous decisions in hit identification and lead selection studies. Therefore, it will be key for future polymyxin drug discovery programs to thoroughly address this issue if they are to be a chance of successfully moving new polymyxin lipopeptides beyond the preclinical stage into the clinic.

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Executive summary

Bad bugs no drugs

- Options for the treatment of multidrug-resistant (MDR) Gram-negative infections are very limited.
- Polymyxin and colistin, old antibiotics discovered over 60 years ago, are the last-line therapy against MDR Gram-negative 'superbugs'.
- Effective use of the polymyxins in the clinic is hampered by their potential to cause nephrotoxicity.
- There is a need to develop new safer polymyxin analogues.

Polymyxins, early medicinal chemistry efforts

- Polymyxins are amphipathic cyclic lipopeptides.
- Early structure–activity relationship studies were limited and mainly focused on the 'outer' parts of the polymyxin scaffold, namely the N-terminal fatty acyl group.
- Early medicinal chemistry efforts provided insights into the polymyxin mechanism of action.

Recent polymyxin drug discovery programs

- The N-terminal fatty acyl groups and positively charged Dab residues have been identified as nephrotoxicity 'hot spots' in the polymyxin scaffold.
- Industry based polymyxin drug discovery programs have continued to focus on modifications to the N-terminal region especially the N-terminal fatty acyl group.
- No new polymyxin analogues have made it beyond Phase I clinical trials.
- It is difficult to structurally 'un-couple' antibacterial activity from nephrotoxicity.
- Understanding of the mechanisms of polymyxin nephrotoxicity is limited and more research in this area will significantly help polymyxin drug discovery programs.
- Examining structural modifications beyond the identified nephrotoxicity 'hot spots' in the polymyxin scaffold may be needed in order to generate new safer polymyxins that have comparable efficacy to polymyxin B and colistin.

References

- Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *P T* 40(4), 277–283 (2015).
- Shlaes DM. Antibiotics: The Perfect Storm conclusions. In: *Antibiotics: The Perfect Storm*. Springer, The Netherlands, 97–99 (2010).
- Shlaes DM, Spellberg B. Overcoming the challenges to developing new antibiotics. *Curr. Opin. Pharmacol.* 12(5), 522–526 (2012).
- Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States (2013). www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf
- Falagas ME, Rafailidis PI, Matthaïou DK, Vrtzili S, Nikita D, Michalopoulos A. Pandrug-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections: characteristics and outcome in a series of 28 patients. *Int. J. Antimicrob. Agents* 32(5), 450–454 (2008).
- Antoniadou A, Kontopidou F, Poulakou G *et al.* Colistin-resistant isolates of *Klebsiella pneumoniae* emerging in intensive care unit patients: first report of a multiclonal cluster. *J. Antimicrob. Chemother.* 59(4), 786–790 (2007).
- Falagas ME, Bliziotis IA. Pandrug-resistant Gram-negative bacteria: the dawn of the post-antibiotic era? *Int. J. Antimicrob. Agents* 29(6), 630–636 (2007).
- Valencia R, Arroyo LA, Conde M *et al.* Nosocomial outbreak of infection with pan-drug-resistant *Acinetobacter baumannii* in a tertiary care university hospital. *Infect. Control. Hosp. Epidemiol.* 30(3), 257–263 (2009).
- Evans ME, Feola DJ, Rapp RP. Polymyxin B sulfate and colistin: old antibiotics for emerging multiresistant gram-negative bacteria. *Ann. Pharmacother.* 33(9), 960–967 (1999).
- Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clin. Infect. Dis.* 40(9), 1333–1341 (2005).
- Landman D, Georgescu C, Martin DA, Quale J. Polymyxins revisited. *Clin. Microbiol. Rev.* 21(3), 449–465 (2008).
- Zavascki AP, Goldani LZ, Li J, Nation RL. Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. *J. Antimicrob. Chemother.* 60, 1206–1215 (2007).
- Velkov T, Roberts KD, Nation RL, Thompson PE, Li J. Pharmacology of polymyxins: new insights into an 'old' class of antibiotics. *Future Microbiol.* 8(6), 711–724 (2013).
- Liu YY, Wang Y, Walsh TR *et al.* Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect. Dis.* 16(2), 161–168 (2016).
- Webb HE, Granier SA, Marault M *et al.* Dissemination of the MCR-1 colistin resistance gene. *Lancet Infect. Dis.* 16(2), 144–145 (2016).
- Choi SK, Park SY, Kim R *et al.* Identification of a polymyxin synthetase gene cluster of *Paenibacillus polymyxa* and heterologous expression of the gene in *Bacillus subtilis*. *J. Bacteriol.* 191(10), 3350–3358 (2009).
- Velkov T, Thompson PE, Nation RL, Li J. Structure–activity relationships of polymyxin antibiotics. *J. Med. Chem.* 53(5), 1898–1916 (2010).
- Clausell A, Garcia-Subirats M, Pujol M, Busquets MA, Rabanal F, Cajal Y. Gram-negative outer and inner membrane models: insertion of cyclic cationic lipopeptides. *J. Phys. Chem. B Condens. Matter Surf. Interfaces Biophys.* 111(3), 551–563 (2007).

- 19 Ofek I, Cohen S, Rahmani R *et al.* Antibacterial synergism of polymyxin B nonapeptide and hydrophobic antibiotics in experimental gram-negative infections in mice. *Antimicrob. Agents Chemother.* 38(2), 374–377 (1994).
- 20 Helander IM, Kilpeläinen I, Vaara M. Increased substitution of phosphate groups in lipopolysaccharides and lipid A of the polymyxin-resistant *pmrA* mutants of *Salmonella typhimurium*: a 31P-NMR study. *Mol. Microbiol.* 11(3), 481–487 (1994).
- 21 Moskowitz SM, Ernst RK, Miller SI. PmrAB, a two-component regulatory system of *Pseudomonas aeruginosa* that modulates resistance to cationic antimicrobial peptides and addition of aminoarabinose to lipid A. *J. Bacteriol.* 186(2), 575–579 (2004).
- 22 Helander IM, Kato Y, Kilpeläinen I *et al.* Characterization of lipopolysaccharides of polymyxin-resistant and polymyxin-sensitive *Klebsiella pneumoniae* O3. *Eur. J. Biochem.* 237(1), 272–278 (1996).
- 23 Clements A, Tull D, Jenney AW *et al.* Secondary acylation of *Klebsiella pneumoniae* lipopolysaccharide contributes to sensitivity to antibacterial peptides. *J. Biol. Chem.* 282(21), 15569–15577 (2007).
- 24 Perez JC, Groisman EA. Acid pH activation of the PmrA/PmrB two-component regulatory system of *Salmonella enterica*. *Mol. Microbiol.* 63(1), 283–293 (2007).
- 25 Adams MD, Nickel GC, Bajaksouzian S *et al.* Resistance to colistin in *Acinetobacter baumannii* associated with mutations in the PmrAB two-component system. *Antimicrob. Agents Chemother.* 53(9), 3628–3634 (2009).
- 26 Beceiro A, Llobet E, Aranda J *et al.* Phosphoethanolamine modification of lipid A in colistin-resistant variants of *Acinetobacter baumannii* mediated by the PmrAB two-component regulatory system. *Antimicrob. Agents Chemother.* 55(7), 3370–3379 (2011).
- 27 Fernandez L, Gooderham WJ, Bains M, McPhee JB, Wiegand I, Hancock RE. Adaptive resistance to the “last hope” antibiotics polymyxin B and colistin in *Pseudomonas aeruginosa* is mediated by the novel two-component regulatory system ParR–ParS. *Antimicrob. Agents Chemother.* 54(8), 3372–3382 (2011).
- 28 Chen JM. Polymyxin derivatives useful as antibacterial agents. Pfizer Inc, US, 2012: WO 2012/168820 A1.
- 29 Leese RA. Antibiotic composition for the treatment of Gram-negative infections. Biosource Pharm, Inc., U.S., 2010: US 2010/0160215 A1.
- 30 Magee TV, Brown MF, Starr JS *et al.* Discovery of Dap-3 polymyxin analogues for the treatment of multidrug-resistant Gram-negative nosocomial infections. *J. Med. Chem.* 56, 5079–5093 (2013).
- 31 Saadi M, Duperchy E, Brown P, Dawson MJ, Wadman SN. Polymyxin Derivatives US, 2013: WO 2013/072695 A1.
- 32 Keith DD, Borders D, Curran W *et al.* Poster F1–1619 synthesis and activity of CB-182,804, a novel polymyxin analog active against clinically relevant Gram-negative bacteria. Presented at: 50th Interscience Conference on Antimicrobial Agents and Chemotherapy, MA, USA, 12–15 September 2010.
- 33 Brown P, Dawson M, Simonvic M, Boakes S, Duperchy E. Polymyxin derivatives and their use in combination therapy together with different antibiotics: WO 2014/188178 A1 (2014).
- 34 Brown P, Dawson M, Simonvic M *et al.* Polymyxin derivatives and their use in combination therapy together with different antibiotics: WO2015/135967 A1 (2015).
- 35 Ali FE, Cao G, Poudyal A *et al.* Pharmacokinetics of novel antimicrobial cationic peptides NAB 7061 and NAB 739 in rats following intravenous administration. *J. Antimicrob. Chemother.* 64(5), 1067–1070 (2009).
- 36 Vaara M. Novel derivatives of polymyxins. *J. Antimicrob. Chemother.* 68(6), 1213–1219 (2013).
- 37 Vaara M, Fox J, Loidl G *et al.* Novel polymyxin derivatives carrying only three positive charges are effective antibacterial agents. *Antimicrob. Agents Chemother.* 52(9), 3229–3236 (2008).
- 38 Vaara M, Sader HS, Rhomberg PR, Jones RN, Vaara T. Antimicrobial activity of the novel polymyxin derivative NAB739 tested against Gram-negative pathogens. *J. Antimicrob. Chemother.* 68(3), 636–639 (2013).
- 39 Vaara M, Siikanen O, Apajalahti J *et al.* A novel polymyxin derivative that lacks the fatty acid tail and carries only three positive charges has strong synergism with agents excluded by the intact outer membrane. *Antimicrob. Agents Chemother.* 54(8), 3341–3346 (2010).
- 40 Vaara M, Siikanen O, Apajalahti J, Frimodt-Moller N, Vaara T. Susceptibility of carbapenemase-producing strains of *Klebsiella pneumoniae* and *Escherichia coli* to the direct antibacterial activity of NAB739 and to the synergistic activity of NAB7061 with rifampicin and clarithromycin. *J. Antimicrob. Chemother.* 65(5), 942–945 (2010).
- 41 Vaara M, Vaara T. The novel polymyxin derivative NAB739 is remarkably less cytotoxic than polymyxin B and colistin to human kidney proximal tubular cells. *Int. J. Antimicrob. Agents* 41(3), 292–293 (2013).
- 42 Vingsbo Lundberg C, Vaara T, Frimodt-Moller N, Vaara M. Novel polymyxin derivatives are effective in treating experimental *Escherichia coli* peritoneal infection in mice. *J. Antimicrob. Chemother.* 65(5), 981–985 (2010).
- 43 Gallardo-Godoy A, Muldoon C, Becker B *et al.* Activity and predicted nephrotoxicity of synthetic antibiotics based on polymyxin B. *J. Med. Chem.* 59(3), 1068–1077 (2016).
- 44 Velkov T, Roberts KD, Nation RL, Wang J, Thompson PE, Li J. Teaching ‘old’ polymyxins new tricks: new-generation lipopeptides targeting gram-negative ‘superbugs’. *ACS Chem. Biol.* 9(5), 1172–1177 (2014).
- 45 Li J, Nation RL, Velkov T, Thompson PE, Roberts KD. Polymyxin derivatives as antimicrobial compounds. Monash University, 2015: WO 2015/149131 A1.
- 46 Huang JX, Blaskovich MA, Cooper MA. Cell- and biomarker-based assays for predicting nephrotoxicity. *Expert Opin. Drug Metab. Toxicol.* 10(12), 1621–1635 (2014).
- 47 Roberts KD, Azad MAK, Wang J *et al.* Antimicrobial activity and toxicity of the major lipopeptide components of polymyxin B and colistin: last-line antibiotics against multidrug-resistant Gram-negative bacteria. *ACS Infect. Dis.* 1(11), 568–575 (2016).