Activity of novel pyrrolocytosine protein synthesis inhibitors against multiresistant Gram-negative bacteria, including carbapenemase producers and those with MCR-1

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BACKGROUND
- Pyrrolocytosines are novel broad-spectrum protein synthesis inhibitors.
- They bind the 50S ribosomal subunit, attacking a site not exploited by other commercially available antimicrobials.
- Development of these compounds at Melinta Therapeutics aimed to maximise target affinity and reduce efflux.
- We evaluated 4 pyrrolocytosine analogues, RX-04 A, B, C and D (Figure 1), against: (i) multiresistant Enterobacteriaceae and non-fermenters with carbapenemases (ii) Enterobacteriaceae with MCR-1 (iii) P. aeruginosa with altered efflux (Table 1)
- MCR-1 is relevant because it reduces the negative charge of lipopolysaccharides, potentially affecting binding of poly-basic molecules such as the RX analogues as well as polymyxins.

METHODS
Organism characterisation
- MIC and carbapenemase genes were detected by PCR or sequencing.
- Efflux levels in P. aeruginosa isolates were inferred by interpretive reading of antibiogram data.

MIC determinations
- MICs of four RX analogues and comparators (amikacin, colistin, meropenem, colistin, meropenem and tigecycline) were determined by CLSI broth microdilution using pre-prepared plates (TreK Diagnostic Systems).

RESULTS
Enterobacteriaceae
- MICs for the 68 Enterobacteriaceae were uniform, with peaks at 1 mg/L for A and B and 2 mg/L for C and D (Figure 2).
- For RX-04A – the most active analogue - 0.768 (96%) MICs were 0.25-2 mg/L.
- For all analogues, MICs were lowest for E. coli and highest for S. marcescens (MICs 8-16 mg/L, were seen for one Enterobacteriaceae).
- MICs of RX-04A for 35/36 (97%) of CPE were within 2-fold of the MIC for E. coli ATCC 25922 (Figure 3). MIC differentials for analogues B-D were similarly small.
- MICs of RX-04A for all MCR-1 isolates (n=14) were within 2-fold of that for E. coli ATCC 25922 (Figure 4). MIC differentials for analogues B-D were similarly small.
- Acquisition of mcr-1 did not raise RX-04 MICs for E. coli DH10B (Table 2).

Non-fermenters
- MIC distributions of RX-04 analogues A-C straddled 1-8 mg/L for the 10 A. baumannii. RX-04A had the lowest MICs, with 7/10 values from 1-2 mg/L, D was the least active analogue (Figure 5).
- MICs for A. baumannii with OXA-31 carbapenemases were mostly higher than carbapenem susceptible isolates, but numbers were small and 3/5 OXA-23 isolates belonged to the same lineage (International Clone II; the other 2 were unique pulstypes).
- RX-04A again was the most active analogue against P. aeruginosa isolates, with MICs from 1-4 mg/L for 19/20 (95%) isolates. By contrast, 43% and 48% of the MICs were >16 mg/L, for analogues C and D, respectively (Figure 6).
- MICs of all analogues tended to be higher for P. aeruginosa with ‘normal’ vs. low efflux, but not further raised for those with elevated efflux.

CONCLUSIONS
- The four analogues had broad activity against Enterobacteriaceae and non-fermenters.
- RX-04A was the most active analogue with MICs mostly 1-2 mg/L for Enterobacteriaceae and A. baumannii and 1-4 mg/L for P. aeruginosa.
- Among Enterobacteriaceae, E. coli was the most susceptible species and S. marcescens the least susceptible.
- MICs for CPE and MCR-1 isolates were only 2-4-fold above an E. coli control.
- Acquisition of MCR-1 did not affect susceptibility to these basic molecules, despite affecting surface charge.
- RX-04A MICs were not raised for P. aeruginosa isolates with elevated efflux.
- MICs were slightly raised against multiresistant A. baumannii.
- Pyrrolocytosines showed promising activity against this challenging collection of multiresistant Gram-negative bacteria.

FUNDING
This study was funded by Melinta Therapeutics, Inc.

TABLE 1. Test panel

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<th>Species</th>
<th>Carbapenem susceptible</th>
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<th>MBL</th>
<th>OXA-23</th>
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<th>Normal Efflux</th>
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TABLE 2. MICs of RX-04A-D and colistin for E. coli DH10B and its mcr-1 transformant

<table>
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<th>Strain</th>
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<th>COL (mg/L)</th>
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<tr>
<td>Rx-04D</td>
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REFERENCES
2. Diameter G, Fedorko D, Antibiotic Carbapenam, 2016, Aug. 1:000:00:00-2:000:00:00.
3. CLSI Approved Standard M100. 2017, Melinta, RX-04A-D, 1:000:00:00-2:000:00:00.