TP-6076 is efficacious in a mouse pneumonia model with carbapenem-resistant Acinetobacter baumannii (CRAB) and retains potency against common tetracycline-resistance mechanisms.

**Introduction**

TP-6076 is a novel, fully synthetic, broad-spectrum tetracycline-like antibiotic that is being developed for the treatment of various and life-threatening bacterial infections, including those caused by pathogens otherwise resistant to current treatment options. It is highly active against difficult-to-treat resistant pathogens in overcoming studies, including carbapenem-resistant Enterobacteriaceae (CRE) and carbapenemase-producing A. baumannii (CRAB). TP-6076 was previously shown to have potent activity in vitro against CRE and CRAB and efficacy against carbapenem-resistant P. aeruginosa in a murine pneumonia model.[1, 2] Here, we report the TP-6076 efficacy and activity against Enterobacteriaceae and A. baumannii expressing tetracycline-resistant mechanisms and demonstrate efficacy against CRAB in a new pneumonia model.

**Methods**

**Clinical isolates**. Parental clinical isolates possessing diverse demographic, genetic, and phenotypic characteristics were obtained from Burdick Medical (Chattanooga, TN) or International Health Management Associates, Inc. (IHMA, Schaumburg, IL). Quality control (QC) strains were used to ensure laboratory standards per guidelines by the Clinical Laboratory Standards Institute (CLSI).[3] The QC strains were obtained from the American Type Culture Collection (Manassas, VA). TP-6076 and comparator were tested against a set of 6 A. baumannii strains with characterized antibiotic susceptibility[4] and a set of well-characterized, tetracycline-resistant strains to further evaluate the impact of gene overexpression on susceptibility.[5] Both sets were obtained from BioLabs (Newnham, UK). The set of 6 A. baumannii clinical isolates with novel tetracycline-resistant mechanisms and sequence variations were identified by comparing to the reference sequence from the NCBI database.

**Minimum inhibitory concentration (MIC)** assays with clinical isolates were performed essentially as described by the CLSI.[3] Parental-pseudogene strains were determined using CLSI breakpoint criteria[6] except for tigecycline, where POA breakpoints were used.[7]

**Susceptibility testing against A. baumannii** expressing overexpression of tetracycline resistance genes. To determine the impact of novel tetracycline resistance genes in A. baumannii, sequence encoding tet(Q), tet(M), tet(A), tet(B), tet(M1), tet(B4), tet(R1), and tet(R2) genes (but not tet(K)) in a negative control strain were replaced with TP-6076 from clinical isolates confirmed by prior sequencing to have these tetracycline-resistance determinants. Strains were cultured on agar plates containing expression system without any tetracycline (positive), or tetracycline (negative) at 100 μg/mL (positive) or 0 μg/mL (negative). Plates were transferred onto cell-ELD001 cells (Gebus, Bielefeld, Germany). Plates were used for an initial study of 2 × 2-DH (Bionostics, Invitrogen, Carlsbad, CA). Plates were transferred onto cell-ELD001 cells (Gebus, Bielefeld, Germany). Strains were tested against TP-6076 with the reported sequence from BioLabs (accession numbers: AE884177, AE907163, AE907164, AE907165, AE907166, AE907167, AE907168, AE907169, AE907170, AE907171). TP-6076 was compared to standard of reference, gentamicin, amikacin, and tobramycin.

**TP-6076 retina potency in vitro with carbapenem-resistant P. aeruginosa.** TP-6076 was found to be active against P. aeruginosa and P. aeruginosa expressing tetracycline-resistant mechanisms and demonstrated efficacy against CRAB in a new pneumonia model.

**Results**

- **Table 1. TP-6076 is potent in vitro against difficult-to-treat Gram-negative pathogens.**
- **Table 2. TP-6076 is efficacious in a mouse pneumonia model.**
- **Table 3. TP-6076 is active in vitro against A. baumannii and other Aminoglycosides In Vivo efficacy.**
- **Table 4. TP-6076 Therapeutic Dose In Vivo: MIC50/90; (Range) with University AIDS Laboratory (UAL) Expressions.**
- **Table 5. In vitro antibacterial activity of TP-6076 and Comparator against Monoclonal Characterized A. baumannii Differentially Resistant 3-clonal isolates Containing High EMR Variations.**

**Conclusions**

- TP-6076 is efficacious in vitro against difficult-to-treat Gram-negative pathogens, including those expressing tetracycline-specific and tetracycline-nonspecific resistance mechanisms, offering a promising new therapeutic option.
- In a mouse pneumonia model challenged with carbapenem-resistant A. baumannii, TP-6076 administration at 10 mg/kg restored lung bacterial burden in line with a 1.4 fold increase (p < 0.05) at 48 hours versus the start of dosing. Higher doses of 15 and 30 mg/kg reduced lung bacterial (p < 0.05) to, or even below, the level of detection by 24 hours, whereas 5 mg/kg of tigecycline did not reach this efficacy.
- TP-6076 continues to demonstrate its potential as a promising antibiotic for the treatment of infections caused by drug-resistant Gram-negative pathogens including carbapenem-resistant Enterobacteriaceae and A. baumannii.