Synergistic peptidomimetic-antimicrobial combinations to combat multidrug-resistant Gram-negative bacterial infections

Baker, Kristin Renee; Jana, Bimal; Franzyk, Henrik; Guardabassi, Luca

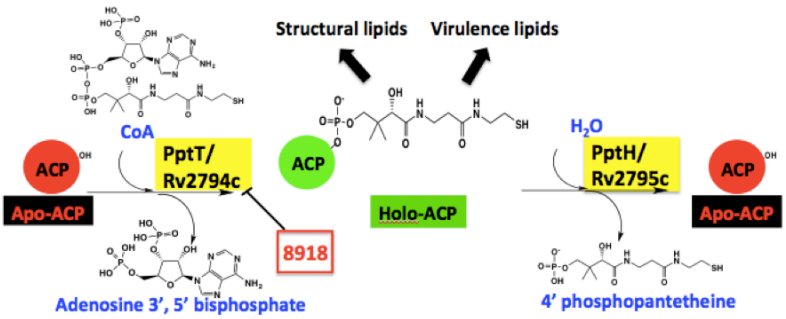
Platform Research Presentation – Wednesday, September 6, 2017 4:45 pm – 5:30 pm

Session Title: **AMR, Regulatory Science, and Drug Development**

Presentation Title: ***Identification of a PptT Inhibitor that Kills M. tuberculosis and a Novel Mechanism of Resistance***

Author Block: **E. Ballinger**[1], J. Mosior[2], K. Burns-Huang[1], B. Gold[1], C. Roubert[3], L. Goullieux[3], I. Blanc[3], S. Lagrange[3], L. Fraisse[3], T. Hartman[1], R. Morris[1], K. Rhee[1], D. Schnappinger[1], J. Sacchettini[2], C. Nathan[1]; [1]Weill Cornell Med., New York, NY, [2]TAMU, College Station, TX, [3]Sanofi, Lyon, France

Abstract Body: **Background:** Mtb is the leading cause of death from infection. Essential enzymes that have not yet been targeted in Mtb include those that synthesize Coenzyme A (CoA) and phosphopantetheinyl transferase (PptT). PptT transfers phosphopantetheine from CoA to acyl carrier proteins (Acps) involved in synthesis of cell wall lipids, siderophores and virulence factors. **Methods:** Screening identified 8918, a mycobactericidal compound related to proguanil, an antimalarial. Proguanil was inactive on Mtb, while 8918 was inactive on *E. coli*, *S. aureus* and human cells. We isolated Mtb clones resistant to 8918, sequenced their genomes for genes of interest, made knockouts, assayed recombinant proteins, solved the co-crystal structure of the target with 8918, and tested 8918's effect on Mtb's metabolome, lipidome and survival in mice. **Results:** Mutation, enzymatic and crystallographic evidence identified PptT (Rv2794c) as the target of 8918. 8918 reduced Mtb's mycolates, mycobactins and phthiocerol dimycocerosates and blocked growth in mice. Resistance was also conferred by mutations in *rv2795c*, encoding an enzyme of unknown function, as phenocopied by deletion of *rv2795c*. Rv2795c reversed the increase in mass that accompanied phosphopantetheinylation of an Acp. **Conclusions:** PptT can be inhibited to a degree that kills Mtb. The mycobactericidal effect of 8918 depends on the action of a new enzyme, hypothesized to be a phosphopantetheinyl hydrolase (PptH) that counteracts PptT. Loss of function in an enzyme that antagonizes the function of the drug target is a new mechanism of antimicrobial resistance.



Platform Research Presentation – Wednesday, September 6, 2017 4:45 pm – 5:30 pm

Session Title: **AMR, Regulatory Science, and Drug Development**

Presentation Title: ***Inhibitors of Nucleotidyltransferase Superfamily Enzymes asa Novel Antibacterial Agent***

Author Block:

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Abstract Body:

Background: Novel antibiotics are urgently needed. The nucleotidyltransferase superfamily (NTS) includes many nucleic acid-processing enzymes. We hypothesized that bacteria would be sensitive to NTS inhibitors.
Methods: We screened for growth inhibition against *E. coli*, *Staphylococcus saprophyticus*, *S. aureus, Acinetobacter baumannii*, *Pseudomonas aeruginosa* and patient-derived multi-drug resistant (MDR) bacteria. Preliminary hits were assessed for minimum inhibitory concentrations (MIC_{80}) and cytotoxicity (CC_{50}) against human hepatoma cells. The top 5 compounds were selected for MIC_{80} evaluation against MDR bacteria. **Results:** 254 NTS compounds were screened. For gram-positive bacteria, compounds #43 (ciclopox-like), #53, 54 (tropolone), #51, 62, 63, 282, 283, 285 (benzoylated tropolones), #46, 262 (α-hydroxytropolones) and #140 (Elvitagravir derivative) inhibited ≥80% growth of *S. aureus* (*methicillin-sensitive,* MSSA) and/or *S. saprophyticus* at <20 µM with CC_{50} >50 µM. Compounds #63 and 285 inhibited 22 *methicillin-resistant Staphylococcus aureus* (MRSA) strains with similar MIC_{80} values as against MSSA strains. For gram-negative rods, #284 and 363 inhibited *E. coli* and MDR-*E.coli*, as well as other MDR-enterobacteriaceae, with MIC_{80} ≤30 µM. #261 inhibited MDR *A. baumanni* with MIC_{80} ≤ 30 µM and CC_{50} >50 µM. The MDR bacteria have variable drug resistant mechanisms. This indicates these inhibitors, and probably similar analogs, act against novel antibacterial target(s). **Conclusions:** NTS inhibitors from multiple chemotypes have antibacterial activity and they inhibit bacterial replication by a mechanism distinct from existing antibiotics.

Platform Research Presentation – Wednesday, September 6, 2017 4:45 pm – 5:30 pm

Session Title: **AMR, Regulatory Science, and Drug Development**

Presentation Title: ***Molecular Bases of Antibiotic Translocation Across Outer Membrane porins***

Author Block: **M. Masi**, J-M. Pagès; Aix-Marseille Univ., Marseille, France

Abstract Body: **Background:** Hydrophilic antibiotics preferentially use non-specific porins, channel-forming proteins in the outer membrane of Gram-negative bacteria, to gain access to the cell interior. Two major porin-based mechanisms for antibiotic resistance have been reported: alterations of the porin expression or altered function due to specific mutations reducing permeability.
In *Escherichia coli*, OmpF is considered a major pathway for the translocation of ß-lactam antibiotics and newer generations of quinolone antibiotics. The X-ray structure of OmpF reveals a homotrimer, with each monomer forming a separate ß-barrel composed of sixteen ß-strands spanning the outer membrane (Fig. 1A and 1B). A key feature in the structure of OmpF is the presence of a constriction region due to loop L3, which folds back into the channel, forming both a steric and electrostatic hindrance (Fig. 1C). This zone is characterized by a strong transversal electric field, generated by the negatively charged residues D113 and E117 on the L3 side facing a cluster of positively charged arginines (R42, R82, and R132). **Methods:** In this work, we investigated the impact of porin structure on antibiotic permeability by using complementary *in vivo* approaches. Specifically, we characterized translocation of ß-lactam antibiotics across the OmpF and OmpC orthologs from four Gram-negative “superbugs”. **Results:** - *E. coli* OmpF and OmpC are good models to study the permeation of β-lactam antibiotics; - Low permeation of compounds across OmpC is mainly due to charge and size penalties; - Our investigation using resazurin-based viability assays in porinless *E. coli* has proven to be a fast and reliable method to study the permeation of unlabeled antibiotics across individual porin. **Conclusions:** Improved understanding of porin specificity, the nature of porin-antibiotic interactions, and the structure-kinetics of porin translocation would facilitate the design of new antibiotics that can reach high intracellular concentration.

Platform Research Presentation – Thursday, September 7, 2017 4:30 pm – 5:15 pm

Session Title: **Global Strategies, Microbiology, PK-PD, and Special Populations**

Presentation Title: Pharmacokinetics and Comprehensive Analysis of Tissue Distribution of Eravacycline Following Intravenous Administration in Rabbits

Author Block: **V. Petraitis**[1], R. Petraitiene[1], B. W. Maung[1], J. Newman[2], M. Olesky[2], A. Mutlib[3], X. Niu[3], M. Satlin[1], T. J. Walsh[1]; [1]Weill Cornell Med. of Cornell Univ., New York, NY, [2]Tetraphase Pharmaceuticals, Watertown, MA, [3]Frontage, Exton, PA

Abstract Body: **Background:** Eravacycline (ERV) (7-fluoro-9-pyrrolidinoacetamido-6-demethyl-6-deoxytetracycline or TP-434) is a novel, fully-synthetic fluorocycline of the tetracycline class that is designed to retain activity against the two main acquired tetracycline-specific resistance mechanisms: drug efflux pumps and ribosomal protection. ERV is particularly active against several life-threatening multidrug-resistant pathogens that are rapidly emerging worldwide, including *Acinetobacter baumannii* and carbapenem-resistant *Enterobacteriaceae*. We characterized the plasma pharmacokinetics and tissue distribution of ERV in rabbits after multiple dosing in order to establish a AUC/MIC relationship to tissue concentrations and to bridge that relationship to human dosing as a foundation for potential investigation in treatment of experimental bacterial infections. **Methods:** ERV was administered to NZW rabbits at 0.5, 1, 2, and 4 mg/kg IV QD with a 5-10 min infusion (n = 20 (4/dosage group)) for 6 days. Plasma PK and tissue concentrations were analyzed at the end of the study. Infusion time was 10 min for all rabbits.

Results:

Dose (mg/kg)	$AUC_{(o-24)}$ (hr·ng/mL)	C_{max} (ng/mL)	CL (L/hr/kg)	V_{ss} (L/kg)
0.5	2,529 ± 338	997 ± 27.7	0.20 ± 0.03	1.39 ± 0.14
1	5,787 ± 854	2,035 ± 462	0.18 ± 0.03	1.19 ± 0.07
2	10,871 ± 428	4,102 ± 283	0.18 ± 0.01	1.10 ± 0.02
4	29,888 ± 1,868	8,695 ± 1,537	0.12 ± 0.01	1.64 ± 0.21

Assuming an MIC of 1 µg/mL, for any one of MDR Enterobacteriaciae, these data indicates that *f*AUC/MIC ratios at 1 or 2 mg/kg Q12 IV of 3.3. to 6.7 would predict potent activity in plasma and in most tissue sites. Highest concentrations of ERV were achieved in kidney, spleen, and liver. Proportional levels of ERV were achieved in all 25 studied tissue/fluid sites. **Conclusion:** The plasma pharmacokinetic profile of 0.5 to 4 mg/kg of in NZW rabbits yields comparable exposure to that of humans and provides a platform for further study of eravacycline against MDR bacterial pathogens.

Platform Research Presentation – Thursday, September 7, 2017 4:30 pm – 5:15 pm

Session Title: **Global Strategies, Microbiology, PK-PD, and Special Populations**

Presentation Title: ***Relationship Between Gepotidacin (GSK1440944) Pharmacokinetics-Pharmacodynamics and Escherichia coli Resistance Amplification Prevention in a Hollow-Fiber Infection Model (HFIM)***

Author Block: **B. D. VanScoy**[1], S. M. Bhavnani[1], H. Conde[1], N. E. Scangarella-Oman[2], P. G. Ambrose[1]; [1]ICPD, Schenectady, NY, [2]GlaxoSmithKline, Collegeville, PA

Abstract Body:

Background: With the increase in drug-resistant pathogens associated with urinary tract infections (UTI), a focus of drug development is to optimally define the drug exposure that prevents the emergence of drug-resistant pathogens for investigational agents. Gepotidacin is a novel triazaacenaphthylene bacterial type II topoisomerase inhibitor in development for the treatment of patients with uncomplicated UTI. Studies using an *in vitro* HFIM were conducted to determine the magnitude of gepotidacin AUC:MIC ratio required to prevent the amplification of drug-resistant *Escherichia coli* subpopulations. This study was supported by GSK and funded through OTA HHSO100201300011C with HHS/BARDA. **Methods:** The challenge isolate, *E. coli* ATCC 13441 (MIC = 2 mg/L), was studied using an inoculum of 10^8 CFU/mL. Free-drug plasma concentration-time human profiles for gepotidacin PO doses administered to healthy volunteers that provided free-drug $AUC_{0\text{-}24}$ values ranging from 9.7 to 811 mg•h/L were simulated and compared to no-treatment and active (meropenem 1 g q8h) controls. Bacterial density was determined by evaluating bacterial populations on drug-free and antibiotic-containing agar plates at 2.5 and 4 x MIC over 10 days. The subset of isolates that grew on drug-containing agar plates was collected for MIC determination, with and without the presence of 40 mg/L of the broad-spectrum pump inhibitor, phe-arg-beta-napthalymide dihydrochloride. **Results:** An inverted-U shaped function described the relationship between drug-resistance amplification and free-drug AUC:MIC ratio for data from both sets of gepotidacin-containing agar plates. MIC values of the isolates collected from the drug-containing plates ranged from 8 to 32 mg/L. MIC values returned to the baseline value when examined in the presence of the broad-spectrum efflux pump inhibitor. Over the 10-day study period, a free-drug AUC:MIC ratio ≥ 275 prevented *E. coli* resistance amplification in the HFIM model. **Conclusions:** These data can be used to support the selection of gepotidacin dosing regimens for the treatment of patients with uncomplicated UTI arising from *E. coli* that minimize the potential for on-therapy drug-resistance amplification.

Platform Research Presentation – Thursday, September 7, 2017 4:30 pm – 5:15 pm

Session Title: **Global Strategies, Microbiology, PK-PD, and Special Populations**

Presentation Title: ***Synergistic Peptidomimetic-Antimicrobial Combinations to Combat Multidrug Resistant Gram-Negative Bacterial Infections***

Author Block: **K. Baker**, B. Jana, H. Franzyk, L. Guardabassi; Univ. of Copenhagen, Copenhagen, Denmark

Abstract Body: New therapeutic strategies are needed to address the growing challenge of antimicrobial resistance in multidrug-resistant (MDR) Gram-negative bacterial pathogens. Synthetic peptidomimetic compounds have high therapeutic potential as they are typically stable *in vivo*, being less subject to proteolytic degradation compared to traditional peptides. Here we employed a screening approach to identify peptidomimetic compounds that in combination induce susceptibility of Gram-negative pathogens to antimicrobials that are primarily used against Gram-positive pathogens. Forty-six synthetic peptidomimetic compounds were screened for synergy with azithromycin or rifampicin in clinical isolates of MDR *Escherichia coli* sequence type 131 (ST131), MDR *Klebsiella pneumoniae* ST258 and *Pseudomonas aeruginosa*. Exposure to three peptidomimetics at 0.5 µM inhibited growth of at least two strains/species in combination with antimicrobial concentrations approximating the clinical breakpoint for Gram-positive species. These three peptidomimetics were further screened with a panel of 22 drugs representing 10 clinically-used antimicrobial classes. However, the concomitant presence of each of these peptidomimetics at ≤1 µM did not reduce the minimal inhibitory concentrations (MIC) below clinical susceptible breakpoints for antimicrobials other than azithromycin and rifampicin. For all three MDR strains, antimicrobial synergy was confirmed by checkerboard assays with fractional inhibitory concentration indices (FICIs) ranging from 0.01 to 0.28 for all three peptidomimetics in combination with rifampicin, and from 0.04 to 0.28 for two peptidomimetics in combination with azithromycin. The third peptidomimetic-azithromycin combination had FICIs ranging from 0.25 to 0.37 in *E. coli*. All synergistic combinations reduced the antibiotic MICs below the clinical resistance breakpoints. Overall, this study identified three peptidomimetics that overcome intrinsic resistance to azithromycin and rifampicin in Gram-negative pathogens, making them *in vitro* susceptible to antibiotic concentrations that are achieved by standard dosage in clinical practice. The identified synergistic combinations will be further investigated to determine their cytotoxicity, strain variability and *in vivo* efficacy.