**56** Antibiotic treatment of exacerbation of chronic airways infection

S. Pattison1, E. Johnston1, D. Gibson2, D. Pappin3, J.S. Elborn1. 1Queen’s University Belfast, CF and Airways Microbiology Research Group, Belfast, United Kingdom; 2Queen’s University Belfast, Centre for Infection and Immunity, Belfast, United Kingdom; 3Cold Spring Harbor Laboratory, NY, United States

**Objectives:** Repeated cycles of pulmonary exacerbation of chronic infection cause tissue damage and progressive drop in lung function for CF patients so making effective treatment and prevention strategies imperative. Our study aims to better understand the response to antibiotics and identify biomarker targets suitable for improved therapeutic or preventative treatments.

**Methods:** Multidimensional LC-MS/MS and relative quantitation was employed to investigate the changes in sputum cellular proteome following antibiotic treatment in 12 CF patients chronically infected with *Pseudomonas aeruginosa*.

**Conclusions:** 1989 human proteins were identified in total. 37 proteins out of the 318 detected in >80% samples were differentially expressed (*p* < 0.05) after antibiotic treatment. Of these, 22 exhibited a strong correlation (*r* > 0.969) between their pre-antibiotic/post-antibiotic ratios and ratios for the same proteins comparing CF/healthy control cohorts, thus suggesting that successful treatment promotes a proteome shift towards a non-CF profile. Ingenuity Pathway Analysis software showed antibiotic treatment to most affect the molecular and cellular functions of Cellular Movement (particularly Immune Cell Traffic), Organismal Injury and Abnormalities, Cell Death and Free Radical Scavenging.

67 *P. aeruginosa* proteins and 17 proteins from other bacterial species were also detected. Bacterial proteins accounted for 0.5−35% of CF sputum cellular protein. Although total cellular protein (per g sputum) decreased following antibiotic treatment, the percentage attributable to bacteria did not alter and no bacterial proteins were detected as differentially expressed.

**57** Azithromycin influence on biofilm formation of *Pseudomonas aeruginosa* isolates from children with cystic fibrosis

V. Chistyakova1, O. Simonova1, L. Katsova2, A. Lazareva2, N. Mayanskiy3. 1Federal State Budgetary Institution Scientific Center of Children’s Health, Pulmonology and Allergology Department, Moscow, Russian Federation; 2Federal State Budgetary Institution Scientific Center of Children’s Health, Microbiology Department, Moscow, Russian Federation; 3Federal State Budgetary Institution Scientific Center of Children’s Health, Laboratory of Experimental Immunology and Virology, Moscow, Russian Federation

Growth of *Pseudomonas aeruginosa* in biofilm makes it more resistant to antimicrobial treatment, and biofilm formation may reduce effectiveness of the antibiotic therapy in children with cystic fibrosis (CF).

**Aim:** To study the biofilm formation ability of *P. aeruginosa* isolates from CF children to optimize azithromycin (AZM) administration.

**Materials and Methods:** Formation of biofilms was studied by determination of the ability of 12 *P. aeruginosa* strains (6 mucoid and 6 non mucoid isolates) to adhesion on the surface of 96-well polystyrol plate. Isolates were incubated in broth for 2 days at 36.8°C, diluted 1/100 and inoculated in a 96-well polystyrol plate (8 replicates for each isolate, in 4 replicates 5µg/ml AZM was added). Biofilms were grown for 2 days at 36.8°C in humid container, then planktonic cells were removed. Biofilms were stained by 0.1% crystal violet, washed 3 times with distilled water. To estimate biofilm formation, after 96% ethanol addition optical density (wave length 540 nm) was measured by a plate-reader.

**Results:** Ability to biofilm formation was identified in 10 out of 12 isolates (83%), 2 isolates did not form biofilm. AZM suppressed biofilm development in 7 of 10 isolates decreasing biofilm density by 1.7−4.2 times. Suppression of biofilm formation by other isolates was not significant.

**Conclusion:** In most cases, *P. aeruginosa* isolates from CF children were able to form biofilm. Suppression of biofilm formation in 2/3 of examined *P. aeruginosa* isolates induced by AZM confirms the need of further research in this field to optimize macrolide antibiotics administration.

**58** No antibiotic cross-resistance after 1 year of continuous aztreonam for inhalation solution (AZLI) in cystic fibrosis (CF) patients (pts) with chronic *Burkholderia (BURK)* infection

J. Burns1, J.J. LiPuma2, G. Retsch-Bogart3, M. Bresnihan4, N. Henig4, M. McKevitt4, S. Lewis5, T. Tullis1. 1University of Washington, Seattle, United States; 2University of Michigan, Ann Arbor, United States; 3University of North Carolina, Chapel Hill, United States; 4Gilead Sciences Inc; Foster City, United States; 5University of Toronto, Toronto, Canada

**Objectives:** A 6 mo double-blind, placebo (PBO)-controlled trial of AZLI with 6 mo open-label (OL) extension was conducted in CF pts chronically infected with BURK to assess the effect of long-term continuous AZLI on *BURK* and *P aeruginosa* (PA) sputum density and antibiotic susceptibility (clinicaltrials.gov #NCT01059565).

**Methods:** Pts were randomized to 6 mo of daily AZLI 75 mg or PBO TID followed by 6 mo of OL AZLI. Expectorated sputum or throat swabs were cultured for CF pathogens and susceptibility testing of *BURK* and *PA* isolates.

**Results:** No long-term suppressive effects on *BURK* and *PA* sputum density were observed in either study group. The BURK MICs of aztreonam and piprofloxacin increased 4-fold from baseline to the end of the randomized period in AZLI pts; no other 4-fold changes were observed (Table). *PA* isolates were more susceptible than BURK isolates to all antibiotics.

<table>
<thead>
<tr>
<th>MICs (µg/ml) at wk 0</th>
<th>AZLI/AZLI</th>
<th>PBO/AZLI</th>
<th>AZLI/AZLI</th>
<th>PBO/AZLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>64/256</td>
<td>128</td>
<td>64</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>256/256</td>
<td>128</td>
<td>64</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>256/256</td>
<td>256</td>
<td>64</td>
<td>32</td>
<td>64</td>
</tr>
</tbody>
</table>

**Table:** *BURK* and *PA* MICs (µg/ml) at wk 0, end of randomized study (wk 24), end of treatment (wk 48)

One year of continuous AZLI did not compromise antibiotic susceptibility of *BURK* or *PA*.

Supported by Gilead Sciences.

**59** Recommended doses of ceftazidime (CAZ) are insufficient to treat less susceptible pathogens in cystic fibrosis (CF) patients

B. Bondue1, K. Schepers2, F. Wolff2, C. Jossart3, C. Knoop4, F. Jacob2. 1Erasmus University Hospital, Adult Cystic Fibrosis Reference Centre, Chest Medicine, Brussels, Belgium; 2Erasmus University Hospital, Infectious Diseases, Brussels, Belgium; 3Erasmus University Hospital, Clinical Chemistry, Brussels, Belgium

**Background:** CF patients present altered b-lactams pharmacokinetics (PKs) and are increasingly infected by less susceptible strain of *P aeruginosa*. The objective of this study was therefore to assess the adequacy of recommended doses of ceftazidime (CAZ) in this context.

**Methods:** We prospectively enrolled CF adults with acute pulmonary exacerbation due to *P aeruginosa* and treated with CAZ (200 mg/kg/day, in 3 injections, adapted to renal function). PKs were calculated from CAZ concentrations measured by HPLC-UV on serum before and 2 hours after the 30-min infusion. The clinical breakpoint for *P aeruginosa* of 8 mg/L, as defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), was used as target minimal inhibitory concentration (MIC). CAZ therapy was defined as adequate if serum concentration remained between 4 and 8 times the target MIC during at least 70% of time.

**Results:** We measured the CAZ serum concentrations in 14 patients (8 males, median age 31y (18−42), median BMI 20 (14−25) at day 2 (median, range 1−14) of treatment. Only 3 patients (21%) had adequate concentrations, 7 (50%) had insufficient and 4 (29%) excessive concentrations. Patients with insufficient concentrations tended to have higher creatinine clearance (22±23 mU/min, mean±SD) than patients with overdose (75±50 mU/min) (p < 0.15). No correlation was observed between CAZ concentrations, plasma protein or BMI. All patients, but one, reached PK targets for most sensitive strains.

**Conclusions:** Although recommended CAZ doses are sufficient to treat susceptible *P aeruginosa* strains, higher doses are probably needed for most patients in case of less susceptible strains.