Avibactam confers susceptibility to a large proportion of ceftazidime-resistant Pseudomonas aeruginosa isolates recovered from cystic fibrosis patients

Hussein Chalhoub1, Michael Tunney2, J. Stuart Elborn2, Anne Vergison3†, Olivier Denis4, Patrick Plésiat2, Barbara C. Kahl6, Françoise Van Bambeke1 and Paul M. Tulkens1*

1Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium; 2CF & Airways Microbiology Research Group, Queen’s University Belfast, Belfast, UK; 3Unité des maladies infectieuses, Hôpital Universitaire des Enfants Reine Fabiola, Brussels, Belgium; 4Laboratoire de microbiologie, Hôpital Erasme, Brussels, Belgium; 5Laboratoire de bactériologie, Hôpital Jean Minjoz, Besançon, France; 6Medical Microbiology, University Hospital Münster, Münster, Germany

*Corresponding author. Tel: +32-2-7647371; E-mail: tulkens@facm.ucl.ac.be
†Present address: Union Nationale des Mutualités Socialistes, Brussels, Belgium.

Keywords: carbapenemases, extended-spectrum β-lactamases, ESBLs, β-lactamase inhibition, P. aeruginosa

Sir, Pseudomonas aeruginosa is the predominant bacterial pathogen in cystic fibrosis (CF) patients and is associated with decline in pulmonary function.1 Due to the chronic persistent nature of infections, CF patients receive frequent antibiotic courses for eradication of potential pathogens, treatment of acute infective exacerbations and as chronic suppressive therapy. Consequently, resistance to antipseudomonal β-lactams is common in the strains collected from CF patients.2,3 Narrowing therapeutic options. Clinicians are therefore forced to use aminoglycosides or polymyxins, increasing the risk of adverse effects.4,5 Therefore, optimizing the activity of β-lactams may help to alleviate this burden. Ceftazidime is a well-established cephalosporin (on the WHO List of Essential Medicines) with an excellent safety profile and an antibacterial spectrum that includes P. aeruginosa. However, ceftazidime is degraded by many β-lactamases, including ESBLs (Ambler classes A and D), cefalosporinases (Ambler class C) and carbapenemases. Avibactam (formerly NXL-104) is a novel non-β-lactam, broad-spectrum β-lactamase inhibitor, with promising inhibitory activity against Ambler class A (including ESBLs and Klebsiella pneumoniae carba-penemases), C and D β-lactamases.6 Combined with ceftazidime, it is currently in Phase III clinical trials for the treatment of complicated intra-abdominal infections, urinary tract infections and healthcare-associated pneumonia (http://clinicaltrials.gov identifiers NCT01499290, NCT01500239, NCT01726023, NCT01644643, NCT01595438 and NCT01808092). In P. aeruginosa from non-CF patients, avibactam has been shown to reverse ceftazidime resistance, bringing MICs to values lower than the EUCAST and CLSI breakpoints.7,8 However, very little is known about the effect of avibactam on ceftazidime activity in P. aeruginosa isolated from CF patients.2 We therefore assembled a collection of 334 non-duplicate P. aeruginosa isolates from 156 patients with a clinically confirmed diagnosis of CF equally distributed between four European countries with a predominance of recent isolates (Belgium (2010), France (1996–2012), Germany (2012) and the UK (2006–09)) and used them to assess the activity of ceftazidime alone or combined with avibactam. MICs were determined by microdilution in cation-adjusted Mueller–Hinton broth following the CLSI methodology for ceftazidime alone (proceded as Glazidim®, the commercial product registered in Belgium for parenteral use; potency, 88.2%; GlaxoSmithKline; Genval, Belgium) and combined with 4 mg/L avibactam (NXL-104, potency 91.7%, batch number AFCH005151; AstraZeneca Pharmaceuticals, Waltham, MA, USA). P. aeruginosa ATCC 27853 (fully susceptible) and K. pneumoniae ATCC 700630 (resistant to ceftazidime by the production of SHV-18 (β-lactamase) were used as quality controls. Correlations between MICs of ceftazidime and ceftazidime/avibactam for individual strains were assessed using quantile density contour analysis (JMP® version 10.0.2, SAS Institute Inc., Cary, NC, USA). Figure 1(a) shows that isolates in this collection had a high MIC90 of ceftazidime (512 mg/L), with only 36% being clinically susceptible (MIC ≤8 mg/L) according to EUCAST or CLSI interpretive criteria. When combined with avibactam, the proportion of susceptible strains increased to 76% and the MIC90 decreased to 64 mg/L. Figure 1(b) shows the fold reduction in MIC observed in the presence of avibactam for these isolates classified according to the MIC of ceftazidime. While the mean reduction in MIC observed for the whole collection was 2.6 dilutions, the amplitude of the effect was clearly dependent on the initial ceftazidime MIC. Thus, when combined with avibactam, the MIC of ceftazidime decreased by 0.6 dilutions for each doubling of ceftazidime MIC in the 1–128 mg/L range (0.6 is the slope value of a linear regression relating the log2 MIC of the combination to the log2 MIC of ceftazidime in that range; R2 = 0.965), which would decrease the MIC to 8–16 mg/L, irrespective of the ceftazidime MIC in that range of concentrations. For more-resistant strains, the amplitude of the avibactam effect plateaued at a reduction of ~4 dilutions in MIC for strains for which the ceftazidime MIC was ~256 mg/L and decreased to a reduction of 3 dilutions for isolates for which the MICs were still higher. This shift in MIC is illustrated for individual strains in Figure 1(c), which shows the correlation between MICs of individual isolates for ceftazidime alone and ceftazidime combined with avibactam. Susceptibility to ceftazidime was restored in 40% of the strains, with avibactam proving more effective for strains for which the MIC was <256 mg/L. In accordance with the conclusion drawn from Figure 1(c), the ceftazidime MIC was now only 4–8 mg/L for most of the affected
strains, a value that is below the EUCAST and CLSI susceptibility breakpoints, extending to CF *P. aeruginosa* isolates the conclusions obtained for pseudomonal isolates of other origins and for other Gram-negative bacteria.

Taken together, these data highlight the potential utility of combining ceftazidime with avibactam for the treatment of *P. aeruginosa* infections, including in clinical situations where resistance rates are high. It also shows that a concentration of 4 mg/L is sufficient to bring into the susceptible range those *P. aeruginosa* strains with a ceftazidime MIC ≤256 mg/L.

### Acknowledgements

We thank AstraZeneca Pharmaceuticals, Waltham, MA, USA, for providing us with avibactam.
**Funding**

This work was supported in part by the Belgian Région Wallonne. H. C. is Boursier of the Belgian Fonds pour la Recherche dans l’Industrie et l’Agriculture (FRIA) and F. V. B. is Maître de Recherches of the Belgian Fonds de la Recherche Scientifique (F.R.S.-FNRS).

**Transparency declarations**

F. V. B. and P. M. T. have received research grants from AstraZeneca Pharmaceuticals for work other than that presented here. All other authors: none to declare.

**References**