

Introduction

Given the problems with antibiotic resistance and the detection of new and emerging pathogens in the CF lung, there is a clear unmet need for novel antimicrobial agents for treatment of respiratory infection in CF patients.

ALX-009, a combination of lactoferrin and hypothiocyanite, has been shown to have *in vitro* activity against a range of CF pathogens, notably *Pseudomonas aeruginosa* and *Burkholderia* isolates.

Aim: To determine if treatment of sputum from CF patients with ALX-009 results in a significant decrease in total sputum microbial load and microbial load of key CF pathogens, *P. aeruginosa* and *Burkholderia cepacia complex* (Bcc).

Methods

- Sputum samples, excess to clinical requirements, were collected from CF patients chronically colonized with *P. aeruginosa* (n=34) or Bcc (n=9). Sputum plugs were selected and homogenised by repeated passage through a 1ml syringe.
- To determine the effect of a single treatment with ALX-009, ALX-009, tobramycin, ALX-009 and tobramycin or Phosphate buffered saline (PBS; control) were added to *P. aeruginosa* (n=24) and Bcc (n=9) positive sputum samples and total viable counts (TVC) measured at 0, 6 and 24 hours. To determine the effect of treating sputum with a second dose of ALX-009, CF sputum samples with a high *P. aeruginosa* load ($\geq 10^5$) (n=10) were used. ALX-009 was added at 0 and 12 hours with TVCs performed at 0, 6, 12, 18, 24 and 34 hours. A reduction, by any of the agents/combinations under test, of the original inoculum by $\geq 3 \log_{10}$ cfu/g sputum was considered bactericidal.
- TVCs for *P. aeruginosa* and Bcc count were performed using selective agar plates, with non-selective blood agar plates used to determine total sputum bacterial load.

Results

- Following a single dose, ALX-009 demonstrated bactericidal activity against *P. aeruginosa* in 18/24 sputum samples (Figure 1A).
- In 10/24 samples the TVC of *P. aeruginosa* was reduced below the detectable limit at 24 hours (Figure 1B); initial *P. aeruginosa* bacterial burden was approximately 10^5 CFU/g sputum in these samples. In contrast, for the remaining 14/24 samples in which *P. aeruginosa* was detected at 24 hours (Figure 1C), initial *P. aeruginosa* bacterial burden was significantly higher at approximately 10^7 CFU/g sputum.
- Treatment with a second dose of ALX-009 resulted in a further reduction in *P. aeruginosa* TVC at 34 hours in comparison to a single dose (Figure 1E). For 7/10 samples, *P. aeruginosa* TVC was below the detectable limit at 34 hours (Figure 1F); in the remaining 3 samples, *P. aeruginosa* TVC was reduced but was still detected.
- Given the excellent activity of ALX-009 against *P. aeruginosa* growing in sputum, it was difficult to determine if there was a synergistic effect when ALX-009 was combined with tobramycin. However, synergistic activity was apparent for 4 samples at 24 hours.
- ALX-009 demonstrated bactericidal activity for 4/9 Bcc samples at 24 hours; however, the TVC of Bcc was not reduced below the detectable limit for any sample at any timepoint (Figure 1D).

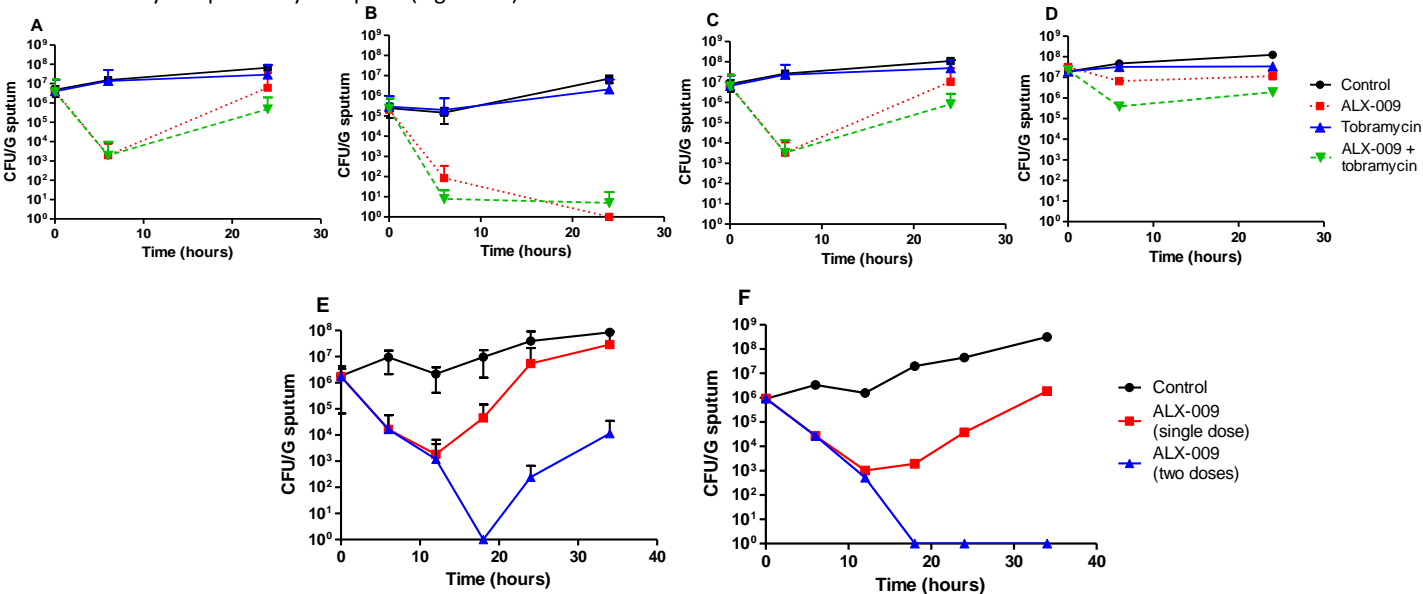


Figure 1. Antimicrobial activity of ALX-009 against (A) *P. aeruginosa* in sputum samples (n=24), *P. aeruginosa* in sputum at an initial bacterial burden of (B) $\sim 10^5$ CFU/g sputum (n=10) and (C) $\sim 10^7$ CFU/g sputum (n=14). (D) Antimicrobial activity of ALX-009 against Bcc in sputum samples (n=9). Activity of ALX-009 (two doses) against (E) *P. aeruginosa* in sputum samples (n=10) and (F) representative graph for reduction of *P. aeruginosa* TVC below the limit of detection following two doses of ALX-009.

Discussion

This study demonstrates that treatment of sputum from CF patients with ALX-009 results in a significant decrease in the microbial load of key respiratory pathogens. Moreover, there was no significant change in the total load of bacteria present in the respiratory microbiota.

A second dose of ALX-009, added within a similar time-frame to the administration of currently used inhaled antibiotics, inhibited the regrowth of *P. aeruginosa* in sputum samples with a high *P. aeruginosa* load.

