THERAPEUTIC POTENTIAL OF INHALED ALX-009 (OSCN/BLF) FOR THE TREATMENT OF ACHROMOBACTER SPP. INFECTIONS IN CYSTIC FIBROSIS

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INTRODUCTION

Recent epidemiologic studies in Europe and USA indicate that prevalence of Achromobacter spp. is increasing within the CF population. ALX-009 is a fixed combination of two endogenous compounds of the innate immune system, OSCN and Lactoferrin (LF), both reduced in CF patients. OSCN disturbs the bacterial machinery while LF either acts as iron chelator and/or by direct interaction with bacterial membranes. According to the current knowledge, particularly from data obtained with Burkholderia spp., the mode of action of ALX-009 may be described as follows:

- High concentration of OSCN penetrates the cell and induces rapid bacterial killing by impairing the enzymes necessary to the respiratory pathways (Carlson et al. Infection and immunity, 1984; 44, 581). This effect last up to 6h.
- At lower concentrations, i.e. from 6-12h, OSCN is still in contact with the surface of the bacterial wall and attacks the proteins involved in membrane transport.
- In the meantime and up to 24h after addition of the compounds to the culture, BLF “relays” OSCN hampering the regrowth of bacteria by direct contact with bacteria (Roseneau et al. Rom J. Biochem. 2010. 47(2):203-209).

The combined effects of OSCN/LF may offer an innovative multi-target strategy to fight resistant pathogens. Achromobacter spp. is a gram negative bacteria able to induce severe lung infections in these patients and for which therapeutic options are limited. In previous reports, Alaxia demonstrated the therapeutic potential of the drug ALX-009 on a large collection of Burkholderia spp. and other emergent bacteria in cystic fibrosis patients. The present study aims at evaluating the bactericidal capacity of ALX-009 against a large number of isolates of the multi-drug resistant bacteria Achromobacter spp.

METHODS

Bacterial clinical isolates

The bacterial clinical isolates were kindly provided by several laboratories and national CF repositories around the world.

Microbiological methods

1. Minimal Inhibitory Concentrations (MIC) for blF, OSCN and ALX-009 were obtained with the microdilution method adapted from the guideline M07-A9.

Each experiment was performed at least three times.

2. Time kill curves for blF, OSCN and ALX-009 were obtained with the microdilution method described in guideline M26-A of CLSI. Each experiment was performed at least three times.

3. Product interaction test was analyzed with the checkerboard method. Each experiment was performed at least three times.

Test compounds

OSCN was produced by enzymatic reaction with Alaxia’s proprietary technology. blF is a pharma grade bovine Lactoferrin produced by Alaxia. Antibiotics used in the control cultures were pure active ingredient powders from Sigma-Aldrich.

RESULTS

The killing pattern of ALX-009 against clinical isolates of Achromobacter spp.

- OSCN inhibits 100% of the tested isolates
- blF inhibits 15% of the tested isolates under the strict CLSI technical conditions
- For Achromobacter spp., the FIC indexes analysis demonstrates that the product interaction is synergistic for 19% (13/68) additive for 67% (63/94), indifferent for 14% (13/94).
- No antagonism was identified.
- Combination MIC is achieved with a reduced OSCN dose (-25%) in presence of blF.

This data confirm the additivity of the product interaction.

- High [OSCN] induces rapid bacterial killing up to -4logs from 0 to 6h after inoculation
- With decreased [OSCN], bacterial killing kinetics slows down maintaining maximal killing capacity up to T12h

In contrast to previous experiments with Burkholderia spp and other bacterial species, the contribution of blF in the killing pattern of ALX-009 against Achromobacter spp. seems limited

CONCLUSIONS AND PERSPECTIVES

- ALX-009 is able to inhibit the growth of a large panel of 94 clinical isolates of Achromobacter spp.
- As demonstrated with other bacteria, ALX-009 killing activity on Achromobacter spp. depends mainly on OSCN; however, blF is required to preserve the killing capacity of the combination over 24h
- The data presented here confirm the potential therapeutic use of ALX-009 for the treatment of multiresistant bacterial infections in CF lung
- The pharmacodynamic profile of ALX-009 supports the enrolment of CF patients with lung infections involving bad bugs such as Achromobacter spp. during the clinical development
- Phase I clinical trial is ongoing (NCT02598999)

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