Cystic Fibrosis (CF) is a genetic disease caused by a complete or partial impairment of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) ion channel at the surface of epithelial cells. As a result, the digestive and lung systems are permanently congested by a sticky mucus causing malnutrition and chronic lung infections. The increased understanding of the physiopathology and etiology of the disease and the improvement on patients management and therapies have changed considerably the CF disease course and survival and nowadays the majority of the patients may reach adulthood. Due to this epidemiology situation, the CF disease faces new challenges. One of these is the increased number of chronic lung infections by bacteria for which the current therapeutic armamentarium offers no or few treatment options to fight against Burkholderia spp., Achromobacter and multiresistant Pseudomonas among others. The development of broad spectrum antibacterial agents with limited potential to induce resistance will contribute to resolve this new challenge and to increase the quality of life and the life expectancy of CF patients.

The data presented here focus on the therapeutic potential of ALX-009, a drug candidate in Phase I composed of Hypothiocyanite (OSCN) and bovine Lactoferrin (bLF) on emergent and multiresistant bacteria in the CF population.

**METHODS**

**Bacterial clinical isolates**

The bacterial clinical isolates were kindly provided by several laboratories and national CF repositories around the world. The tested isolates belongs to the genus Achromobacter (n = 15), Burkholderia (n = 185), Cupriavidus (n = 3), Hafniala (n = 8), Pandoraea (n = 6), Prevotella (n = 6), Pseudomonas (n = 20), Ralstonia (n = 5).

**Microbiological methods**

1. **Minimal Inhibitory Concentrations (MIC) for bLF, OSCN and ALX-009** were obtained with the microdilution method described in guideline M07-A9 of CLSI with slight modifications. Each experiment was performed three times.
2. **Time kill curves for bLF, OSCN and ALX-009** with the microdilution method described in guideline M06 of CLSI. Each experiment was performed three times.
3. **Product interaction test** was analyzed with checkerboard method as described elsewhere. Each experiment was performed three times.
4. **Biofilm Minimal Inhibitory (BIC) concentration for bLF, OSCN and ALX-009** with established biofilms produced with the MBEC™ assay (Innovatech, Vancouver, Ca).
5. **Induction of resistance** was performed by 20 successive passages with increased selection pressure for each compound and the combination followed by 10 passages without selection pressure on Burkholderia cenocepacia and Staphylococcus epidermidis clinical isolates.
6. **Cross resistance** was tested by comparing the MIC value for each isolate before the induction of resistance with the MIC value of each isolate obtained after the 20 successive passages. Each experiment was performed 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 and in the cross resistance tests were pure active ingredient powder from Sigma-Aldrich.

**RESULTS**

**1. IN VITRO EFFICACY**

![B. multivorans 10699 Growth control](image1)

- **OSCN/bLF (ALX-009)** is a broad spectrum antibacterial combination with a dose-isolate dependency irrespective of the bacterial species.
- **ALX-009** is active against multidrug resistant and emergent CF clinical isolates.
- The MIC values range from 13-117 µg/ml for OSCN. Only few isolates (20/298) where inhibited by bLF alone within a range of 0.25-64 mg/ml.
- In the MIC tests, presence of bLF induces a reduction up to 65% of OSCN on the inhibitory values according to the isolate and the bacterial species.
- In 50% of the strains, the combination shows a synergistic or additive interaction.

- **bLF induced a partial acquired resistance in Bcc strain 8149 but no in S. epidermidis**
- **OSCN did not induce any resistance level in any of the two isolates tested**
- A cross resistance with Colistin was identified in the OSCN and bLF stressed strain indicating a possible interaction of these compounds with a target common to Polymyxins.
- The combination reduces the level of acquired and cross resistance to other antibiotics.
- Taken together these results indicates a low resistance/cross resistance potential of ALX-009.

**2. RESISTANCE**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Cross resistance Bcc strain 8149</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original culture</td>
<td>bLF</td>
</tr>
<tr>
<td>Colistin (Polymyxin)</td>
<td>4-8</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>4-8</td>
</tr>
<tr>
<td>Achromobacter</td>
<td>2-8</td>
</tr>
<tr>
<td>Burkholderia</td>
<td>2-8</td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td>1-2</td>
</tr>
</tbody>
</table>

**3. PUTATIVE MODE OF ACTION**

- **High concentration of OSCN may penetrate 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 lasts up to 6h.**
- **At lower concentrations of OSCN, it may be in contact with the surface of the bacterial wall and may only attack the proteins involved in membrane transport (Michalos, M. et al. Gen.2h Microbiol. 1966, 43, 31-43) from 6-12h.**
- **At the same time and during the last step, bLF may “relay” OSCN impeding the regrowth of bacteria by direct contact with bacteria (Riosena et al. Rom. J. Biochem. 2010, 47(2):203-209) up to 24h after addition of the compounds to the culture.**

**CONCLUSION AND PERSPECTIVES**

- **ALX-009** is able to inhibit the growth of a large panel of clinical, laboratory and environmental multiresistant strains.
- In time killing experiments, **ALX-009 killing activity depends mainly on OSCN**. However, bLF is required to preserve the killing capacity over 24h.
- **The proposed mode of action suggests a competitive and sequential biocide activity of each compound.**
- **Resistance experiments suggest that each component of the combination “blocks” the induction of resistance.**
- **Preliminary results indicates that ALX-009 may induce only cross resistance with polymyxins.**
- **ALX-009 phase I clinical trial authorization granted in July 2015.**

Acknowledgments to Peter Vandamme (University of Gent, Belgium), John Lufta (University of Michigan, USA), Pavel Drvinkov (Charles University of Prague, Czech Republic), David P. Spert (University of British Columbia, Canada) and Jose Diogo (University of Buenos Aires) for kindly providing their bacterial isolates. Thanks to Michel Tunnely and Stuart Elibon to perform tests on some of their clinical isolates.

[ALX-009](http://www.alx-pharma.eu)