# BACTERICIDAL POTENTIAL OF OSCN$^-$, BOVINE LACTOFERRIN AND THEIR COMBINATION ON CLINICAL ISOLATES OF *Burkholderia* spp.

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## INTRODUCTION

*Burkholderia cepacia* complex (Bcc) bacteria are important opportunistic pathogens in cystic fibrosis (CF). Bcc pulmonary infections are associated with a rapid decline of lung function. Bcc lung infections are difficult to treat due to Bcc inherent resistance to most of the clinically available antibiotics, including aminoglycosides, quinolones, polymyxins, and ß–lactams. Identification of new antimicrobials targeting *Burkholderia* spp. are needed to address this unmet medical need.

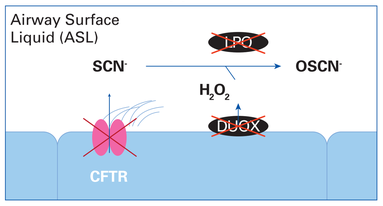
The aim of this *in vitro* study was to test the antibacterial efficacy of Hypothiocyanite (OSCN$^-$) and bovine Lactoferrin (bLF) and OSCN$^-$/bLF combination (ALX-009) on Bcc CF-clinical isolates.

## OSCN$^-$/bLF (ALX-009): Scientific and Medical Rationale

***In CF patients***

1. **Defective / abstent CFTR**
   - **Impaired SCN$^-$ transport**
2. **Defective Lactoperoxidase system**
   - **Decreased OSCN$^-$ level in lower airways**
3. **Decreased Lactoferrin level / activity**

SCN$^-$ hypothesis

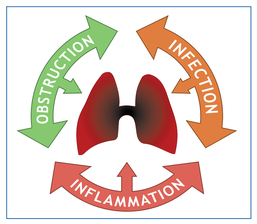



***CF epithelia***
*adapted from Fischer (2009) [1]*

**ALX-009 administered by inhalation**

**OSCN$^-$/bLF compensates defective host protection against microorganisms and provides to CF patients a therapeutic option to fight lung infections**

**Potentially breaking the vicious circle leading to lethal conditions**




***Thick mucus + defective mucociliary clearance***

[1] Fischer H. Mechanisms and function of DUOX in epithelia of the lung. Antioxid & Redox Signal. 2009 Oct; 11(10):2453-2465. doi: 10.1089/ars.2009.2558

## METHODS

***Burkholderia* spp. CLINICAL ISOLATES:**

155 *Burkholderia* spp. clinical isolates from different geographical origins (Figure 1) and representing the genomovars prevalence in CF patients (Figure 2) were studied.

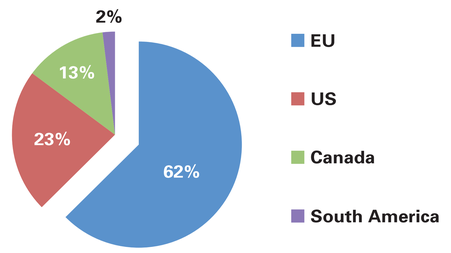



**Figure 1:** Geographical origin of *Burkholderia* spp. clinical isolates.

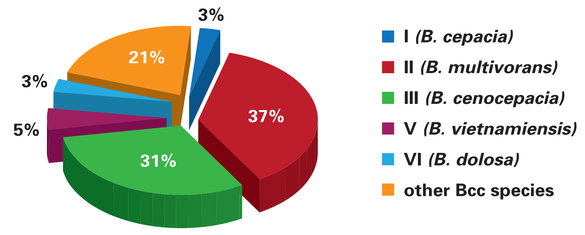



**Figure 2:** Prevalence of Bcc genomovars in *Burkholderia* positive CF patients as reported by LiPuma (2010)[2].

**Test compounds:** OSCN$^-$ was produced by enzymatic reaction with the Alaxia's proprietary technology. bLF is a pharma grade bovine Lactoferrin produced by Alaxia.

**MICROBIOLOGY ASSAYS*:**

| | Methods | Active ingredients and dose range |
|---|---|---|
| **1. MIC: OSCN$^-$ or bLF alone** | Broth microdilution method CLSI guideline M07-A9 | bLF: 0.125 to 64 mg/mL<br>OSCN$^-$: 25 to 150 µg/mL |
| **2. FIC Index: OSCN$^-$ and bLF combined** | Microdilution method | bLF: 0.125 to 32 mg/mL<br>OSCN$^-$: 25 to 125 µg/mL |
| **3. Time kill curves** | Macrodilution method CLSI guideline M26-A | Time points: 0, 2, 4, 6, and 24h<br>OSCN$^-$+bLF: $MIC_{OSCN+bLF}$ and ± 25% $MIC_{OSCN+bLF}$<br>OSCN$^-$: alone at $MIC_{OSCN+bLF}$ value<br>bLF: alone at $MIC_{OSCN+bLF}$ value |

*Each assay was performed in triplicate

[2] Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. Clin Microbiol Rev. 2010 Apr; 23(2):299-323. doi: 10.1128/CMR.00068-09

## RESULTS

### 1 MIC: OSCN$^-$ or bLF alone

| GENOMOVAR | Isolates (n=155) | MIC OSCN$^-$ (µg/mL) 50% | 90% | 100% | MIC bLF (mg/mL) |
|---|---|---|---|---|---|
| I *(B. cepacia)* | 8 (5%) | 70 | 88 | 89 | > 96 |
| II *(B. multivorans)* | 39 (25%) | 68 | 93 | 101 | |
| III *(B. cenocepacia)* | 66 (43%) | 71 | 105 | 125 | |
| V *(B. vietnamiensis)* | 6 (4%) | 64 | 83 | 89 | |
| VI *(B. dolosa)* | 9 (6%) | 81 | 116 | 117 | |
| Other Bcc spp. | 27 (17%) | 63 | 101 | 157 | |

**Table 1:** Values of $MIC_{50}$, $MIC_{90}$ and $MIC_{100}$ obtained with Bcc clinical isolates in presence of OSCN$^-$ or bLF.

- OSCN$^-$ showed growth inhibitory activity on 100% of tested isolates
- No inhibitory activity was observed with bLF alone except for 9 isolates over the 155 tested. MIC values for these susceptible isolates were between 0.25 and 32 mg/mL

### 2 FIC index : OSCN$^-$ and bLF combined

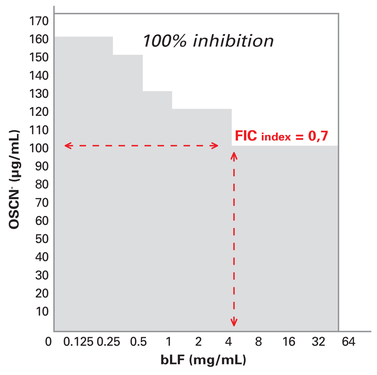



**Figure 3:** Graphical representation of OSCN$^-$/bLF (ALX-009) combinations.

- OSCN$^-$/bLF (ALX-009) combination is able to inhibit the growth of 100% of the tested isolates
- bLF decreases up to 40% the concentration of OSCN$^-$ required to inhibit growth when assayed alone (MIC value)
- 8% of combinations were synergistic and 58% were additive

### 3 TIME KILL CURVES

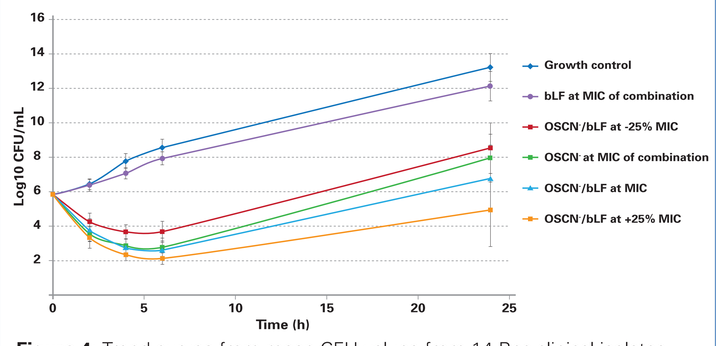



**Figure 4:** Trend curves from mean CFU values from 14 Bcc clinical isolates, selected from their low, medium or high susceptibility to OSCN$^-$ and/or bLF.

- OSCN$^-$/bLF (ALX-009) has a strong bactericidal effect
- After 6h, OSCN$^-$/bLF (ALX-009) induces up to 4-log reduction compared to the starting inoculum
- After 24h, a strong inhibition was maintained with 6- to 8-log reduction compared to the growth control

## CONCLUSIONS

**OSCN$^-$/bLF (ALX-009):**

- **Is able to inhibit the growth of 155 geographic and genetic-diverse CF *Burkholderia* spp.**
- **Has a strong antibacterial potential with a maximal activity after six hours, however benefit lasts up to 24h**
- **Has promising efficacy to fight against *Burkholderia* spp. lung infection in CF patients, a high unmet medical need**

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