

Successful *Burkholderia* spp. eradication with hypothiocyanite/Lactoferrin. *In vitro* study evidence over a worldwide collection of clinical strains.

Yasmine Sonmez*, Camille Bechetoille*, Sandrine Perrotto*, Alban Payet-Burin* and Victor Juarez-Perez**
 *Alaxia SAS Lyon, France **Stragen France SAS Lyon, France

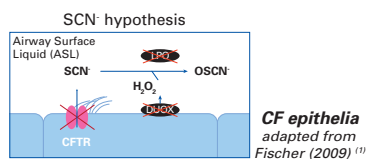
INTRODUCTION

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 β -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

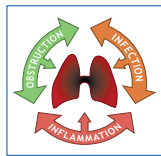
1. Defective / absent CFTR
 ▶ Impaired SCN⁻ transport
2. Defective Lactoperoxidase system
 ▶ Decreased OSCN⁻ level in lower airways
3. Decreased Lactoferrin level / activity



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:

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

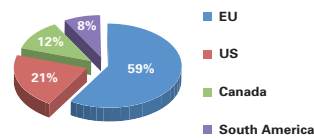


Figure 1: Geographical origin of *Burkholderia* spp. clinical isolates.

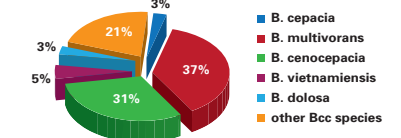


Figure 2: Prevalence of Bcc genomovars in *Burkholderia* positive CF patients as reported by LiPuma (2010)².

Test compounds: OSCN⁻ was produced by enzymatic reaction with the Alaxia's proprietary technology. LF is a pharma grade bovine Lactoferrin produced by Alaxia.

⁽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

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 OSCN ⁻ : 25 to 150 μ g/mL
2. FIC Index: OSCN ⁻ and bLF combined	Microdilution method	bLF: 0.125 to 32 mg/mL OSCN ⁻ : 25 to 125 μ g/mL
3. Time kill curves	Macrodilution method CLSI guideline M26-A	Time points: 0, 2, 4, 6, and 24h OSCN ⁻ +bLF: MIC _{OSCN+bLF} and \pm 25% MIC _{OSCN+bLF} OSCN ⁻ : alone at MIC _{OSCN+bLF} value bLF: alone at MIC _{OSCN+bLF} value

*Each assay was performed in triplicate

RESULTS

1 MIC: OSCN⁻ or bLF alone

Burkholderia species	Isolates (n=165)	MIC OSCN ⁻ (μ g/mL)			MIC bLF (mg/mL)
		50%	90%	100%	
<i>B. cepacia</i>	8 (5%)	70	88	89	> 96*
<i>B. multivorans</i>	39 (24%)	68	93	101	
<i>B. cenocepacia</i>	68 (40%)	72	109	149	
<i>B. stabilis</i>	11 (7%)	57	68	70	
<i>B. dolosa</i>	9 (6%)	81	116	117	
<i>B. vietnamiensis</i>	6 (4%)	64	83	89	
Other Bcc	23 (14%)	72	120	157	
<i>B. gladioli</i>	1 (<1%)	na	na	144	

Table 1: Values of MIC_{50%}, MIC_{90%} and MIC_{100%} obtained with *Burkholderia* clinical isolates in presence of OSCN⁻ or bLF.

- OSCN⁻ showed growth inhibitory activity on 100% of tested isolates
- bLF showed growth inhibitory activity against only 8 (5%) of the tested isolates.

* No inhibitory activity was observed with bLF alone except for 8 isolates over the 165 tested. MIC values for these susceptible isolates were between 0.25 and 96 mg/mL

2 FIC index : OSCN⁻ and bLF combined

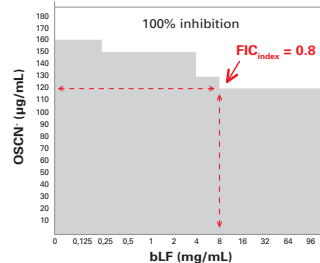


Figure 3: Graphical representation of OSCN/bLF (ALX-009) combinations.

- OSCN/bLF (ALX-009) combination is able to inhibit the growth of 100% of the tested isolates
- bLF decreases up to 25% the concentration of OSCN⁻ required to inhibit growth when assayed alone (MIC value)
- 13% of combinations were synergistic and 60% were additive

3 TIME KILL CURVES

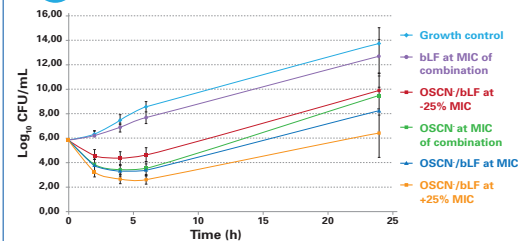


Figure 4: Trend curves from mean CFU values from 21 *Burkholderia* clinical isolates, selected from their low, medium or high susceptibility to OSCN⁻ and/or bLF.

- OSCN/bLF (ALX-009) has a strong bactericidal effect
- After 6h, OSCN/bLF (ALX-009) induces up to 3-log reduction compared to the starting inoculum
- After 24h, a strong inhibition was maintained with 4- to 8-log reduction compared to the growth control

CONCLUSIONS

OSCN⁻/bLF (ALX-009):

- Is able to inhibit the growth of 165 geographic and genetic-diverse CF *Burkholderia* spp.
- Has a strong antibacterial potential with a maximal activity after six hours, however activity 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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ALAXIA SAS

Bâtiment Adenine, 60 Avenue Rockefeller, F-69008 Lyon

Phone: +33 4 37 53 26 40, Email: contact@bioalaxia.eu, www.alaxia-pharma.eu

