THERAPEUTIC POTENTIAL OF INHALED ALX-009 (OSCN-/ BLF) FOR EMERGENT AND MULTIRESISTANT BACTERIAL INFECTIONS IN CYSTIC FIBROSIS

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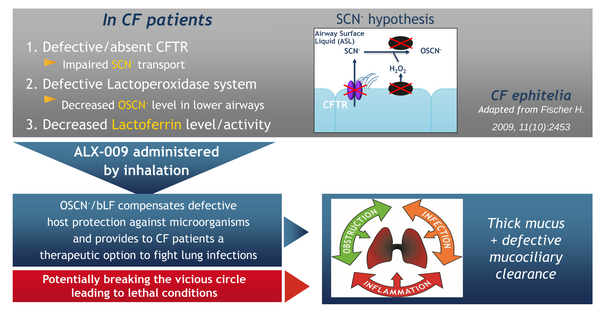
INTRODUCTION

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 armamentum offers no or few treatment options to fight against *Burkholderia* spp. *Achromobacter* and multidrug resistant *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.

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



Fischer H. 2009 Oct; 11(10):2453-2465.

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), *Pandorea* (n=6), *Prevotela* (n=6), *Pseudomonas* (n=50), *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 macrodilution method described in guideline M26 of CLSI. Each experiment was performed three times.
3. Product interaction test was analyzed with the checkerboard method as described elsewhere. Each experiment was performed three times.
4. Biofilm Minimal Inhibitory (BIC) concentration for bLF, $OSCN^-$ and ALX-009 with stablished biofilms produced with the MBEC™ assay (Innovatech, Vancuver, 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 epidermis* 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.

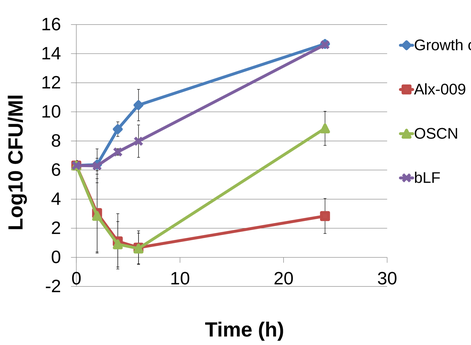
RESULTS

1. *IN VITRO* EFFICACY

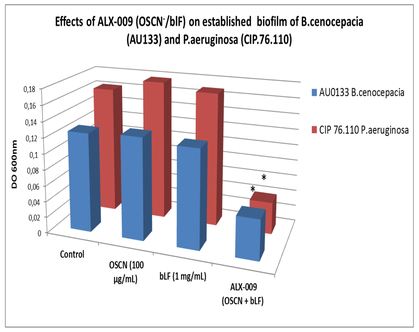
	Bacteria	Susceptible isolates
Gram-	*Achromobacter xylosoxidans*	15/15
	Burkholderia spp.	185/185
	Cupriavidus spp.	3/3
	Hemophilus influenzae	5/5
	Pandorea spp	6/6
	Prevotella spp	5/5
	*Pseudomonas aeruginosa**	50/50
	Ralstonia spp.	5/5
	Yersinia pestis	2/2
Gram+	*Bacillus* spp.	3/3
	MRSA**	0/5
	Streptococcus spp.	4/4
	Staphylococcus spp.	5/10

*Includes the complete *P. aeruginosa* International reference panel (de Soyza, MicrobiologyOpen 2013; 2(6): 1010–1023)
**MRSA = Methicillin Resistant *Streptococcus aureus*

- ✓ $OSCN^-$/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



- ✓ $OSCN^-$/bLF killing profile demonstrates a rapid reduction of CFU from 0-6h after inoculation
- ✓ In the absence of bLF, $OSCN^-$ killing potential decreases at 24h post-incubation in comparison with ALX-009 (average -6Logs)
- ✓ Similar killing profiles are obtained for all the species tested



- ✓ Preliminary results demonstrated that $OSCN^-$/bLF combination (Marquis et al., Antimicrob Chemother. 2015; 70(1):160) is able to hampers the development of biofilms and disrupt established biofilms.
- ✓ Here we show that ALX-009, a $OSCN^-$/bLF enhanced formulation of this combination is able to disrupt established biofilms alone.
- ✓ Additional testing is being conducted with ALX-009 to study the development and disruption of established biofilms alone or in combination with antibiotics.

2. RESISTANCE

Species	$OSCN^-$	bLF	ALX-009
Bcc 8149	2x	32x*/16x**	3x
S. epidermis	2x	2x	-

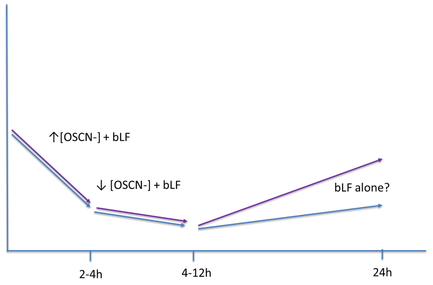
Resistance level of Bcc 8149 and S. epidermis
**After 20 passages. **After 20+10 passages*

Antibiotic	*Cross resistance Bcc strain 8149*			
	Original culture	bLF	$OSCN^-$	ALX-009
Cefepime (Cephalosporin)	≤0.125-0.25	-	-	-
Colistin (Polymixin)	4-8	8x	16x	8x
Tobramycin (Amynoglycoside)	1-2	-	-	-
Aztreonam (β-lactam)	≥128	-	-	-
Ceftazidime (β-lactam)	4-8	-	-	-
Temocillin (β-lactam)	128-64	-	-	-
Meropenem (Carbapenem)	≤0.125-0.25	-	-	-

Cross resistance level of Bcc 8149 after 20 passages

- ✓ bLF induced a partial acquired resistance in Bcc strain 8149 but no in *S. epidermis*
- ✓ $OSCN^-$ 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 compound with a target common to Polymixins
- ✓ 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

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 last up to 6h,
- ✓ At lower concentrations of $OSCN^-$, it may be only in contact with the surface of the bacterial wall and may only attacks the proteins involved in membrane transport (Mickelson, M. *et al.* Gen.2h Microbiol. 1966, 43, 31-43) from 6-12h;
- ✓ At the mean time and during the last step, bLF may "relay" $OSCN^-$ hampering the regrowth of bacteria by direct contact with bacteria (Roseneau 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 contributive 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 polymixines*
- ✓ *ALX-009 phase I clinical trial authorization granted in July 2015*

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