

ALX-009

A promising antimicrobial therapy for Respiratory Infections



Member of iABC consortium

Program supported by

PRESENTATION OVERVIEW

- About ALAXIA
- ALX-009 for respiratory infections
 - *ALX-009, a First in class antimicrobial drug candidate*
 - *Cystic Fibrosis as first target*
 - *Combination Rationale and Efficacy*
 - *Safety profile*
 - *Clinical Trial status*
 - *ALX-009 Description of the product*
- Bronchiectasis as a second target
- Conclusion

ABOUT ALAXIA

- ▶ Founded in 2008, **ALAXIA** is a private SME biotech company located in Lyon (France)
- ▶ Development of ALX-009 innovative program
 - ALX-009 targeting infectious respiratory diseases based on our peroxidase platform

ALX-009
OSCN-/Lactoferrin

ALX-009, A FIRST IN CLASS ANTIMICROBIAL DRUG CANDIDATE (1/2)

- Innovative concept and program supported by BPI France, Cystic Fibrosis Foundation (USA) and member of [iABC](#) consortium, Innovative Medicines Initiative (EU) and AMR Syndicate (CF Trust/MD Catapult)
 - Association of 2 endogenous substances (OSCN/Lactoferrin) with antimicrobial properties mimicking the innate immune system
 - ➡ symptomatic treatment of lung infections via a solution for inhalation
- ALX-009 is protected by several patents and designated Orphan Drug Status by EMA and FDA in Cystic Fibrosis
- ALX-009 targets antibiotic multiresistant Gram(-) bacteria that are not killed by current antimicrobials; the so called “bad bugs”
 - First target population in western countries : Cystic Fibrosis
 - Second target, more global : Bronchiectasis

ALX-009, A FIRST IN CLASS ANTIMICROBIAL DRUG CANDIDATE (2/2)

- Multi-target mode of action limiting emergence of resistance:
 - OSCN: non specific oxidant of thiol groups,
 - LF: bacteriostatic
- Bactericidal effect maintained in complex matrices (biofilm and sputum) without requiring significant product dose increase
- Standalone therapy &/or adjunctive to antibiotics
- Phase Ib in Cystic Fibrosis and Bronchiectasis patients ongoing

ALX-009, CF LUNG INFECTIONS AS 1ST TARGET INDICATION

- ▶ Cystic Fibrosis (CF) is an Orphan Disease and ALX-009 is already designated Orphan Drug Status by EMA and FDA in this indication

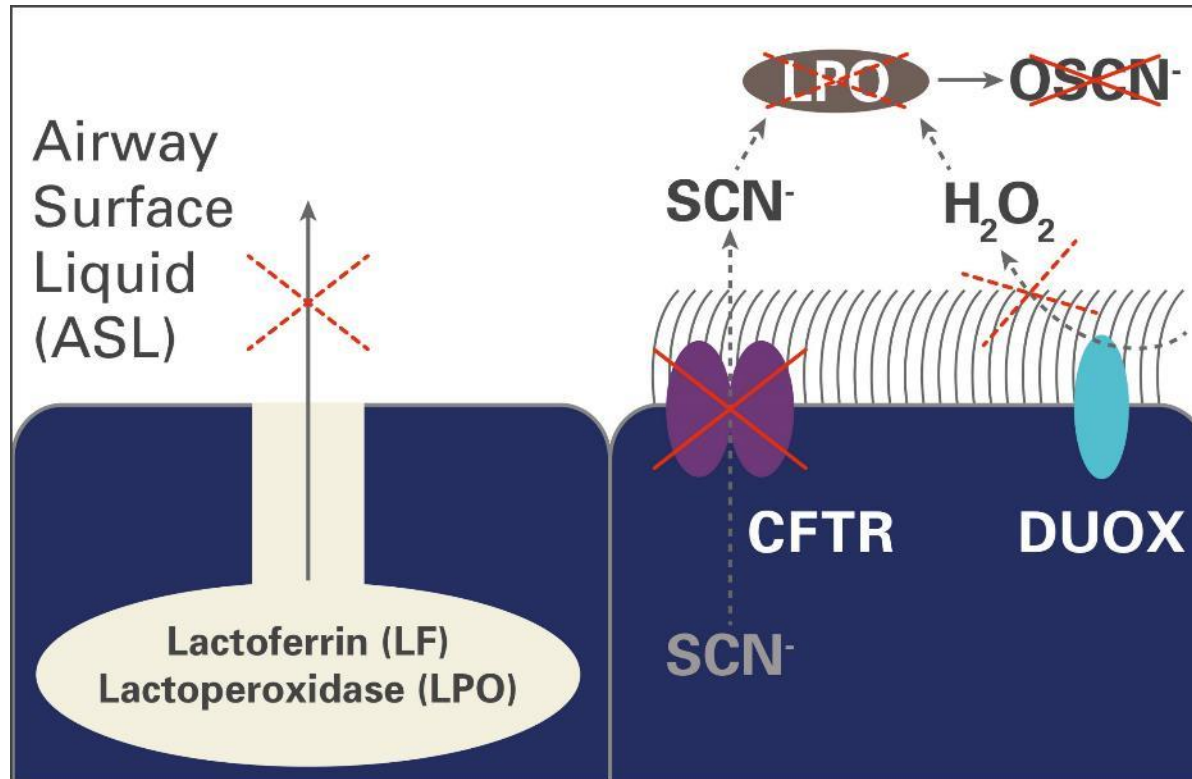
- ▶ 100 000 CF patients (EU & NA) suffer from recurrent bacterial lung infections

Lung infections → irreversible lung damage → reduced life expectancy

- ▶ 15-20% of bacterial infections are caused by “Bad Bugs” such as *Burkholderia* spp., *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia*, MDR *Pseudomonas aeruginosa*,...No treatments available
- ▶ Emergence of multiresistant bacteria results in unmet medical needs for lung disease management

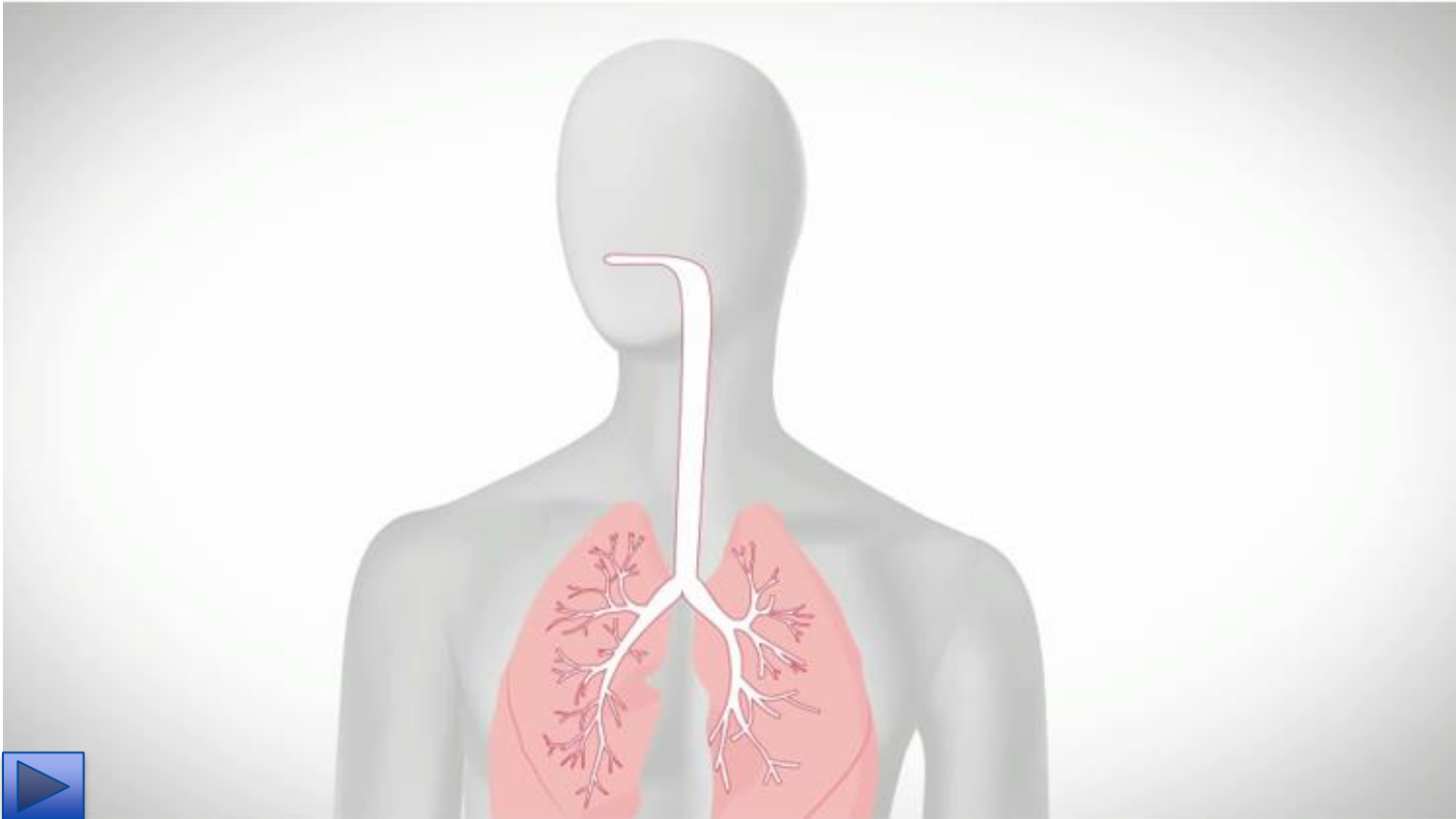
SCIENTIFIC AND MEDICAL RATIONALE

IMPAIRED OSCN⁻ AND LACTOFERRIN IN CF PATIENTS LUNGS' DEFENSE



SCIENTIFIC AND MEDICAL RATIONALE

ALX-009 MIMICKS INNATE IMMUNE SYSTEM



ALX-009 FOR RESPIRATORY INFECTIONS

- **Bacterial Infections including bad bugs (Gram-)**
 - MIC and TK supporting data
 - Effects maintained in biological matrices
 - Innovative MoA limiting emergence of resistance
 - OSCN⁻: non specific oxidant of thiol groups,
 - LF: bacteriostatic
- Other infectious agents
 - Non Tuberculosis Mycobacteria (NTM), Virus

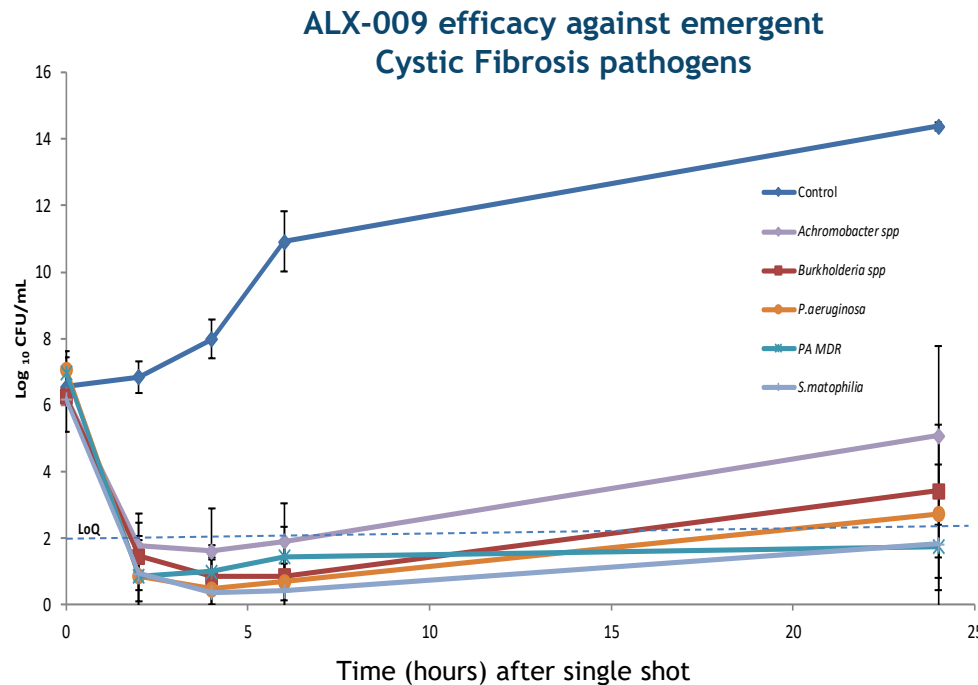
EFFICACY : BACTERIAL STRAINS SUSCEPTIBILITY* TO ALX-009

Bacteria		Number of isolates Susceptible vs Tested
Gram ⁻	<i>Achromobacter</i> spp.	114/114
	<i>Burkholderia</i> spp.	189/189
	<i>Cupriavidus</i> spp.	3/3
	<i>Escherichia</i> spp.	2/2
	<i>Haemophilus influenzae</i>	5/5
	<i>Pandorea</i> spp.	11/11
	<i>Prevotella</i> spp.	5/5
	<i>Pseudomonas aeruginosa</i>	72/72
	<i>Pseudomonas aeruginosa</i> MDR	91/91
	<i>Ralstonia</i> spp.	10/10
	<i>Stenotrophomonas maltophilia</i>	116/116
	<i>Yersinia pestis</i>	2/2
Gram ⁺	<i>Bacillus</i> spp.	3/3
	<i>Streptococcus</i> spp.	5/7
	<i>Staphylococcus</i> spp.	4/27
	<i>Staphylococcus aureus</i> MRSA	3/28

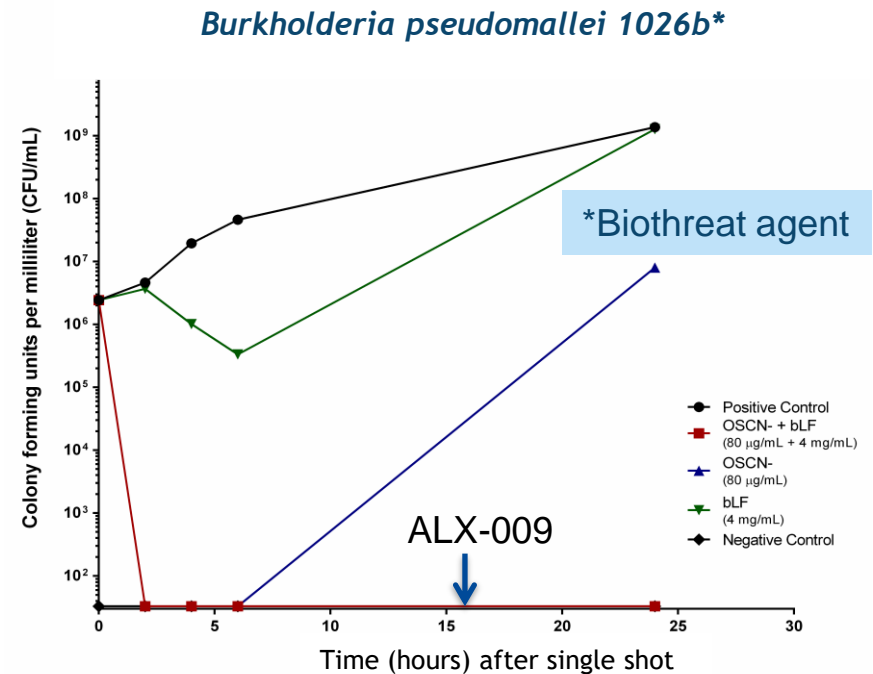
100% Efficacy demonstrated on Gram(−) of interest including antibiotic/multidrug resistant strains

ALX-009 (OSCN-/LF): COMBINATION RATIONALE

- ▶ Cooperative mode of action of OSCN⁻ and LF ➡ OSCN⁻ induces rapid bacterial killing during the first 6-8h and then LF hampers regrowth up to 24h after exposure
- ▶ Added-value of combining OSCN⁻ and LF demonstrated



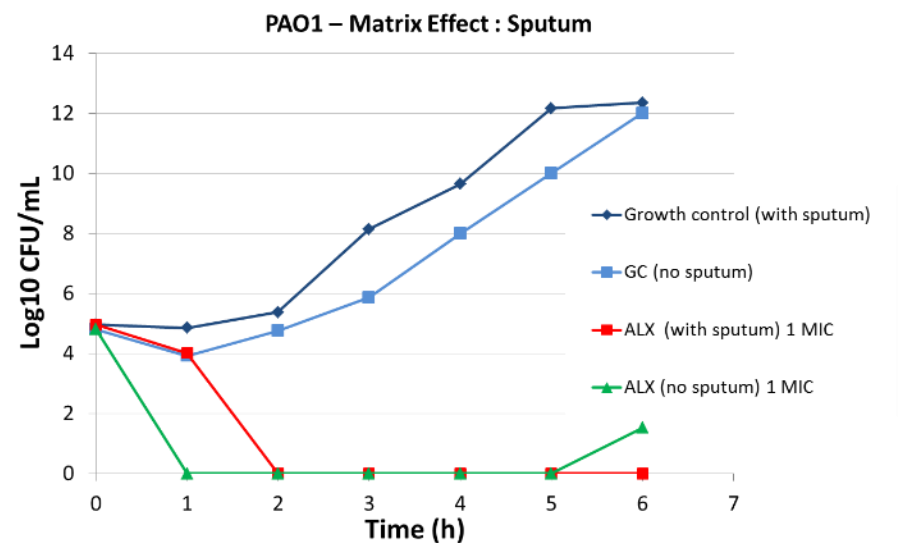
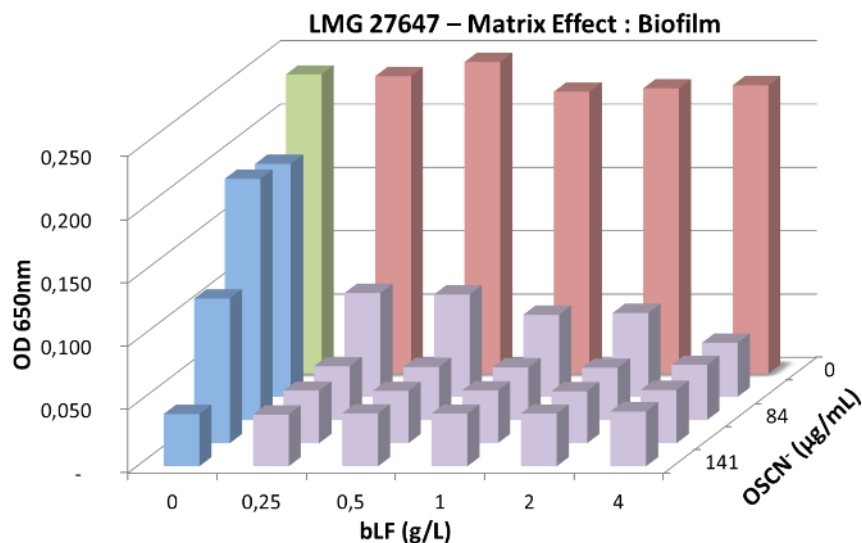
The results correspond to the compiled average values of 9 *Burkholderia* spp, 9 *Achromobacter* spp, 9 *Pseudomonas aeruginosa*, 2 MDR *Pseudomonas aeruginosa* and 9 *Stenotrophomonas maltophilia* clinical isolates. Each isolate was tested at least three times. Vertical bars show the standard variation for the group at the given time point. CFU: Colony Forming Unit. LoQ, Lower level of quantification



CSU, H. Schweizer BSL3 Lab

ALX-009 – BACTERICIDAL EFFECT IN BIOLOGICAL MATRICES

- ▶ In contrast to antibiotics, ALX-009 activity is not altered by complex structures such as biofilm and/or sputum that are present in lung infections



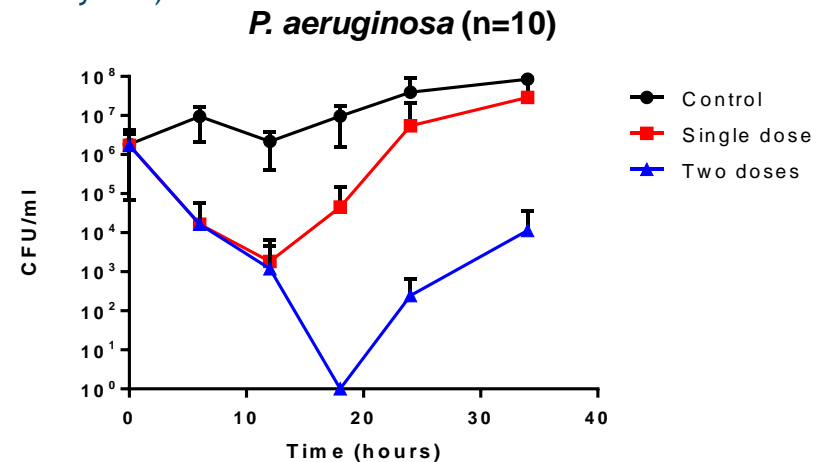
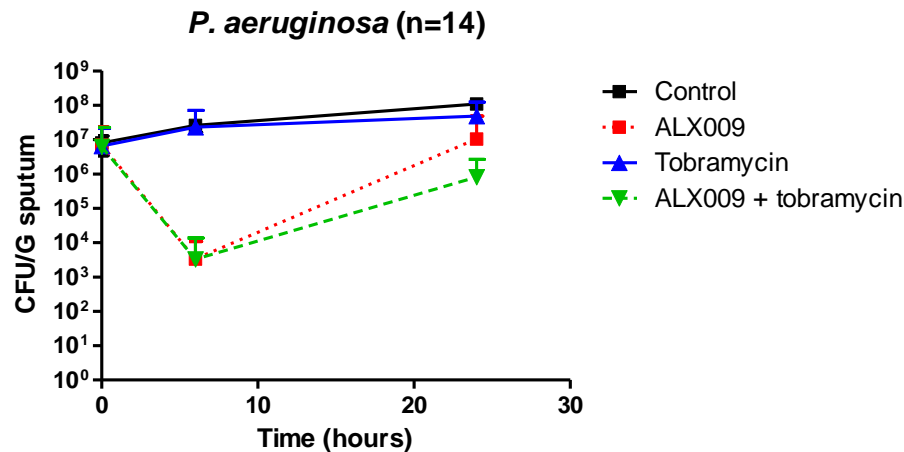
- ▶ ALX-009 kills bacteria embedded in *P. aeruginosa* biofilms at concentrations similar to planktonic cultures. Presence of LF allows decreasing OSCN⁻ dose

ALX-009 ACTIVITY IN SPUTA OF CF PATIENTS

Average bacterial counts after ALX-009 treatment¹

- ALX-009 was tested at MIC₉₀ dose for a collection of 450 clinical isolates as single and twice a day administration
- Tobramycin was tested at an average dose of 6 x MIC of the published values for the *P. aeruginosa* species in the single administration test

(Queen's University Belfast, M Tunney Lab)

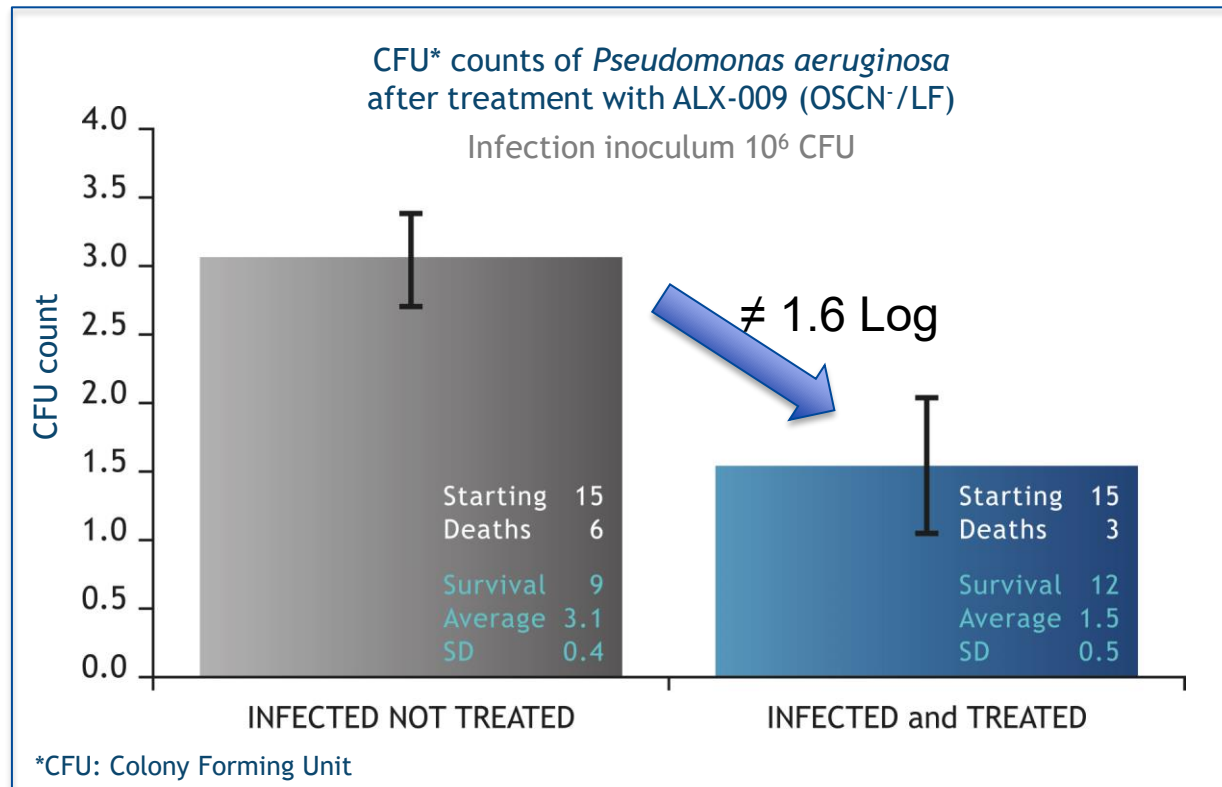


- Under the same experimental conditions, tobramycin shows no activity alone
- Treatment of infected sputum with ALX-009 results in a significant decrease in the microbial load of *P. aeruginosa*.
- A twice a day *in vitro* application improves efficacy of ALX-009

IN VIVO EFFICACY AGAINST *PSEUDOMONAS AERUGINOSA*

IN VIVO TESTS

Demonstration
of efficacy on female mice,
previously infected
with mucoid
Pseudomonas aeruginosa



Animal received intra-tracheal ALX-009 instillation treatment at 24h and 48h post infection. Bacterial counts were performed at 72h post infection

- ▶ CFU count reduction of 1.6 Log
- ▶ Improved survival by 50%

ALX-009 – A MULTI-TARGET MoA LIMITING EMERGENCE OF RESISTANCE

- ▶ ALX-009 potential for inducing resistance was studied on 2 strains (Gram+ and Gram-) according to a typical protocol¹ :
 - 20 induction passages with increased selection pressure
 - 10 passages with no selection pressure
- ▶ No induction of resistance by the combination
- ▶ ALX-009 cross-resistance with available antibiotics was also tested
 - ▶ No cross-resistance identified for Cephalosporins, Carbapenems, Betalactams, Aminoglycosides, Polymyxins

ALX-009 FOR RESPIRATORY INFECTIONS

- **Bacterial Infections including bad bugs (Gram-)**
 - MIC and TK supporting data
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 - OSCN⁻: non specific oxidant of thiol groups,
 - LF: bacteriostatic
- **Other infectious agents**
 - Non Tuberculosis Mycobacteria (NTM), Virus

ALX-009 FOR RESPIRATORY INFECTIONS

- **Other infectious agents**

- Non Tuberculosis Mycobacteria (NTM)

- One addition (T_0) : Bacteriostatic effect
 - Several additions ($T_0, T_{24}, T_{48}, \dots$) : To be studied

- Viruses

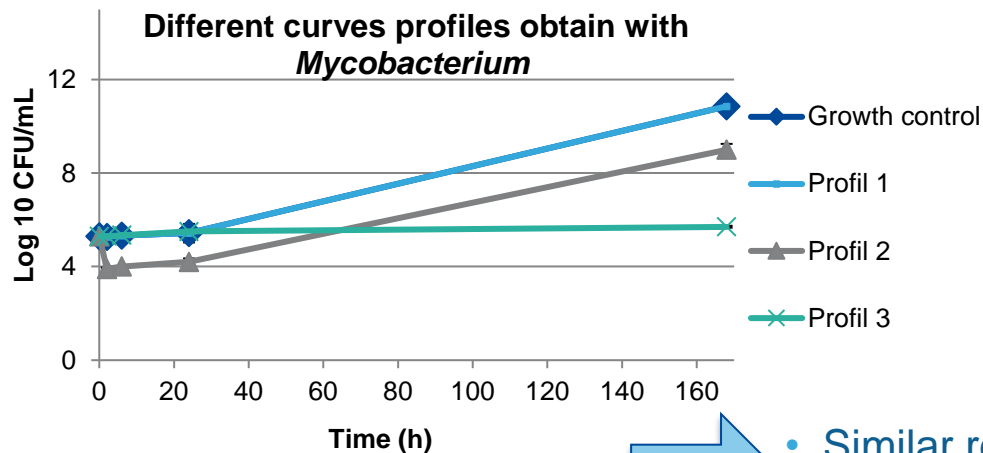
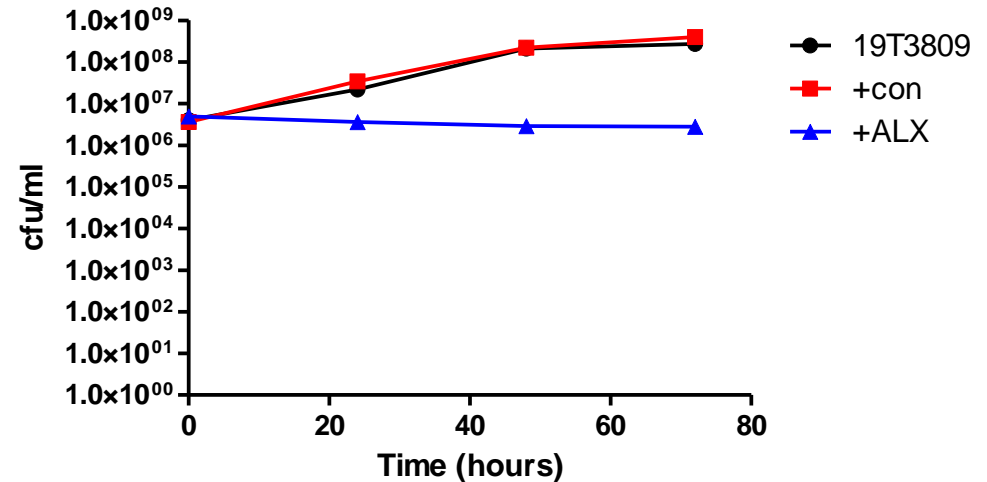
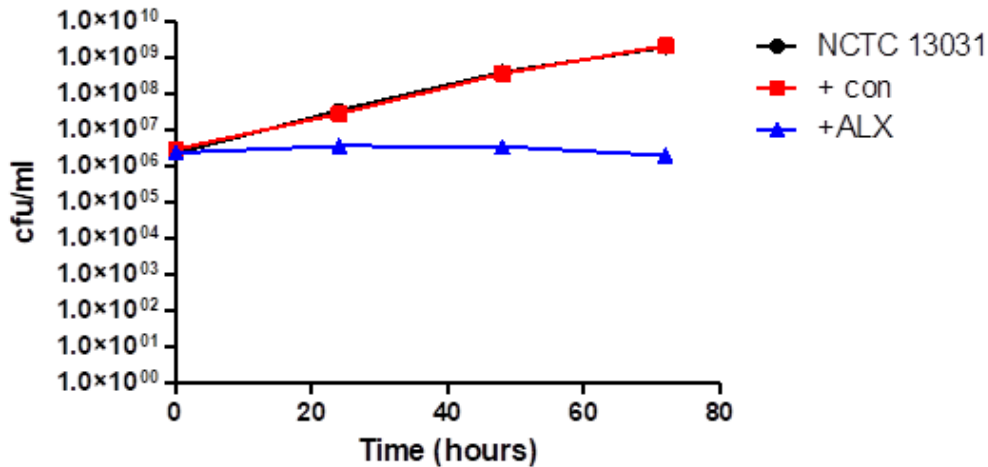
- H1N1 Influenza virus 2009 pandemic¹ : virucidal
 - rVSV-S (pCoV2) and SARS-CoV-2² : virucidal

¹Cegolon L, Salata C, Piccoli E, Juarez V, Palu G, Mastrangelo G, et al. In vitro antiviral activity of hypothiocyanite against A/H1N1/2009 pandemic influenza virus. *Int J Hyg Environ Health*. 2014;217(1):17–22.

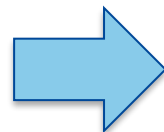
²Cegolon L, Mirandola M, Salaris C, Salvati MV, Salata C, Mastrangelo G. Hypothiocyanite and Hypothiocyanite/Lactoferrin mixture exhibit virucidal activity in vitro against SARS-CoV-2. *Pathogens* 2021, 10, 233. <https://doi.org/10.3390/pathogens10020233>

MYCOBACTERIUM – TIME KILL CURVE

QUB ALX (OSCN⁻ 265µg/mL - LF 8g/L) on two strains *M. abscessus*
T24h, T48h and T72h



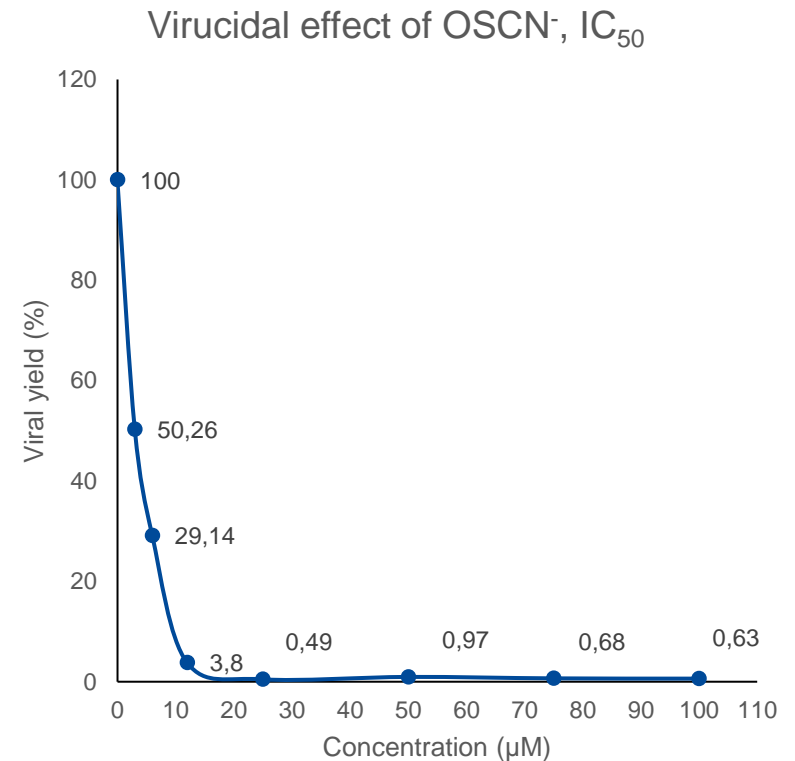
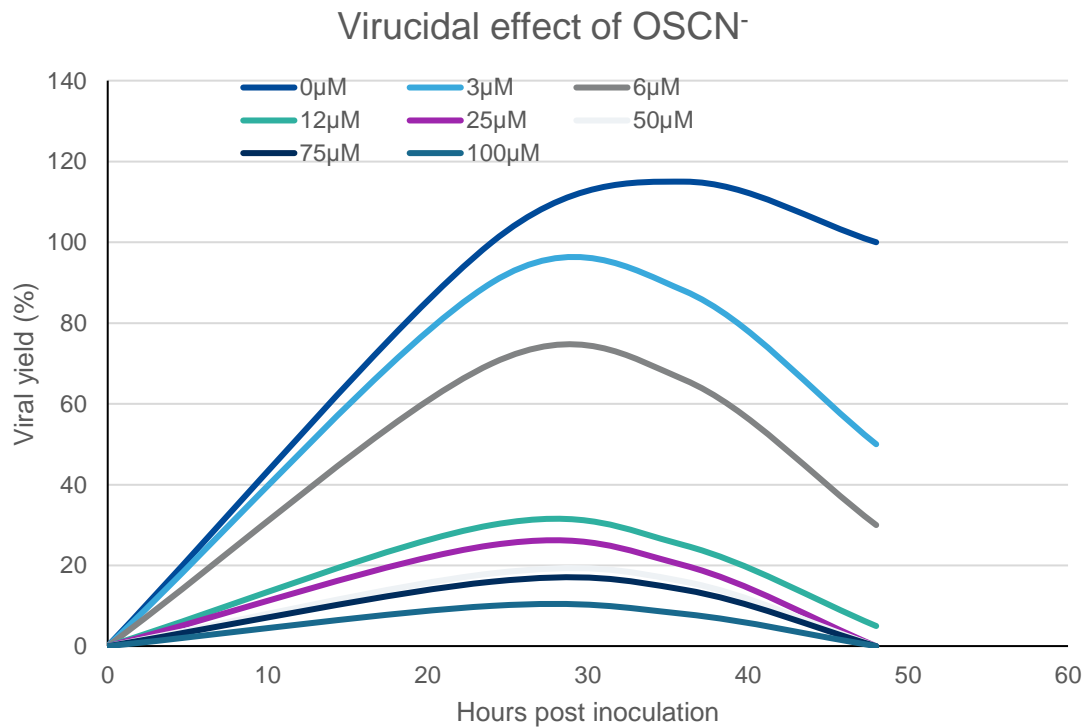
ALAXIA: 10 strains tested in duplicate
(9 *M. abscessus* ; 1 *M. fortuitum*)
Tested conditions combo:
OSCN⁻ 155µg/mL - LF 4g/L
T2h, T6h, T24h and T7days



- Similar results between Queen's University Belfast and Alaxia
- Bacteriostatic effect after single treatment

EFFICACY ON VIRUS - INFLUENZA VIRUS

- A/H1N1 Influenza virus¹,
 - No citotox observed on MDCK cells
 - 2 μM OSCN⁻ (0.1 $\mu\text{g/mL}$) strong enough to reduce viral infectivity by 50% (IC_{50})



EVALUATION OF VIRUCIDAL EFFECT AGAINST VSV-S

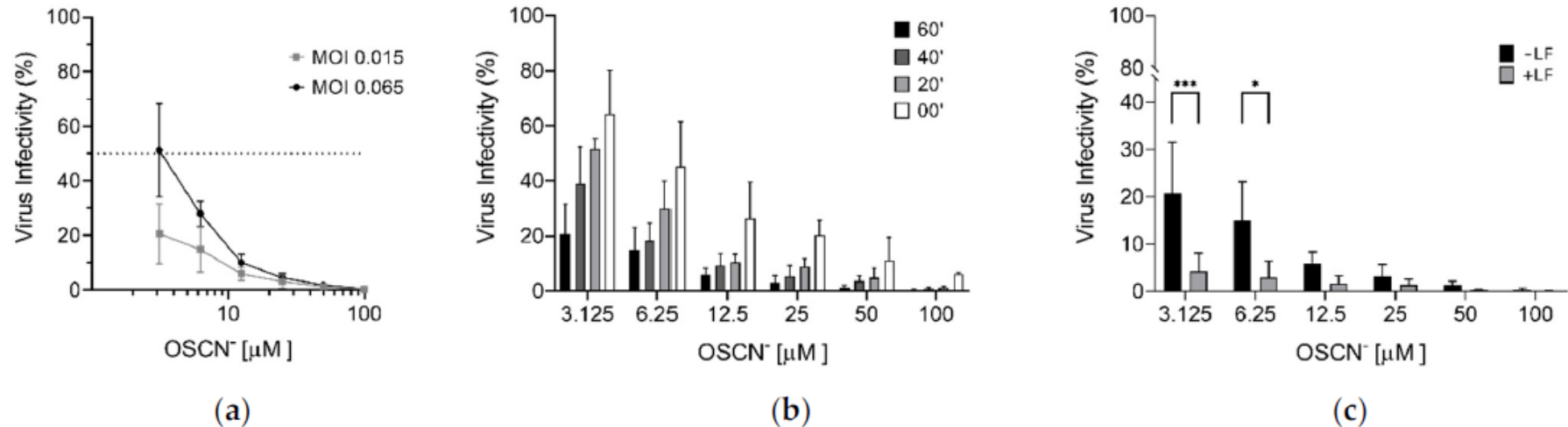


Figure 1. OSCN⁻ and OSCN⁻/LF virucidal activity against the pseudovirus VSV-S diluted in Minimum Essential Medium (MEM). Infection of Vero cells was evaluated measuring the activity of the VSV-S encoded luciferase. (a) Efficiency of pseudovirus infection at MOI 0.065 and 0.015 FFU/mL after preincubation with different OSCN⁻ concentrations for 1 h at 37 °C; (b) evaluation of the virucidal activity of OSCN⁻ after pseudovirus treatment for 0, 20, 40, and 60 min at 37 °C before the infection of target cells at MOI 0.015; (c) comparison between OSCN⁻ and OSCN⁻ + LF virucidal activity after 1 h of preincubation of VSV-S and before cell infection at MOI 0.015. Data (mean ± SD, N = 3, experiments in duplicate) are percentages of no drug, set as 100% (* = $p < 0.05$; *** = $p < 0.001$).

EVALUATION OF VIRUCIDAL EFFECT AGAINST SARS-CoV-2

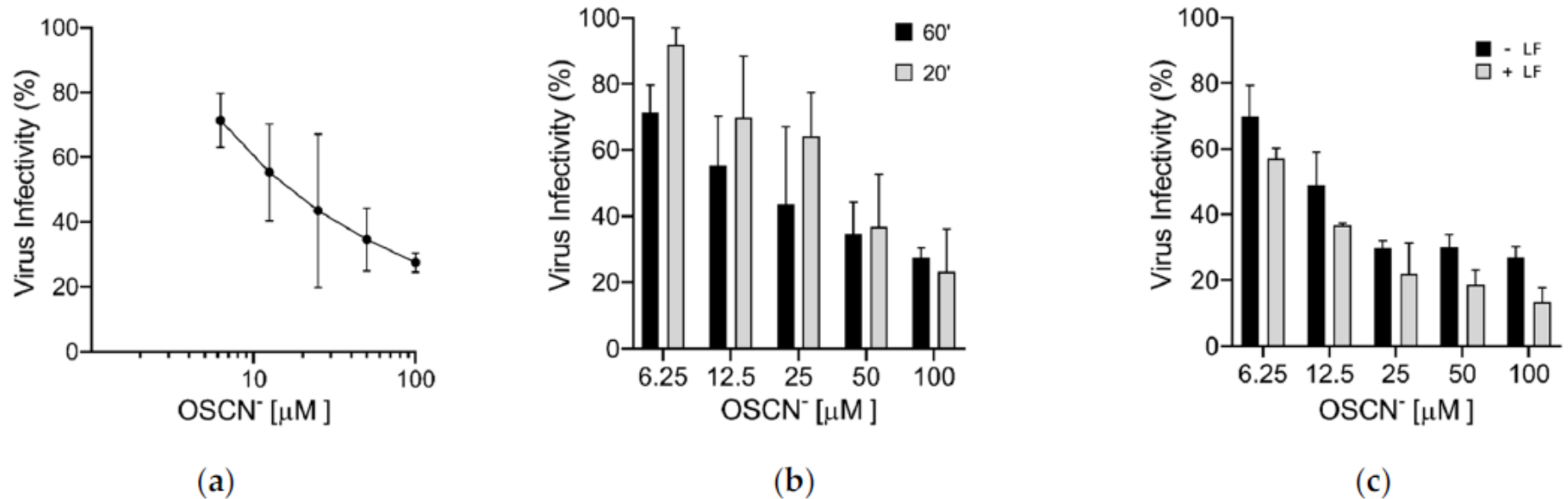


Figure 3. Virucidal activity of OSCN⁻ and OSCN⁻/LF against SARS-CoV-2. SARS-CoV-2 was diluted in MEM and incubated for 1 h at 37 °C with only OSCN⁻ or supplemented with LF before infection of Vero-E6 cells. The reduction of infectivity was evaluated by plaque assay. (a) Virucidal effect of OSCN⁻; (b) comparison between different times of virus-OSCN⁻ exposure on the efficiency of the virucidal activity; (c) evaluation of the combination OSCN⁻ + LF on the virucidal activity. Data (mean ± SD, N = 3, experiments in duplicate) are percentages of no drug, set as 100%.

ALX-009 – NO LIMITING TOXICITY IDENTIFIED IN ANIMAL STUDIES

- ▶ Regulatory toxicology package conducted in rat and dog by inhalation
- ▶ 28-days studies: no clinical signs or changes in hematology, blood chemistry or urinalysis reported in toxicology studies
- ▶ Lung events reported dose dependent and reversible
- ▶ No significant cardiac, respiratory and CNS toxicity
- ▶ No genotoxicity
- ▶ Long term toxicity studies in support to Phase II already performed: no new findings

Favorable safety profile to support clinical studies

WHERE ARE WE ?

ALX-009 - CLINICAL TRIAL PHASE I - STUDY STATUS

COMPLETED

Part I - Single dose in Healthy Volunteers (n=42)
3 cohorts - bLF or OSCN⁻ separately vs placebo (n=6/6/2)



COMPLETED

Part II - Multiple doses in Healthy Volunteers (n=16),
TID during 7 days 2 cohorts – ALX-009 vs placebo (n=6/2)



COMPLETED

Part III - Multiple doses in CF patients (completed) / Healthy Volunteers
(n=21), BID during 7 days
3 cohorts (n=3/3/1 each) - bLF or OSCN⁻ or placebo



COMPLETED

Part IVa - Multiple doses in healthy volunteers (n=12) BID during 7 days
3 cohorts (n=3/1 each) - ALX-009 vs placebo

ONGOING

Part IVb - Multiple doses in **CF/BE** patients (n=12) BID during 7 days
3 cohorts (n=3/1 each) - ALX-009 vs placebo

ALX-009 : DESCRIPTION OF THE PRODUCT

OSCN⁻ Solution

Highly reactive ion (chemical $\frac{1}{2}$ life < 2h)
→ Extemporaneous production required

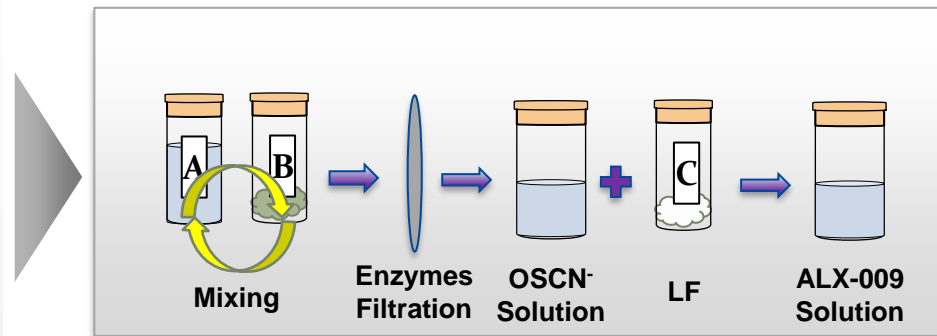
LACTOFERRIN (LF)

Purified from cow milk

ALX-009

Association of two key **endogenous** microbiocide substances for lung innate immune response against infections

Extemporaneous production in a dedicated equipment, followed by an administration by inhalation:



ALX-009 PRODUCTION: DESCRIPTION OF THE DEDICATED EQUIPMENT

- Composed of 2 parts:
 - An electrical and automated production unit (re-useable)
 - A single-use sterile fluidic kit carrying on dialysis micromodule
- Intended to be used at home:
 - By lay users: the patient himself or its relative
 - At patient's bedside, just before administration
 - Using 4 sterile single-use vials
- Designed to:
 - Be robust
 - Avoid misuses (built-in misuse detection system)
 - Be user-friendly (ease of use, production time ~ 15 min)
with online demo videos, VR training
 - Ensure users' and patients' safety



1st machine producing drug on site AI compatible
for personalized medicines

[EOLEASE in Real Life](#)

BRONCHIECTASIS AS A SECOND TARGET

- Lung disease very close to CF disease
- Lung Infection is the major issue in BE
- Prevalence is estimated between 5,3 to 56.6/10.000 depending on age and countries
- Older persons are more affected
- World wide indication, over 10 Millions patients suffer from BE
No antimicrobial product registered in EU/US,
- Alaxia, partner of Inhaled Antibiotics in Bronchiectasis and Cystic Fibrosis [iABC project](#), an IMI project

CONCLUSION

- ALX-009, First in class inhaled antimicrobial drug, is developed by Alaxia to be used in Cystic Fibrosis and Bronchiectasis indications
- ALX-009 is currently running last part of Clinical Trial Phase I in CF and BE patients in EU and UK
- Next Step, Running Clinical Trial Phase IIa in CF and BE patients

CLINICAL TRIAL PHASE IIA IN CF AND BE PATIENTS

Looking for New partner, ready to support Alaxia, especially CT PhaseIIA
due to Alaxia main shareholder health concerns

Alaxia and/or ALX-009 rights to be discussed accordingly

ALX-009 FOR CYSTIC FIBROSIS AND BEYOND



Early stage, Chronic,
Stand alone/combination
with antibiotics on various
types of infections
including multidrug
resistant one's

RECOMMENDED READING

- ▶ **Singh PK, Parsek MR, Greenberg EP, Welsh MJ.** A component of innate immunity prevents bacterial biofilm development. *Nature*. 2002 May 30; 417(6888):552-5.
- ▶ **Geiszt M, Witta J, Baffi J, Lekstrom K, Leto TL.** Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defenses. *The FASEB J*. 2003; 17(11):1502-4.
- ▶ **Wijkstrom-Frei C, El-Chemaly S, Ali-Rachedi R, Gerson C, Cobas MA, Forteza R, Salathe M, Conner GE.** Lactoperoxidase and human airway host defense. *Am. J. Respir. Cell Mol. Biol*. 2003 Aug; 29(2):206-12.
- ▶ **Rogan MP, Geraghty P, Greene CM, O'Neill SJ, Taggart CC, McElvaney NG.** Antimicrobial proteins and polypeptides in pulmonary innate defence. *Respir. Res*. 2006; 7:29.
- ▶ **Moskwa P, Lorentzen D, Excoffon KJ, Zabner J, McCray PB Jr, Nauseef WM, Dupuy C, Bánfi B.** A novel host defense system of airways is defective in CF. *Am. J. Respir. Crit. Care Med*. 2007 Jan 15; 175:174-83.
- ▶ **Conner GE, Wijkstrom-Frei C, Randell SH, Fernandez VE, Salathe M.** The lactoperoxidase system links anion transport to host defense in CF. *FEBS Lett*. 2007 Jan 23; 581(2):271-8.
- ▶ **Rada B, Lekstrom K, Damian S, Dupuy C, Leto TL.** The pseudomonas toxin pyocyanin inhibits the dual oxidase based antimicrobial system as it imposes oxidative stress on airway epithelial cells. *J Immunol*. 2008 Oct 1; 181(7):4883-93.
- ▶ **Fischer H.** Mechanisms and function of DUOX in epithelia of the lung. *Antioxid*. 2009 Oct; 11(10):2453-65.
- ▶ **Chandler JD, Day BJ.** Thiocyanate: a potentially useful therapeutic agent with host defense and antioxidant properties. *Biochem Pharmacol*. 2012 Dec 1;84(11):1381-7.
- ▶ **Chandler JD, Nichols DP, Nick JA, Hondal RJ, Day BJ.** Selective metabolism of hypothiocyanous acid by mammalian thioredoxin reductase promotes lung innate immunity and antioxidant defense. *J Biol Chem*. 2013 Jun 21;288(25):18421-8.
- ▶ **Cegolon L, Salata C, Piccoli E, Juarez V, Palu G, Mastrangelo G, Calistri A.** In vitro antiviral activity of hypothiocyanite against A/H1N1/2009 pandemic influenza virus. *Int J Hyg Environ Health*. 2014 Jan;217(1):17-22.
- ▶ **Moreau-Marquis S, Coutermarsh B, Stanton BA.** Combination of hypothiocyanite and lactoferrin (ALX-109) enhances the ability of tobramycin and aztreonam to eliminate *Pseudomonas aeruginosa* biofilms growing on cystic fibrosis airway epithelial cells. *J Antimicrob Chemother*. 2014 Sep 11. pii: dku357.
- ▶ **Tunney MM, Payne JE, McGrath SJ, Einarsson GG, Ingram RJ, Gilpin DF, Juarez-Perez V, Elborn JS.** Activity of hypothiocyanite and lactoferrin (ALX-009) against respiratory cystic fibrosis pathogens in sputum *J Antimicrob Chemother*. 2018 Dec 1;73(12):3391-3397. doi: 10.1093/jac/dky357
- ▶ **Cegolon L, Mirandola M, Salaris C, Salvati MV, Salata C, Mastrangelo G.** Hypothiocyanite and Hypothiocyanite/Lactoferrin mixture exhibit virucidal activity in vitro against SARS-CoV-2. *Pathogens* 2021,10, 233. <https://doi.org/10.3390/pathogens10020233>

ALAXIA ORAL COMMUNICATIONS AND POSTERS



- ▶ **Post-antibiotic effect of OSCN⁻, Lactoferrin and ALX-009 on clinical strains isolated from Cystic Fibrosis patients**
L. Jubeau, V. Juarez-Perez. *ECFS Belgrade, Serbia, 41st annual congress, June 7-9, 2018*
- ▶ **Non clinical safety of ALX-009, An antimicrobial therapy for Cystic Fibrosis lung infections**
V. Juarez-Perez, P. Bordeau, P. Gaillard. *NACFC Indianapolis, Indiana, 31st annual congress, Nov 2-4, 2017*
- ▶ **Effect of multiple doses of ALX-009, A novel combination of Hypothiocyanite and Lactoferrin on microbial load in Cystic Fibrosis (CF)**
J. Payne, R. Ingram, S. Elborn, D. Gilpin, V. Juarez-Perez, M. Tunney. *ECFS Sevilla, Spain, 40th annual congress, June 7-10, 2017*
- ▶ **Activity of ALX-009, A novel combination of Hypothiocyanite and Lactoferrin against clinical Cystic Fibrosis respiratory pathogens**
J. Payne, R. Ingram, S. Elborn, D. Gilpin, V. Juarez-Perez, M. Tunney. *NACFC Orlando, Florida, 30th annual congress, Oct 27-29, 2016*
- ▶ **ALX-009 (OSCN⁻/bLF) efficacy against emergent Cystic Fibrosis pathogens**
C. Bechetoille, L. Jubeau, V. Juarez-Perez. *NACFC Orlando, Florida, 30th annual congress, Oct 27-29, 2016*
- ▶ **Therapeutic Potential of Inhaled ALX-009 (OSCN⁻/ LF) for the treatment of *Achromobacter* spp. infections in Cystic Fibrosis**
C. Bechetoille, Y. Sonmez, L. Jubeau and V. Juarez-Perez. *ECFS Basel, Switzerland, 39th annual congress, June 8-11, 2016*
- ▶ **Therapeutic potential of inhaled ALX-009 (OSCN⁻/LF) for emergent and multiresistant bacteria infections in Cystic Fibrosis**
Y. Sonmez, C. Bechetoille, S. Perrotto and V. Juarez-Perez. *NACFC Phoenix, Arizona, 29th annual congress, Oct 8-10, 2015*
- ▶ **Successful *Burkholderia* spp eradication with OSCN/Lactoferrin. In vitro study evidence over a worldwide collection of clinical strains**
Y. Sonmez, C. Bechetoille, S. Perrotto, A. Payet-Burin, V. Juarez-Perez. *ECFS Brussels, Belgium, 38th annual congress, June 10-13, 2015*
- ▶ ***Burkholderia* spp. Resistances strategies. OSCN⁻/LF (ALX-009), Is its mode of action a bulwark against bacterial resistance?**
V. Juarez-Perez, C. Bechetoille, Y. Sonmez and S. Perrotto. *IBCWG Vancouver, Canada, 19th annual congress, April 15-18, 2015*
- ▶ **Bactericidal potential of OSCN⁻, bovine Lactoferrin and their combination on clinical isolates of *Burkholderia* spp.**
Y. Sonmez, C. Bechetoille, S. Perrotto, A. Payet-Burin, V. Juarez-Perez. *NACFC Atlanta, Georgia, 28th annual congress, Oct 9-11, 2014*
- ▶ **Bactericidal potential of OSCN⁻, bovine Lactoferrin and their combination over clinical strains of *Burkholderia* spp.**
Y. Sonmez, C. Bechetoille, S. Perrotto, A. Payet-Burin, V. Juarez-Perez. *IBCWG Nimes, France, 18th annual congress, April 8-12, 2014*
- ▶ **ALX-109 potentiates the effect of inhaled antibiotics at killing *Pseudomonas aeruginosa* biofilms on human airway cells**
S. Moreau-Marquis, J.D. Drexinger, V. Juarez Perez, B.A. Stanton. *NACFC Orlando, Florida, 26th annual congress, Oct 11-13, 2012*
- ▶ **Feasibility study of OSCN⁻ and Lactoferrin (Meveol®) nebulization for Cystic Fibrosis patients**
S. Perrotto, S. Le Guellec, L. Vecellio, E. Fichant, P. Stordeur, P. Bordeau, JP. Perraudin. *ECFS Hamburg, 34th congress, June 8-11, 2011*

ALX-009

www.alaxia-pharma.eu

Member of iABC consortium



innovative
medicines
initiative



Program supported by



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