Efficacy of Inhaled ALX1 in a Model of Chronic Pseudomonas Aeruginosa Infection

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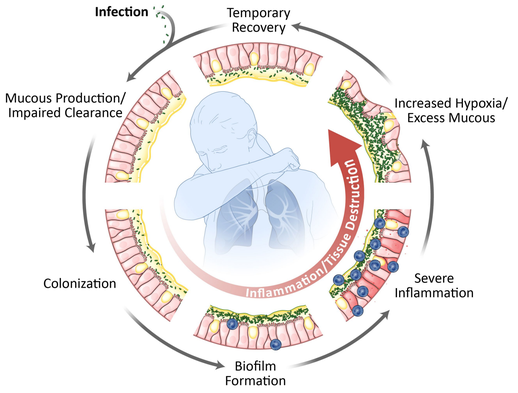
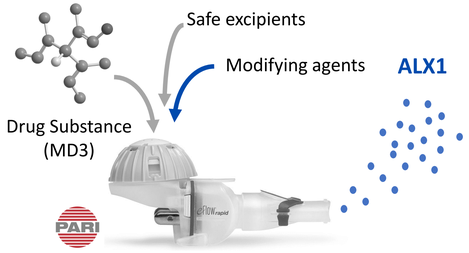
Introduction

Bronchiectasis is a disease based on dysregulated inflammation that leads to enlarged airways (bronchi), mucus build-up, and bacterial infections. Recurring respiratory infections cause significant morbidity and mortality.



Pseudomonas aeruginosa biofilms and related infections are particularly problematic with a 30-50% 5-year mortality rate. The airway mucus and biofilm matrix both frustrate the immune response and mitigate the effectiveness of antibiotics to *P. aeruginosa*.

The objective of this study was to evaluate the efficacy and pharmacokinetics of a novel inhaled nitric oxide-releasing formulation, ALX1, to treat *P. aeruginosa* in a chronic rodent model of infection.

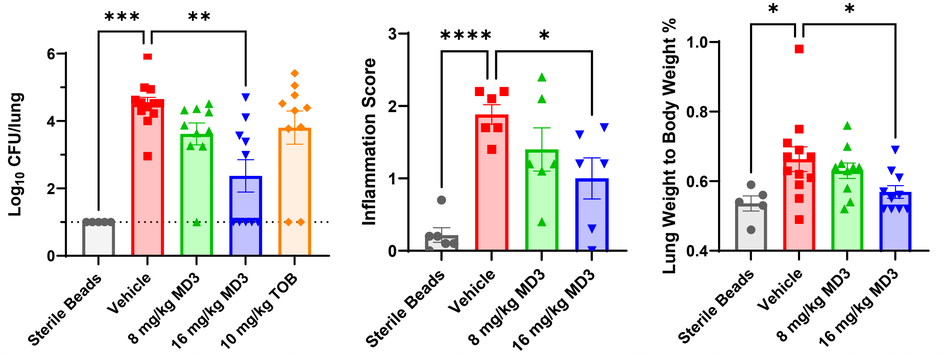


Precise droplet size control for enhanced lung deposition

Efficacy of Inhaled ALX1 in a Rodent Model of Chronic *P. aeruginosa* Infection (DDR-026)

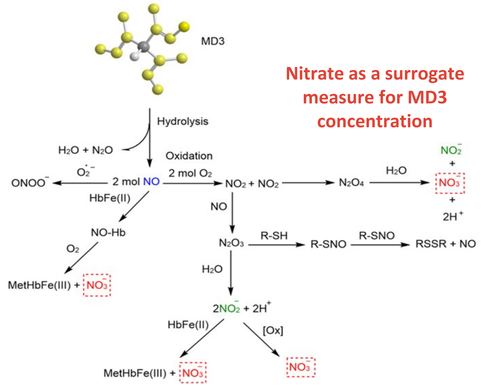
In vivo dose responsive reduction in bacterial load superior to Tobramycin

- **>99% *P. aeruginosa* reduction for 16 mg/kg**
  - **50% of animals had complete eradication**
- **Well-tolerated based on clinical observations & body weight**



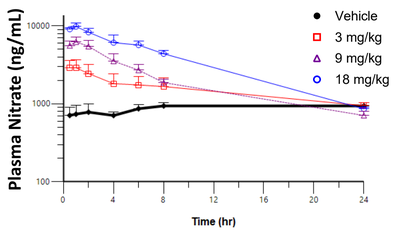
Mean *P. aeruginosa* Log_{10} CFU/lung, inflammation scores, and lung-to-body weight ratios of infected rats after 7 d of once daily treatment with nebulized vehicle, MD3, or Tobramycin. Each data point represents a single animal, with error bars representing the standard error of the mean. Significance: *=p<0.05, **=p<0.01 relative the P. aeruginosa-infected vehicle and ***=p<0.001 , ****=p<0.0001 relative sterile beads calculated with one-way ANOVA.

Single Dose Pharmacokinetic Study of Inhaled ALX1 in Male Rats (ADME-001)

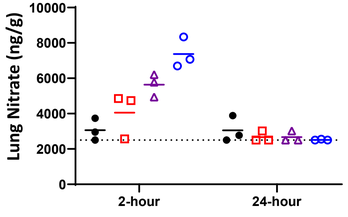


Dose (mg/kg)	T_{max} (h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	T_{last} (h)	AUC_{last} (h*ng/mL)	AUC_{INF} (h*ng/mL)	Cl/F (mL/min/kg)	Vz/F (L/kg)
3	0.50	2190	2.3	24	15800	15800	3.2	0.642
9	1.0	5510	2.6	8.0	23800	27400	5.5	1.25
18	1.0	9060	5.7	8.0	46100	74300	4.0	1.98

Dose proportional increase in C_{max}



Predictive Pharmacokinetics



Conclusions

The therapeutic potential of an inhalable NO-releasing formulation, ALX1, was successfully demonstrated in a pre-clinical model of chronic *P. aeruginosa* respiratory infection with superior performance to that of Tobramycin (10 mg/kg). Given the predictive pharmacokinetics following inhalation, these results suggest the ALX1 drug product is a promising candidate for treating chronic *P. aeruginosa* respiratory infections.

Methods

DDR-026: *P. aeruginosa* was embedded in agarose beads ($6.5x10^7$ CFU/mL) and used to infect male Sprague Dawley (SD) rats via oropharyngeal administration as reported previously. The resulting infection was non-lethal, with moderate-to-severe inflammation and a high proportion of neutrophils in the lungs. Infected animals (n=16 per group) were dosed once daily via inhalation on Study Day -1 through Study Day 6 with test article (ALX1 at two concentrations of the NO-releasing MD3 prodrug in phosphate buffer), vehicle control (phosphate buffer), or a positive control (Tobramycin at 10 mg/kg). ALX1 and vehicle aerosolized solutions were delivered using a Pari E-Flow Mesh Nebulizer with an open reservoir attached to a 3-tier nose-only flow-past inhalation chamber (total air flow rate of 20 L/min). The delivered dose of ALX1 (8 or 16 mg/kg/day) to the animals was controlled by altering the nebulization time (up to 80 min). Animals receiving vehicle only were dosed with isotonic phosphate buffer (without MD3) for 80 min. Tobramycin (10 mg/kg/day) was dosed daily by oropharyngeal delivery (validated for use as positive control, DDR-012). At Day 7, a portion (n=10) of the rodents were sacrificed, and their lungs homogenized for enumeration of bacteria. For histopathological analysis of lung inflammation, nine lung cross sections from each of the remaining rodents (n=6) were processed via H&E staining and scored by a blinded veterinary pathologist on a 5-point scale (absent 0, minimal 1, mild 2, moderate 3, marked 4, severe 5).

ADME-001: In a separate pharmacokinetic study using the same nebulization apparatus/conditions, lung and blood tissue samples were collected following a single inhaled ALX1 dose to SD rodents (n=6 per group). The test article was delivered via inhalation at a single dose of 3, 9, or 18 mg/kg MD3, or vehicle alone. Serial blood samples were collected for 24 hours following treatment. Lungs were terminally collected at either 2- or 24-hours following the dosing period (n=3 per time point). Plasma and tissue samples were stored frozen (-20 °C) until analyzed by mass spectrometry for nitrate concentrations as the most stable metabolite of nitric oxide (BAM0640). PK analyses were carried out by Phoenix WinNonlin non-compartmental analysis using the linear trapezoidal rule for AUC calculations and sparse data analysis function. Concentrations below the limit of quantitation were set equal to zero. Change from baseline was calculated by first averaging the endogenous nitrate plasma concentration values for vehicle treated animals at each time point and then subtracted from the mean value from each dosing group at a given time point.