Inhibition of the Ion Channel TMEM16A with Niclosamide Inhalation Powder Reduces Inflammation, Bronchoconstriction, and Airway Hyperresponsiveness in a Large Animal Model of Asthma

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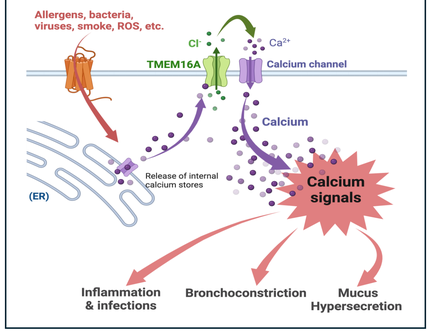
INTRODUCTION AND METHODS

Introduction: Severe asthma is poorly controlled with existing therapies. Core disease features include smooth muscle constriction, mucus hypersecretion, inflammation, and infections. The calcium-activated chloride channel, TMEM16A (ANO1), is a unique drug target capable of alleviating all these causes of difficult breathing in asthmatics, that also holds promise for the treatment of IPF, PAH, COPD, CF, and as a broad-spectrum anti-infective. TMEM16A's role in disease pathogenesis has been validated by gene silencing and knockout studies[1-3]. We previously determined from a high throughput screen of over 580,000 compounds that the approved drug niclosamide is a potent TMEM16A inhibitor[4]. Niclosamide, however, has poor oral bioavailability[5] making it unsuitable for treating lung disorders. To overcome this challenge, we produced a novel dry powder formulation for inhalation and describe herein its pharmacokinetics and efficacy in vivo.

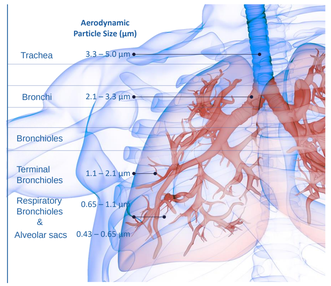
Methods: A Supercritical Precipitation (SCP) solvent-antisolvent system was used to engineer niclosamide dry particles suitable for inhalation. SD rats were exposed to aerosolized SCP-niclosamide dry powder (AR-001) by nose-only inhalation, while a canine face mask inhalation exposure system was used for studies with beagle dogs. A rotating brush generator (Palas) was used for aerosolization of the dry powder. To determine the efficacy of daily inhaled doses of AR-001 versus vehicle control, beagle dogs with pre-existing asthmatic-like responses[6] were challenged with ragweed and dust-mite allergens and changes in lung resistance, methacholine (Mch) airway hyper-responsiveness (AHR) and lung inflammation was measured.

TMEM16A, a Ca^{2+}-activated Cl^- channel that amplifies activation-induced calcium signaling in the lungs



Characteristics of Niclosamide Dry Powder Generated by Supercritical Precipitation

Stable crystalline dry powder of small particle size (0.5 – 5.0 µm) and density for deposition throughout the airways that show excellent performance from clinical dry powder inhaler

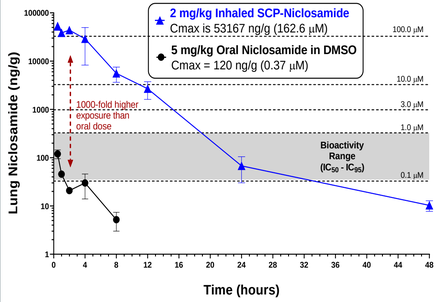


Pulmonary Deposited Dose and Mass Median Aerodynamic Diameter (MMAD) after Aerosolization

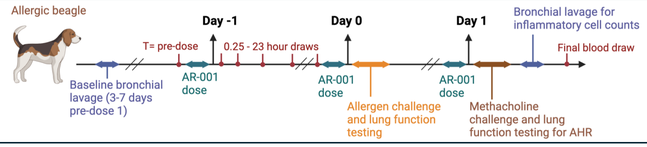
Study	Group	Ave. AR-001 Deposited Dose	Ave. AR-001 MMAD (µm)	GSD
Rat PK	NA	2.04 mg/kg	3.38	1.57

Beagle Efficacy	Vehicle	(air only, as AR-001 has no excipients)		
	Low Dose	0.49 mg/kg	3.32	1.70
	High Dose	6.22 mg/kg	3.65	1.56

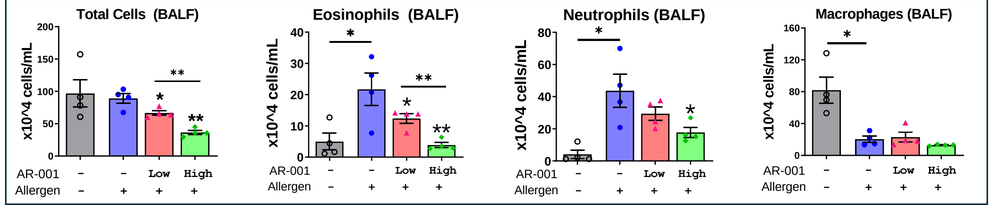
Inhaled SCP-Niclosamide (AR-001) Provides Far Greater Lung Exposure in Rats than Oral Dosage



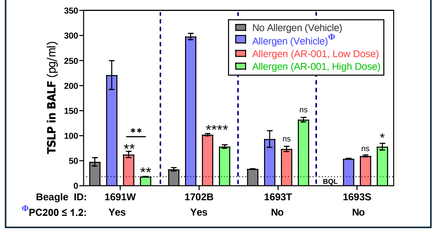
Allergic Beagle Study Design. A cross-over inhalation study with three arms: vehicle control, low dose and high dose of AR-001



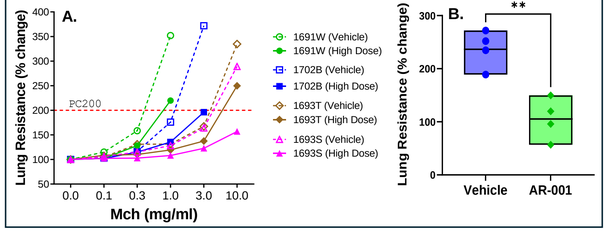
AR-001 Reduces Lung Inflammation



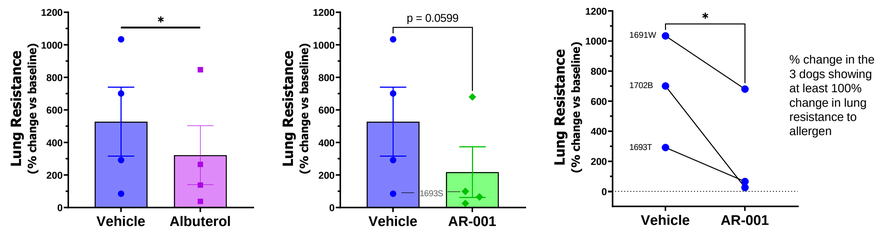
AR-001 Reduces BAL Fluid TSLP



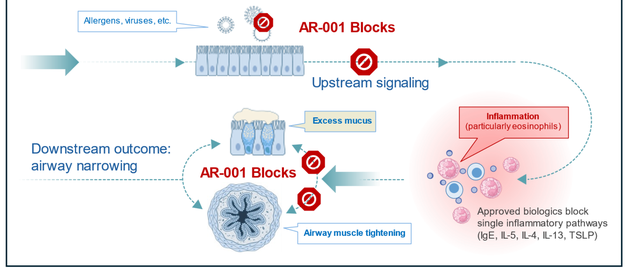
AR-001 Decreases Mch AHR (A) and Maximum Response to Mch (B)



AR-001 (High Dose) Reduces the Acute Bronchoconstrictive Early-Phase Response to Allergen



AR-001 Improves Breathing in Asthma by Blocking Both the Upstream and Downstream Signals That Cause Airway Obstruction



CONCLUSIONS

- Inhaled AR-001 provided higher and more sustained lung drug concentrations than oral dosing, allowing once-daily dosing
- AR-001 reduced both inflammation and bronchoconstriction in a large animal model of mixed granulocytic asthma
- The dose-dependent reduction in BAL eosinophils & neutrophils is predicted to be beneficial in poorly controlled severe asthma[7]
- AR-001 impact on eosinophils was comparable to the anti-IL5 biologic mepolizumab in a cynomolgus monkey model of asthma[8]
- The important upstream epithelial cytokine TSLP was also decreased in beagle dogs that showed the greatest AHR
- AR-001 reduced the early bronchoconstrictive response to allergen and the late asthmatic response leading to Mch AHR
- No adverse effects noted after inhaled delivery of low and high doses of AR-001 to beagle dogs

REFERENCES

1. I. Cabrita et al. (2021) Am J Respir Cell Mol Biol 64, 50-58.
2. R. Benedetto et al. (2019) FASEB J. 33, 4502-4512.
3. P. Wang et al. (2018) J Allergy Clin Immunol 141, 1259-1268 e1211.
4. K. Miner et al. (2019) Front Pharmacol 10, 51.
5. M. T. Schweizer et al. (2018) PloS one 13, e0198389.
6. T. K. Redman et al. (2001) Experimental Lung Research 27, 433-451.
7. W. C. Moore et al. (2014)J Allergy Clin Immunol 133, 1557-1563.e1555.
8. T. K. Hart et al. (2001) J Allergy Clin Immunol 108, 250-257.

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