Abstract (revised)

Background: RSV is the most common cause of lower respiratory tract disease and hospitalization of US infants. It is also an important pathogen of the elderly and immunocompromised. Existing prevention and treatment strategies are limited. RNAi based therapy via small interfering RNAs (siRNA) utilizes endogenous cellular mechanisms to catalyze the targeted degradation of mRNA in a sequence specific manner. Therapeutic and prophylactic administration of an siRNA against the RSV N-gene (ALN-RSV01) reduces RSV in vitro and in vivo. Intranasally aerosolized single doses of 2mg/kg reduce RSV in mouse lungs by 3-4 logs. Synthetic siRNAs have not been previously evaluated in man except for direct administration to the eye.

Objective: To evaluate the aerosolizability and clinical safety of an siRNA delivered to the respiratory tract. To develop a safe, reproducible and robust human experimental infection model for testing RSV antiviral proof of concept in man.

Design/Methods: 1. ALN-RSV01 was characterized pre, during, and post nebulization (PAR) aerosol nebulization in vitro. Intranasal sprays of ALN-RSV01 were administered in single and multiple rising doses to healthy adults in randomized, double blind, placebo (saline) controlled trials. 2. Increasing quantities of a wild type GMP-produced RSV strain were administered intranasally to cohorts of healthy volunteers. Safety and clinical data and nasal washes were collected.

Results: A novel RSV experimental human infection model has been developed which will now allow rapid and efficient evaluation of novel RSV antivirals.

Conclusions: ALN-RSV01 can be aerosolized in a manner predictive of substantial lung deposition while retaining its structure and function. It appears safe in early clinical evaluation when administered intranasally. A robust RSV experimental human infection model has been developed which will now be used to test proof of concept antiviral efficacy.

Figure 1. Phase I Study Results: Most Common Adverse Events

Figure 6. Comparison of Quantitative RSV Assays from All Nasal Washes

Figure 5. Experimental Infection summary Table

Figure 4. Selected Individual Subject Data Sets

Figure 2. Characterization of aerosolized ALN-RSV01

Figure 3. aerosolized ALN-RSV01 can be aerosolized in a manner predictive of substantial lung deposition

Poster No. 227
Early Clinical Evaluation of an RNA interference (RNAi) Based Therapy for Respiratory Syncytial Virus (RSV) Infection
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Summary of Phase I Intranasal Studies

- ALN-RSV01 appears safe when administered in relevant doses to human respiratory epithelium
- Adverse event profile comparable to placebo
- No drop outs
- No serious adverse events
- No laboratory or EKG abnormalities
- Pharmacokinetics consistent with in vitro exposure
- Cumulative clinical experience with 65 exposed subjects is encouraging

Plan for Clinical Proof of Concept

To develop and manufacture a safe, low passage, wild type RSV strain
- Original nasal aspirate from hospitalised infant with known high RSV load (RSV infection identified by FDA approved method)
- Plaque purified on Vero GMP cell line from nasal aspirate (2 passages)
- 5 subsequent passages in Vero GMP cell line
- RSV density by sequence, culture, IFA and electron microscopy
- No other adversarial agents identified
- PCR negative for other human pathogens
- Sterility testing completed and passed
- Manufactured and individually validated under GMP

ALN-RSV01 was cultured from the nasopharynx, before nebulization and collected in a nasal wash chamber after nebulization. Aerosolization using a nebulizer and PAR offered aerosolization similar results. Both cultures were (A) analyzed by ion exchange HPLC (B) analyzed in vitro in RSV plaque reduction by transfection into Vero cells subsequently infected with RSV A2. (C) The aerosol particle size distribution was determined by laser diffraction and cascade impaction methods (MMD: mass median diameter, GSD: geometric standard deviation, RF: respirable fraction).

Plan of Clinical Study

- Nasal wash samples run in duplicate on each 96 well plate having its own internal standard curve. Only data sets where at least one of the duplicate samples were positive by PCR were included in this graph.
- Quantitative PCR was performed on parallel aliquots of these nasal washes after freezing at -80°C for 20 minutes.
- Quantitative PCR was performed on frozen aliquots. All viral and disease assessments were stopped on study day 12. Safety evaluations continued through day 28.

Conclusions

- RNA interference (RNAi) is a natural process existing in all cells
- ALN-RSV01 is an siRNA utilizing this RNAi pathway targeting RSV and achieves ≥4 log reductions in virus in vivo with a single 2mg/kg dose delivered topically to the respiratory tract.
- ALN-RSV01 is the first RNAi based therapy to be tested in humans that targets an infectious disease
- ALN-RSV01 can be aerosolized into respirable particles while maintaining its structure and function
- ALN-RSV01 administered intranasally appears safe and well tolerated at doses up to 150µg (2mg/kg)
- Experimental wild type RSV infection model has been established achieving safe and robust viral quantitative detection and percent infection
- This experimental RSV infection model will be used in a proof of concept study designed to show antiviral effect in man.