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Evaluation of the safety, tolerability and pharmacokinetics of ALN-RSV01, a novel RNAi antiviral therapeutic directed against respiratory syncytial virus (RSV)

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Abstract

Small interfering RNAs (siRNAs) work through RNA interference (RNAi), the natural RNA inhibitory pathway, to down-regulate protein production by inhibiting targeted mRNA in a sequence-specific manner. ALN-RSV01 is an siRNA directed against the mRNA encoding the N-protein of respiratory syncytial virus (RSV) that exhibits specific *in vitro* and *in vivo* anti-RSV activity. The results of two safety and tolerability studies with ALN-RSV01 involving 101 healthy adults (65 active, 36 placebo, single- and multiple dose, observer-blind, randomized dose-escalation) are described. Intranasal administration of ALN-RSV01 was well tolerated over a dose range up through 150 mg as a single dose and for five daily doses. Adverse events were similar in frequency and severity to placebo (normal saline) and were transient, mild to moderate, with no dose-dependent trend. The frequency or severity of adverse events did not increase with increasing ALN-RSV01 exposure. All subjects completed all treatments and assessments with no early withdrawals or serious adverse events. Physical examinations, vital signs, ECGs and laboratory tests were normal. Systemic bioavailability of ALN-RSV01 was minimal. ALN-RSV01 appears safe and well tolerated when delivered intranasally and is a promising therapeutic candidate for further clinical development.

Keywords: Respiratory syncytial virus; RNA interference; Small interfering RNA; RNA-induced silencing complex; ALN-RSV01; Antiviral

1. Introduction

Respiratory syncytial virus (RSV) infects nearly 70% of all infants within their first year of life (Glezen et al., 1986) and causes lower respiratory tract disease in a large percentage of these children (Glezen and Denny, 1973; Fisher et al., 1997). In addition to significant morbidity in the outpatient setting, RSV infection is the single most common cause of hospitalization of US infants (Leader and Kohlhase, 2002). In adults, RSV typically produces a mild upper respiratory illness. In immunocompromised adolescents and adults, individuals with underlying lung diseases, or the elderly, RSV can produce severe respiratory disease, including pneumonia (Falsey and Walsh, 2000; Glezen et al., 2000). Current prevention strategies are used primarily in a small percentage of high-risk infants. These rely on monoclonal antibodies that are only partially effective (The Impact-RSV Study Group, 1998; American Academy of Pediatrics, 2003). No RSV vaccine is available. The only approved antiviral therapy for RSV (ribavirin) is rarely used in the European or the US pediatric population due to its potential teratogenicity and its limited effectiveness (American Academy of Pediatrics, 1996; Kimpen and Schaad, 1997; DeVincenzo, 2000). Thus, there is a major unmet medical need for an effective therapy for RSV infection in the pediatric and adult population.

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RNA interference (RNAi) is a natural process of protein down-modulation that is mediated through the sequence-specific degradation of cytoplasmic mRNA. Small interfering RNAs (siRNAs) initiate and direct this process. siRNAs have a characteristic structure of two 21 nucleotide strands with 19 nucleotides existing as a staggered duplex with a 2-nucleotide overhang at each end. siRNAs can be synthesized to target any endogenous mRNA sequence in a cell or an exogenous sequence carried by a virus. Upon cell entry siRNAs incorporate into a naturally occurring cytoplasmic protein complex, the RNA-induced silencing complex (RISC) where the two strands separate. The antisense strand of the siRNA can then bind in a sequence-specific manner to existing mRNAs in the cytoplasm. Once bound, the siRNA-RISC complex cleaves and then releases the inactivated fragments of the complementary mRNA. The antisense strand of the siRNA remains bound to RISC and continues to bind and cleave other copies of the complementary mRNA in a catalytic fashion. This process interrupts the synthesis of the corresponding protein (Fire et al., 1998; Tuschl et al., 1999; Elbashir et al., 2001). ALN-RSV01 is an siRNA designed to inhibit the replication of RSV by interrupting the synthesis of the viral nucleocapsid protein (N-protein).

In humans, RSV replicates almost exclusively in the single outermost layer of cells of the respiratory epithelium (Zhang et al., 2002; Johnson et al., 2007). These cells (ciliated pseudostratified columnar epithelial cells) form the lining of the nasal passages, the trachea, and the deeper branches of the bronchial tree. Delivering ALN-RSV01 topically to these cells of the respiratory tract reduces RSV replication in animal models of infection (Meyers et al., 2007); thus ALN-RSV01 may be an effective antiviral treatment in humans. We therefore delivered ALN-RSV01 topically to a histologically representative and easily observable area of the human respiratory tract, the nasopharynx, to evaluate its safety and tolerability in healthy volunteers. ALN-RSV01 is the first siRNA targeting a microbial pathogen to be tested in humans and is the first siRNA to be administered to the human respiratory tract. We describe the results of the first two placebo-controlled healthy volunteer studies evaluating the safety and tolerability of ALN-RSV01 delivered as a nasal spray to the epithelium of the upper respiratory tract.

2. Materials and methods

Two studies were conducted in healthy volunteers: Study 101 was a randomized, placebo-controlled, observer-blind, single dose, dose-escalation trial conducted in the United States, while Study 102 was a randomized, placebo-controlled, observer-blind, single- and multiple dose dose-escalation trial conducted in the European Union. Both studies employed the intranasal route of administration. Subjects enrolled were healthy males aged 18–45 years; key inclusion and exclusion criteria are listed in Table 1. As is common practice for first-in-man Phase 1 studies, females were excluded. Appropriate approval and informed consent was obtained prior to the conduct of any study-related procedures.

Table 1
General selection criteria for Studies 101 and 102

Inclusion criteria
• Male, 18–45 years of age, inclusive
• Good general health
• Able to read, understand and sign Informed Consent Form and Instructions
• Agreement to use appropriate contraception for a period of 14 days after administration of study medication
Exclusion criteria
• Any major organ system disease, or evidence of any acute illness at time of examination prior to study drug administration
• Any history of frequent nose bleeds
• Evidence of acute sinusitis or history of chronic sinusitis, a history of active allergic rhinitis (AR), history of perennial allergic rhinitis (PAR), or current seasonal allergic rhinitis (SAR), or recent viral rhinitis within 2 weeks prior to study drug administration
• Any nasopharyngeal abnormality that may have interfered with nasal absorption, distribution, or study-related evaluations of signs or symptoms (e.g., polyps, septal deviation)
• Blood or plasma donation within 30–60 days of screening

In Study 101 five sequential cohorts of healthy volunteers (five active, two placebo) received escalating single doses of 1.5, 5, 15, 50, or 150 mg of ALN-RSV01 or placebo (normal saline). Subjects randomized to each sequential cohort received the assigned dose of ALN-RSV01 or placebo as a nasal spray (BD Accuspray™, Becton Dickinson Medical Pharmaceutical Systems, Franklin Lakes, NJ), then were observed for at least 2 weeks after dosing to assess safety prior to exposing the next cohort to the next higher dose of study drug. In Study 102, Part A, sequential cohorts of subjects (five active, two placebo) received ALN-RSV01 or placebo as single intranasal doses (5, 25, or 150 mg), delivered as a nasal spray (as above). In Part B multiple doses of ALN-RSV01 (5, 25, or 150 mg) or placebo were administered once daily as a nasal spray to three sequential dose-escalating cohorts of 14 subjects each (nine active, five placebo) for 5 consecutive days. Safety review of data from each cohort was completed before the next cohort initiated dosing. Fig. 1 illustrates the final distribution of subjects across dose cohorts in the two studies.

2.1. Safety assessments

Clinical assessments were performed at regular intervals after administration of study drug in both studies, as shown in Figs. 2 and 3. Vital signs were determined with the subject in the sitting position for 1 min. Baseline physical examinations included specific examination of head, eyes, ears, nose, and throat, as well as vital signs and a complete body system-based examination.

Adverse events (AEs) were considered to be any untoward medical event occurring in a subject after administration of the study drug, whether or not the event was considered related to the study drug. AEs could have been volunteered spontaneously by the subject, or discovered as a result of general questioning by the study staff, by physical examination, or through procedures or laboratory testing. At each evaluation the subject was

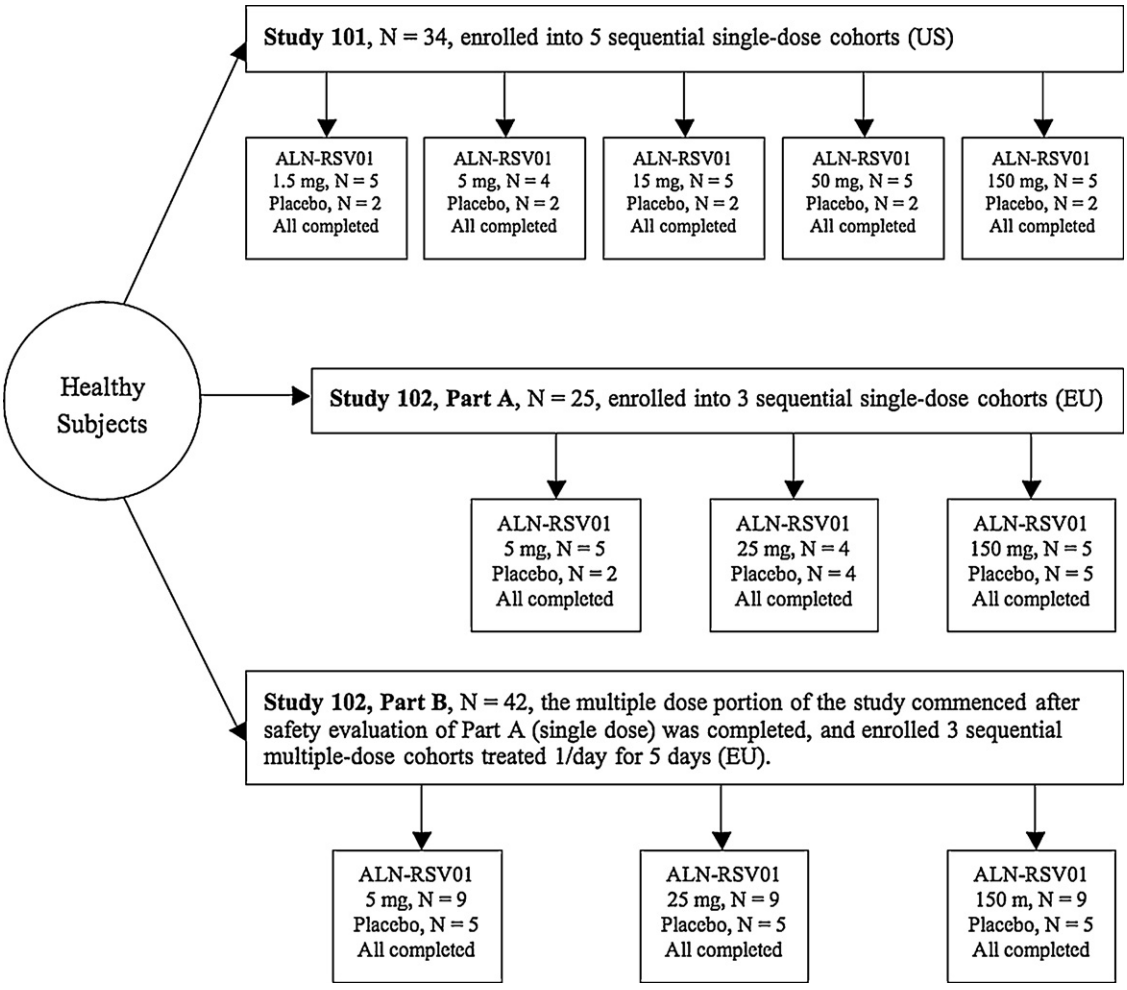

Fig. 1. Subject disposition in Studies 101 and 102. The disposition of subjects across dose levels (cohorts and treatments (ALN-RSV01 or placebo) are illustrated for both studies. Note that dosing in Part B of Study 102 was not initiated until a complete safety review of data from Part A was completed.

asked, "Have you experienced any problems or changes since the last evaluation?" Each AE reported was recorded and graded according to modified World Health Organization (WHO) Toxicity Criteria. Modifications to the criteria allowed more precise descriptions of respiratory AEs by imposing more conservative grading across more specific categories of respiratory events.

A focused examination of the head and neck involved use of a numeric scale for rating all findings (from absent to severe on a 0–3 scale) for elicited symptoms of headache, congestion, itchy or watery eyes, ringing in the ear, post-nasal drip, decreased sense of smell, sore throat, and nose pain, and for objective signs of conjunctival injection, erythematous tympanic membrane, or

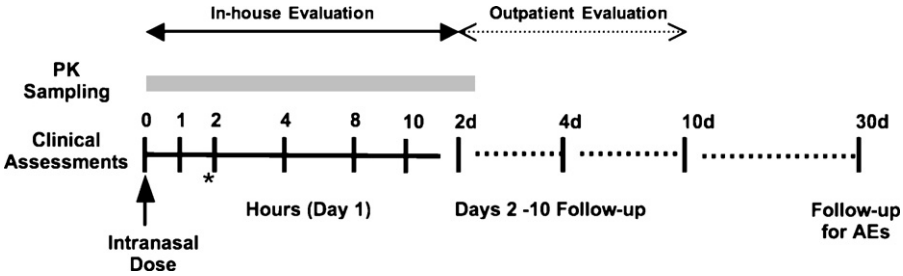

Fig. 2. Schematic of Study 101. Study 101 was a randomized, placebo-controlled, single dose, dose-escalation study in which subjects were enrolled in five sequential cohorts of six to seven subjects each who received a single dose level of either ALN-RSV01 (1.5, 5, 15, 50 or 150 mg) or placebo. Each cohort underwent the same assessments over the 10-day study period with a follow-up visit at Day 30. Subjects meeting eligibility criteria were admitted to the clinical research unit and received a single intranasal dose (solid vertical arrow) of ALN-RSV01 or placebo on Day 0. Safety assessments were performed over the next 24 h (solid horizontal line and solid horizontal arrow) with subjects released from the clinical research unit after the 24-h sample in the morning of Day 2. Subjects returned to the clinic for assessment on Days 4 and 10; day 10 completed the outpatient-monitoring phase (dotted horizontal line and dotted horizontal arrow). Subjects were followed for a total of 30 days after the dose of study medication for adverse events (dotted horizontal line). *Note*: spacing of events is not to scale. *Pharmacokinetic samples (grey bar) were obtained at 2, 4, and 10 min, and at 2 and 24 h after dosing. Urine concentrations were assessed at 1–2 and 24 h after study drug dosing.

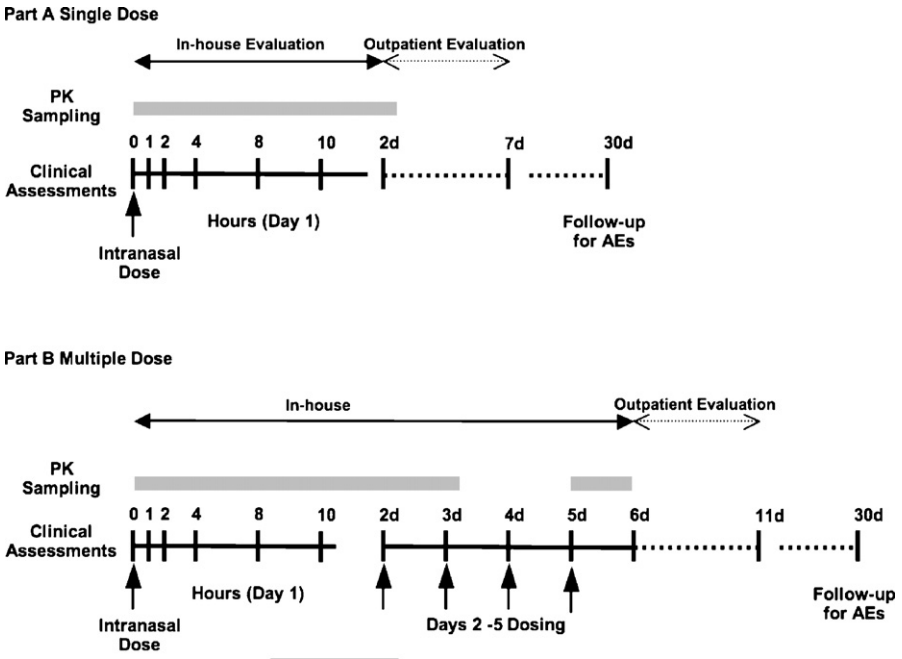

Fig. 3. Schematics of Study 102, Parts A and B. Study 102 Part A (upper panel) was a randomized, placebo-controlled, single dose, dose-escalation study similar to Study 101 in which subjects were enrolled in three sequential cohorts of 7–10 subjects each who received a single dose level of either ALN-RSV01 (5, 25 or 150 mg) or placebo. Study 101 Part B (lower panel) was a randomized, placebo-controlled, multiple dose, dose-escalation study in which subjects were enrolled in three sequential cohorts of 14 subjects each who received multiple doses of ALN-RSV01 (5, 25 or 150 mg) or placebo once per day for 5 days. Each cohort underwent the same assessments over the 11-day study period with a follow-up visit at Day 30. Subjects meeting eligibility criteria were admitted to the clinical research unit (solid horizontal arrow) and received their first intranasal dose (solid vertical arrow) of ALN-RSV01 or placebo on Day 0. Safety assessments were performed and subjects received their daily intranasal dose of study medication (solid vertical arrows) each morning (dotted grey bar). Subjects were released from the clinical research unit after the 24-h sample was obtained on Day 6 and returned to the clinic for assessment on Day 11; day 11 completed the outpatient-monitoring phase (dotted horizontal line and dotted horizontal arrow). Subjects were followed for a total of 30 days after the dose of study medication for adverse events (dotted horizontal line). Note that spacing of events is not to scale. *Pharmacokinetic samples (grey bar) were obtained pre-dose and at 2, 4, and 10 min and 1 and 24 h post-dose on Day 0 (Part A). Part B (multiple dosing) blood samples were obtained pre-dose and at 2, 4, and 10 min and 1 and 24 h post-dose on Days 0 and 4, and pre-dose on Days 2 and 3. A single morning urine sample was obtained on Day 5 (24 h post-last dose) in Part B of Study 102.

lymphadenopathy. Nasal examinations were conducted using a nasal scope according to a standardized procedure. To minimize inter-examiner variability, the same two individuals performed the pre-dose and all post-dose examinations on an individual subject. Each naris (including the nasal turbinates) was examined for visible hemorrhage, swelling, erythema, rhinorrhea, discharge, changes in color, bleeding, ulceration, crusting, and polyps or deviations of the septum. Observations were graded as none, mild, moderate, or severe. Any other signs of irritation, inflammation, or other abnormality were noted and recorded. Findings that represented a worsening from baseline, regardless of their clinical relevance or relatedness to study medication, were reported as an adverse event.

2.2. Pharmacokinetic analysis

In Study 101, pharmacokinetic blood samples were drawn at 2, 4, and 10 min, and at 2 and 24 h after study drug dosing. In Part A (single dose) of Study 102, blood samples were obtained pre-dose and at 2, 4, and 10 min and 1 and 24 h post-dose on Day 0; and in Part B (multiple dose), blood samples were obtained pre-dose and at 2, 4, and 10 min and 1 and 24 h post-dose on Days 0 and 4, and pre-dose on Days 2 and 3. Urine pharmacokinetic levels were assessed at 1–2 and 24 h after study drug dosing in Study 101, and a single sample of morning urine was obtained on Day 5 (24 h- post-last dose) in Part B of Study 102. Concentrations of ALN-RSV01 in plasma and urine were analyzed by a hybridization/ligation reaction, followed by detection using an enzyme-linked immunosorbent assay (ELISA). The lower limit of quantitation (LLOQ) for the assay was 1.5 ng/mL of plasma (Yu et al., 2002).

2.3. Statistical Procedures

Continuous and categorical variables were summarized descriptively. Missing or invalid data was not imputed. Fisher's exact test was used to compare frequencies of adverse events between treatments, while analysis of variance (ANOVA) was employed to evaluate dose-related trends in adverse events. All comparisons were two-tailed using $\alpha = 0.05$.

3. Results

3.1. Subject disposition

A total of 101 subjects were enrolled in the two studies (65 to various single and multiple doses of ALN-RSV01, and 36 to single and multiple doses of placebo), as shown in Fig. 2.

Table 2
Most common adverse events for Study 101

Body system[a] preferred term	ALN-RSV01						Placebo $N=10$
	1.5 mg $N=5$	5 mg $N=4$	15 mg $N=5$	50 mg $N=5$	150 mg $N=5$	All active $N=24$	
Respiratory, thoracic, mediastinal disorders	4 (80.0)	4 (100.0)	4 (80.0)	5 (100.0)	5 (100.0)	22 (100.0)	9 (90.0)
Nasal edema	2 (40.0)	3 (75.0)	4 (80.0)	4 (80.0)	3 (60.0)	16 (66.7)	6 (60.0)
Nasal mucosal disorder (erythema)	3 (60.0)	2 (50.0)	0 (0)	4 (80.0)	3 (60.0)	12 (50.0)	5 (50.0)
Nasal dryness	1 (20.0)	1 (25.0)	0 (0)	0 (0)	0 (0)	2 (8.3)	2 (20.0)
Rhinorrhea	1 (20.0)	0 (0)	1 (20.0)	0 (0)	0 (0)	2 (8.3)	2 (20.0)
Nasal congestion	1 (20.0)	0 (0)	1 (20.0)	1 (20.0)	0 (0)	3 (12.5)	0 (0)
Epistaxis	1 (20.0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.2)	1 (10.0)
Pharyngeal erythema	0 (0)	0 (0)	0 (0)	1 (20.0)	0 (0)	1 (4.2)	1 (10.0)
Post-nasal drip	0 (0)	0 (0)	2 (40.0)	0 (0)	0 (0)	2 (8.3)	0 (0)
Nasal discomfort	0 (0)	0 (0)	1 (20.0)	0 (0)	0 (0)	1 (4.2)	0 (0)
Pharyngolaryngeal pain	0 (0)	0 (0)	0 (0)	1 (20.0)	0 (0)	1 (4.2)	0 (0)
Upper respiratory tract infection	0 (0)	1 (25.0)	1 (20.0)	0 (0)	2 (40.0)	4 (16.7)	0 (0)

[a] Body system reported by more than one subject in any treatment group.

There were no dropouts or early withdrawals in either study. All subjects completed their assigned treatments and all follow-up assessments.

3.2. Adverse events

The most common AEs are listed in Tables 2–4. The overwhelming majority of AEs originated from observations made during the nasopharyngeal examinations; few AEs were based on subject-reported symptoms or illnesses. The majority of AEs were mild with a few AEs reported of moderate severity. No serious adverse events occurred in any subject. Occasional AEs occurred outside of the upper respiratory system and were distributed equally amongst the ALN-RSV01 and placebo subjects. These AEs were mild and included vomiting, headache, myalgia, and other nonspecific events. Four subjects in Study 101 experienced wintertime upper respiratory infections; one began at baseline prior to dosing, and the other three occurred at Days 19, 20, and 23 after drug administration (Table 2). The character, frequency and severity of AEs in the ALN-RSV01 group were similar to those of the placebo group. There was no evidence of trends in AE frequency or severity with increasing dose or increasing number of doses in any treatment group. The collection of nasal aspirates was associated with epistaxis in a minority of subjects. Table 2 provides the treatment-emergent adverse events and their frequencies in Study 101, while Tables 3 and 4 provide similar data for single and multiple dose exposures in Study 102.

3.3. Nasopharyngeal examinations

Due to the heavily vascularized nature of the nasal mucosa, well-known natural fluctuations in blood flow over time, as well as responsiveness to temperature and other environmental conditions, we expected to observe nasopharyngeal examination changes frequently in these studies. Serial nasal examinations revealed changes from baseline generally equal in frequency and character in the subjects receiving ALN-RSV01 and those receiving placebo. Frequently observed changes in examination signs included mucosal edema and mucosal erythema. There were rare reports of nasal discharge, pharyngeal erythema, mucosal petechiae and pinpoint bleeding, also of similar frequency and character in the ALN-RSV01 and placebo groups. There were no patterns or trends in the examination findings

Table 3
Most common adverse events for Study 102, Part A (single dose)

Body system[a] preferred term	ALN-RSV01				Placebo $N=11$ N (%)
	5 mg $N=5$ N (%)	25 mg $N=4$ N (%)	150 mg $N=5$ N (%)	All active $N=14$ N (%)	
Respiratory, thoracic, mediastinal disorders	5 (100)	4 (100)	5 (100)	14 (100)	11 (100)
Nasal oedema	5 (100)	3 (75)	2 (40)	10 (71)	8 (73)
Rhinorrhea	0 (0)	1 (25)	4 (80)	5 (36)	6 (55)
Nasal mucosal discoloration	5 (100)	1 (25)	1 (20)	7 (50)	5 (46)
Epistaxis	0 (0)	4 (100)	1 (20)	5 (36)	4 (36)
Cough	0 (0)	0 (0)	0 (0)	0 (0)	2 (18)
Nasal disorder	0 (0)	1 (25)	0 (0)	1 (7)	2 (18)
Nasal congestion	1 (20)	1 (25)	0 (0)	2 (14)	1 (9)
Nasal discomfort	0 (0)	0 (0)	1 (20)	1 (7)	1 (9)
Throat irritation	0 (0)	0 (0)	0 (0)	0 (0)	1 (9)

N is the number of subjects exposed per treatment. Each recorded event is the number (and percentage) of subjects that experienced the AE per treatment.
[a] Body system reported by more than one subject in any treatment group.

Table 4
Most common adverse events for Study 102, Part B (multiple dose)

Body system[a] preferred term	ALN-RSV01				
	5 mg $N=9$ N (%)	25 mg $N=9$ N (%)	150 mg $N=9$ N (%)	All active $N=27$ N (%)	Placebo $N=15$ N (%)
Respiratory, thoracic, mediastinal disorders	9 (100)	8 (89)	9 (100)	26 (96)	15 (100)
Rhinorrhea	8 (89)	2 (22)	5 (56)	15 (56)	9 (60)
Epistaxis	4 (44)	4 (44)	3 (33)	11 (41)	6 (40)
Nasal mucosal discoloration	7 (78)	2 (22)	3 (33)	12 (44)	6 (40)
Nasal edema	8 (89)	5 (56)	8 (89)	21 (78)	6 (40)
Nasal disorder	3 (33)	0 (0)	4 (44)	7 (26)	4 (28)
Pharyngolaryngeal pain	0 (0)	0 (0)	2 (22)	2 (7)	4 (28)
Intranasal numbness	0 (0)	0 (0)	0 (0)	0 (0)	1 (7)
Nasal septum ulceration	0 (0%)	0 (0%)	0 (0%)	0 (0)	1 (7%)

N is the number of subjects exposed per treatment. Each recorded event is the number (and percentage) of subjects that experienced the AE per treatment.
[a] Body system reported by more than one subject in any treatment group.

to suggest any effects due to increasing study drug dose or treatment allocation.

3.4. Other safety assessments

General physical examination findings were unchanged from baseline for all subjects in both studies. While there were some variations from normal, no abnormal vital signs or changes in vital signs were reported as AEs in either study. Pulse rate, blood pressure, body temperature and respiration rate showed no dose-related trends, and no patterns were suggestive of a relationship to study drug, or any other changes of clinical relevance. No abnormal laboratory results or changes in laboratory results were reported. There was no apparent dose relationship and no consistent difference in the incidence of out-of-range values observed between placebo subjects and those administered ALN-RSV01. No electrocardiographic abnormalities occurred in any of the subjects.

3.5. Pharmacokinetic analysis of ALN-RSV01

The study medication was administered topically to the nasopharynx, thus it was anticipated that little or no ALN-RSV01 would be absorbed systemically and be detected in plasma or urine. Samples for pharmacokinetic analysis were obtained from all subjects in both studies. In keeping with the limited surface area of the trials' topical delivery method, ALN-RSV01 was not detectable in plasma and urine for the vast majority of samples and time points. In both studies, plasma concentrations of ALN-RSV01 were below the LLOQ (<1.5 ng/mL) of the assay at all time points and for all doses, except for a subset of the 150 mg cohort in the Study 102 (Table 5). There was no evidence of drug accumulation with multiple dosing. No subjects had detectable plasma levels beyond the 10-min collection time point. In Study 101, all but five urine concentrations evaluated were below LLOQ. All five urine pharmacokinetic values that were detectable were from the first void post-dosing sam-

Table 5
Plasma pharmacokinetics of ALN-RSV01[a] in Study 102

	Pre-dose (Day 0/2/3 for Part B)	Post-dose (Day 0 for Part A, Day 1/4 for Part B)				
		2 min	4 min	10 min	1 h	24 h
Part A (single dose)						
Placebo	–	–	–	–	–	
5 mg	–	–	–	–	–	–
25 mg	–	–	–	–	–	–
150 mg	–	–	1.6–1.7[b]	–	–	–
Part B (multi dose)						
Placebo	–/–/–	–/–	–/–	–/–	–/–	–/–
5 mg	–/–/–	–/–	–/–	–/–	–/–	–/–
25 mg	–/–/–	–/–	–/–	–/–	–/–	–/–
150 mg	–/–/–	–/2.0[c]	1.8–2.6[d]/1.6–4.8[e]	1.7–1.8[f]/2.6–3.9[g]	–/–	–/–

–: Concentrations below the limit of quantification.
[a] Concentrations are measured in ng/mL and are listed as ranges of detectable concentrations.
[b] Range of three positive subjects (out of five total subjects at this dose).
[c] One positive subject (out of nine total subjects at this dose).
[d] Range of three positive subjects (out of nine total subjects at this dose).
[e] Range of three positive subjects (out of nine total subjects at this dose).
[f] Range of two positive subjects (out of nine total subjects at this dose).
[g] Range of three positive subjects (out of nine total subjects at this dose).

ples among the subjects in the final cohort receiving the highest dose of study drug (150 mg of ALN-RSV01). The highest urine concentration was 13.88 ng/mL at 111 min post-dose, and the lowest concentration was 2.13 ng/mL. For Study 102, a total of 42 urine samples were obtained approximately 24 h after the final intranasal dose and the concentration of ALN-RSV01 was below the LLOQ in all samples.

4. Discussion

We have evaluated ALN-RSV01, an RSV-specific RNAi therapeutic, in two randomized, placebo-controlled intranasal safety and tolerability studies. While a number of other antiviral siRNAs have been tested *in vitro* and *in vivo* (Capodici et al., 2002; Jiang and Milner, 2002; Hamasaki et al., 2003; Jia and Sun, 2003), ALN-RSV01 is the first (antiviral) siRNA to be delivered to the human respiratory tract.

Prior to these clinical trials, ALN-RSV01 had been extensively tested *in vitro* and *in vivo*. It demonstrates a potent antiviral activity with specificity for RSV, and a robust safety profile in both rodents and monkeys (DeVincenzo et al., 2007; Meyers et al., 2007). These animal studies revealed ALN-RSV01 to be rapidly cleared from circulation when delivered either intranasally or by intravenous dosing. This finding is consistent with the low systemic exposure observed in the studies reported here.

In these two trials, we evaluated the effects of ALN-RSV01 on a directly observable area of respiratory epithelium that is histologically similar to that lining the deeper respiratory tract—the ultimate target system in naturally infected patients. ALN-RSV01 was delivered using the BD Accuspray™, a device designed to deliver live attenuated intranasal vaccines to the nasopharynx without exposing the lower respiratory tract. When delivered in a single dose, or in five consecutive daily doses, ALN-RSV01 exhibited a side effect profile that was similar to placebo (normal saline). There were no apparent dose-related increases in the frequency or severity of side effects up to a dose of 150 mg (approximately 2 mg/kg), a dose corresponding to an effective antiviral dose identified in an *in vivo* animal model of RSV infection (Meyers et al., 2007).

The results of these studies contribute to our understanding of the safety and pharmacology of siRNAs. They also provide the foundation for further clinical evaluation of ALN-RSV01.

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