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MONOGRAPH

ALN-RSV01

siRNA Targeting RSV Nucleocapsid N Gene
Treatment of RSV Infection

Small interfering RNA (siRNA) targeting the respiratory syncytial virus (RSV) nucleocapsid *N* gene

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ABSTRACT

Respiratory syncytial virus (RSV) is one of the most frequent causes of lower respiratory tract infections. RSV, a negative-sense, single-stranded RNA virus of the family Paramyxoviridae, is associated with significant morbidity, especially in children and the immunocompromised. The lack of an effective drug therapy for RSV has stimulated the search for other treatments. ALN-RSV01, a small interfering RNA (siRNA) that affects the ability of the virus to replicate, is one of the latest developments in RSV treatment. In a randomized, double-blind, placebo-controlled phase II trial ALN-RSV01 proved to be safe and well tolerated and showed antiviral efficacy, with an approximately 40% reduction in RSV infection rate and a 95% increase in infection-free subjects.

BACKGROUND

Respiratory syncytial virus (RSV) is one of the most frequent causes of lower respiratory tract infections in children and the leading cause of pediatric hospitalizations in the U.S. (125,000 hospitalizations per year) (1). Bronchiolitis caused by RSV is a risk factor for the development of asthma in children (2). RSV is also a major infectious disease in the elderly and in adults with compromised immune systems (175,000 hospitalizations per year in the U.S.). No vaccine has proven effective against RSV and available drug therapies have not shown significant benefit (3). Hence, RNA interference (RNAi) has been developed as a potential therapeutic for RSV.

RNAi, a process in which post-transcriptional gene silencing occurs, was first described by Fire et al. in the nematode *Caenorhabditis elegans* (4). They observed that injection of double-stranded RNA (dsRNA) resulted in a pronounced decrease in endogenous transcription. RNAi-dependent gene silencing was thought to be impossible because the introduction of large dsRNA molecules is known to cause an innate immune response in mammalian cells. However, Tuschl et al. (5) demonstrated that small interfering RNA (siRNA) with a characteristic structure of two 21-nucleotide strands with 19 nucleotides existing as a duplex with a 2-nucleotide overhang at each end was effective in mammalian cells, without evidence of an innate immune response. This finding led to the development of siRNAs for various gene targets.

ALN-RSV01 is an siRNA specifically designed to inhibit RSV replication by targeting the nucleocapsid *N* gene of the RSV genome, a gene that is required for the replication of RSV. This inhibition interrupts the synthesis of the viral nucleocapsid protein (N protein), thereby reducing the ability of the virus to replicate. DeVincenzo et al. (6) developed a human RSV infection model to demonstrate the therapeutic effect of ALN-RSV01, which has been shown to be effective intranasally (7). In this review, we describe preclinical and clinical studies with ALN-RSV01.

PRECLINICAL PHARMACOLOGY

Prophylactic administration of siNS1 plasmid in the form of nanoparticles decreased the incidence of RSV infection in BALB/c mice (8). The siNS1 plasmid was complexed with a nanochitosan polymer referred to as nanogene 042 (NG042) to generate siRNA in situ. Viral titers were significantly decreased in siNS1-treated mice compared with controls. Prophylaxis with siNS1 enhanced cellular immunity and attenuated RSV infection.

Bitko et al. (9) investigated whether siRNAs that were active ex vivo could be effective in vivo. Ex vivo, 21-nucleotide dsRNAs were generated against a specific messenger RNA (mRNA) of an RSV phosphoprotein that is essential in the translation of viral proteins (10). Cells were then transfected with anti-phosphoprotein dsRNA and infected with RSV. Immunoblot analysis showed a 90% reduction in phosphoprotein levels and dsRNAs did not cause an interferon response. siRNAs were complexed with TransIT-TKO reagent and administered intranasally to BALB/c mice, a well-established animal model for RSV infection. Four hours later, each animal was challenged with 10^7 plaque-forming units (pfu) of RSV or control parainfluenza virus (PIV) administered intranasally. After 6 days, it was found that siRNAs that had been effective ex vivo were also highly effective in vivo. siRNA significantly inhibited ($P < 0.05$) pulmonary viral titers and virus inhibition was highly specific for anti-RSV; siRNA had no inhibitory effect on PIV. There were no adverse events, such as respiratory distress or change in appetite.

The design and characterization of ALN-RSV01, a potent RSV nucleocapsid gene-specific siRNA, were subsequently described (11, 12).

D. Devuni, G.Y. Wu*.
Department of Medicine, Division of Gastroentoerology-Hepatology, University of Connecticut Health Center, Farmington, CT 06030, USA. *Correspondence: wu@nso.uchc.edu.

ALN-RSV01 had an IC_{50} of 0.7 nM in in vitro RSV plaque assays. The siRNA target site was completely conserved in 89 of 95 isolates and ALN-RSV01 demonstrated antiviral activity against all isolates, including those with single mismatch mutations. In vivo, intranasal administration of ALN-RSV01, either prophylactically or therapeutically, in BALB/c mouse models resulted in potent antiviral efficacy, with 2.5-3.0 log reductions in RSV lung concentrations on both single-dose and multiple-dose regimens. ALN-RSV01 lacked measurable immunostimulatory effects and did not induce interferon alfa or TNF-α. The mechanism of action of RNAi was demonstrated by capture of the site-specific cleavage product of the RSV mRNA via rapid amplification of complementary DNA ends both in vitro and in vivo. This siRNA was directed against the N protein of the nucleocapsid region, a protein that is absent from the outer surface of RSV and part of the most highly conserved part of the RSV genome, which could be one of the reasons for its considerable efficacy.

PHARMACOKINETICS AND METABOLISM

To determine the distribution and disposition of ALN-RSV01, De Vincenzo et al. (13) conducted two studies in healthy volunteers. One was a randomized, placebo-controlled, observer-blind, single-dose, dose-escalation trial conducted in the U.S. and the other was a similar single- and dose-escalation trial conducted in Europe. A total of 101 volunteers were enrolled in the two studies; 65 received ALN-RSV01 at doses up to 150 mg as a single daily dose and for 5 days, and 36 were administered placebo. Pharmacokinetic blood samples were drawn predose and at 2, 4 and 10 min and 2 h after administration of study drug on day 0 in the single-dose study. In the multiple-dose study, samples were taken on days 0 and 4 and predose on days 2 and 3. Urine drug levels were assessed at 1, 2 and 24 h after study drug dosing in the single-dose study and a single sample was obtained on the morning of day 5 in the multiple-dose study. Concentrations of ALN-RSV01 in plasma and urine were analyzed by enzyme-linked immunosorbent assay (ELISA). ALN-RSV01 was undetectable in the majority of the plasma and urine samples. Plasma concentrations were < 1.5 ng/mL except in the 150-mg multiple-dose cohort. All detectable urine samples were from the 150-mg cohort; concentrations were < 1.5 ng/mL in the other urine samples. These studies demonstrated low systemic exposure of ALN-RSV01 following intranasal administration.

SAFETY

In two studies in which ALN-RSV01 was administered intranasally to healthy volunteers, no abnormal vital signs or changes in blood pressure, body temperature or respiration were observed and ALN-RSV01 had a similar side effect profile to placebo. Adverse events were transient, mild to moderate in intensity and were not dose-dependent. The most frequently reported events were nasal edema, nasal mucosal discoloration, rhinorrhea and epistaxis. Nonrespiratory events included vomiting, headache and myalgia (13).

CLINICAL STUDIES

A randomized, double-blind, placebo-controlled phase II trial (GEMINI) was designed to evaluate the safety and antiviral activity of ALN-RSV01 in 88 experimentally infected patients. ALN-RSV01 or placebo was administered intranasally for 5 consecutive days, 2 days prior to and 3 days after viral inoculation. Efficacy was measured by infection rate and effects on viral dynamics and clinical symptoms. ALN-RSV01 was safe and well tolerated and had statistically significant antiviral efficacy, with an approximately 40% reduction in RSV infection rate and a 95% increase in infection-free subjects compared with placebo. This study has yet to be published and more detailed results are awaited (14).

Results from a randomized, double-blind phase II study comparing inhaled ALN-RSV01 with placebo in adult lung transplant patients infected with RSV at 11 sites in 4 countries have been reported (15). Twenty-four lung transplant patients with confirmed RSV infection were randomized to receive inhaled ALN-RSV01 (n = 16) or placebo (n = 8) once daily for 3 consecutive days. There were no drug-related serious adverse events and no clinically significant differences in the overall adverse event profile compared to placebo. There was no increase in immune-mediated cytokine levels and no difference in lung function secondary to administration of the drug. There was no evidence of disease exacerbation related to the administration of ALN-RSV01. At the 90-day endpoint, all patients had survived and the incidence of intubation, new respiratory tract infections and acute rejection was comparable across the ALN-RSV01 and placebo groups. Exploratory data on bronchiolitis obliterans syndrome (BOS) were also collected at 90 days. ALN-RSV01 treatment was associated with a statistically significant decrease in the total incidence of new or progressive BOS at 90 days compared with placebo; 50% of patients who received placebo had new or progressive BOS compared with only 7.1% of ALN-RSV01-treated patients.

SOURCES

Alnylam Pharmaceuticals, Inc. (US); partnered with Cubist Pharmaceuticals, Inc. (US) worldwide except Asia and with Kyowa Hakko Kirin for Asia.

DISCLOSURE

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REFERENCES

1. Centers for Disease Control and Prevention. *Respiratory Syncytial Virus Infection (RSV).* http://www.cdc.gov/rsv/about/faq.html
2. Sigurs, N., Bjarnason, R., Sigurbergsson, F., Kjellman, B. *Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7.* Am J Respir Crit Care Med 2000, 161(5): 1501-7.
3. DeVincenzo, J.P. *Therapy of respiratory syncytial virus infection.* Pediatr Infect Dis J 2000, 19(8): 786-90.
4. Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., Mello, C.C. *Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans.* Nature 1998, 391(6669): 806-11.
5. Tuschl, T., Zamore, P.D., Lehmann, R., Bartel, D.P., Sharp, P.A. *Targeted mRNA degradation by double-stranded RNA in vitro.* Genes Dev 1999, 13(24): 3191-7.
6. DeVincenzo, J., Lambkin-Williams, R., Cehelsky, J. et al. *Development of a human experimental infection model of respiratory syncytial virus (RSV) and evaluation of an RNA interference (RNAi) therapeutic for safety and anti-viral efficacy in man.* J Allergy Clin Immunol [Annu Meet Am Acad

Allergy Asthma Immunol (AAAAI) (March 14-18, Philadelphia) 2008] 2008, 121(2, Suppl.): Abst 1037.

7. Meyers, R.E., Alvarez, R., Tripp, R. et al. *ALN-RSV01, an RNAi therapeutic for the treatment of respiratory syncitial virus (RSV) infection.* Annu Meet Pediatr Acad Soc (May 5-8, Toronto) 2007, Abst 6295.12.
8. Zhang, W., Yang, H., Kong, X. et al. *Inhibition of respiratory syncytial virus infection with intranasal siRNA nanoparticles targeting the viral NS1 gene.* Nat Med 2004, 11(1): 56-62.
9. Bitko, V., Musiyenko, A., Shulyayeva, O., Barik, S. *Inhibition of respiratory viruses by nasally administered siRNA.* Nat Med 2004, 11(1): 50-5.
10. Bitko, V, Barik, S. *Phenotypic silencing of cytoplasmic genes using sequence-specific double-stranded short interfering RNA and its application in the reverse genetics of wild type negative-strand RNA viruses.* BMC Microbiol 2001, 1: 34.
11. Meyers, R., Alvarez, R., Tripp, R. et al. *Design, screening, and evaluation of small interfering RNAs (siRNAs) for prevention and treatment of respiratory syncytial virus (RSV) infection.* Annu Meet Pediatr Acad Soc (April 29-May 2, San Francisco) 2006, Abst 4130.3.
12. Alvarez, R., Elbashir, S., Borland, T. et al. *RNA interference-mediated silencing of the respiratory syncytial virus nucleocapsid defines a potent antiviral strategy.* Antimicrob Agents Chemother 2009, 53(9): 3952-62.
13. DeVincenzo, J., Cehelsky, J.E., Alvarez, R. et al. *Evaluation of the safety, tolerability and pharmacokinetics of ALN-RSV01, a novel RNAi antiviral therapeutic directed against respiratory syncytial virus (RSV).* Antiviral Res 2008, 77(3): 225-31.
14. de Fougerolles, A., Vornlocher, H.P., Maraganore, J., Lieberman, J. *Interfering with disease: A progress report on siRNA-based therapeutics.* Nat Rev Drug Discov 2007, 6(6): 443-53.
15. *Alnylam and Cubist report complete data from phase II study of ALN-RSV01 in lung transplant patients naturally infected with respiratory syncytial virus.* Alnylam Pharmaceuticals, Inc. Press Release, July 20, 2009.