aragen.com

Preclinical evaluation of vaccines and antivirals in RSV challenge rodent models

Prepared by: Nagendra Ningaraj, PHD, MBA, CCRP
Senior Director, Scientific Affairs

Executive Summary

Presently, there is no targeted treatment available for human respiratory syncytial virus (hRSV) infection other than the supportive care to mitigate the signs of and symptoms. Antiviral drug development and RSV vaccine is expected to play a critical role in overall reduction of RSV infections globally. Continued effort in RSV research published in high profile scientific journals has invigorated efforts by larger and smaller pharmaceutical and biotechnology companies to develop antivirals and RSV vaccines. Preclinical development of vaccines is hindered by the lack of clinically relevant rodent models. More importantly, the safety of new vaccines, preclinical pharmacological, safety and toxicology studies should be conducted prior to initiating the clinical studies. Aragen will enable successful IND-enabling preclinical RSV therapeutic development studies by providing appropriate study designs, right animal models, and confirming effective immune response by performing *in vivo* and *in vitro* assays. Our scientists have and will continue to establish the identity, purity, safety, and potency of the vaccine or antivirals. In this white paper, we present several case studies performed in clinically relevant mouse and rat models to assess the critical characteristics of potential RSV therapeutics. In summary, this article includes in-house as well as client-sponsored study data developed over several years using rodent models. These studies generated clinically pertinent information for proper screening of new vaccines and antivirals. Aragen will continue to support your RSV therapeutic development effort by providing reliable and effective preclinical services in this important infectious disease area.

Introduction

As per Centers for Disease Control and Prevention (CDC), human respiratory syncytial virus (hRSV) is a common respiratory virus that usually causes mild, cold-like symptoms (1). RSV and its chimeric strains affect infants, adults, elderly worldwide and cause significant morbidity. Aragen continues to serve pharmaceutical, biotechnology and SMEs in their preclinical efficacy and safety studies. Appropriate in vivo rodent models enable development of anti-RSV antibodies, small molecules, and vaccines for the treatment of respiratory disease.

RSV is a leading cause of infant hospitalization. RSV infection causes cold-like symptoms and often progresses to bronchiolitis and pneumonia in infants. The cold-like symptoms seen in RSV infected patients appears to be like common flu in infants. However, infants and older adults exhibit symptoms differently. In infants, the RSV infection might occur at age 2 and remain unnoticed until infant exhibits severe RSV symptoms whereas in adults RSV symptoms are milder and less easy to identify. There are no targeted antivirals or vaccines readily available to treat or prevent RSV infection. Several pharmaceutical and biotechnology companies are striving to develop effective RSV vaccines.

Treatment

Typically, RSV infections go away on their own in a week or two. Virus is contagious, spreads from infected people to others living in close contact with infected persons or areas. There are few steps taken to relieve symptoms, such as managing fever and pain, giving enough fluids, and contacting healthcare provider if the symptoms persist. There is no specific treatment for RSV infection, though efforts are on to develop vaccines and antivirals. Palivizumab is a monoclonal antibody recommended by the American Academy of Pediatrics (AAP) is prescribed to prevent severe RSV illness in certain infants and children.

Present Status of RSV Vaccines

Unfortunately, no vaccine is available against RSV for more than 50 years of effort. Main hurdle for developing RSV vaccine appears to be its multiple mechanisms of evading immunity, hence it reinfects people throughout life with relatively low genetic variation compared to retro (RNA) viruses. The recent availability of structure and antigenic content of the fusion (F) glycoprotein in its metastable untriggered prefusion form (pre-F) and the stable rearranged post fusion form (post-F) gave an impetus to develop vaccine strategies (2). Hence, novel live-attenuated and chimeric virus vaccine candidates and other novel approaches to deliver vaccine antigens have been developed as vaccine products to fight RSV infection (2). Fight against RSV starts with maternal or infant immunization. RSV vaccine should reduce elderly mortality, which is still high. In both populations, vaccine safety is highly critical (3). Palivizumab currently used for treatment of high-risk infants with RSV infection. Developing RSV mice models to measure the RSV-specific neutralizing hu-MoAbs after RSV infection is crucial for early detection and therapeutic intervention (4) in infants.

Preclinical RSV models for vaccine development

The RSV mouse model is the preferred choice for most preclinical for most immunological studies, ranging from simple vaccine testing to the intricate assessment of fundamental immunopathogenic responses. An important preclinical study showed the generation of RSV-specific neutralizing hu-MoAbs after intravenous injection of immunoglobulins and Palivizumab, a ‘humanized’ anti-RSV-F MoAb (4). In last few years, neonatal mice (aged <7 days at time of initial infection) model is being validated to understand RSV infection in infants (4). Researchers showed that downregulating IL-4Rα allows for the survival of Th1 cells in the presence of Th2 cytokines such as IL-4/IL-13. This finding demonstrates the importance of the age of initial infection of RSV in determining disease outcome. Therefore, the key to a successful vaccine lies in administration of immunomodulatory agents at the time of vaccination (4). Cotton rat is considered a more relevant animal model for preclinical studies on RSV infection than BALB/c mice. Consequently, cotton rats are used to study RSV pathogenesis, anti-RSV drugs, and RSV vaccine efficacy and safety. For example, the cotton rat model was used for pre-clinical evaluation of unglycosylated recombinant E. coli produced G protein (REG) as a potential RSV vaccine (3). A preclinical study showed that bacterially produced REG could provide an economical, safe and effective broad protective vaccine against RSV disease (3).

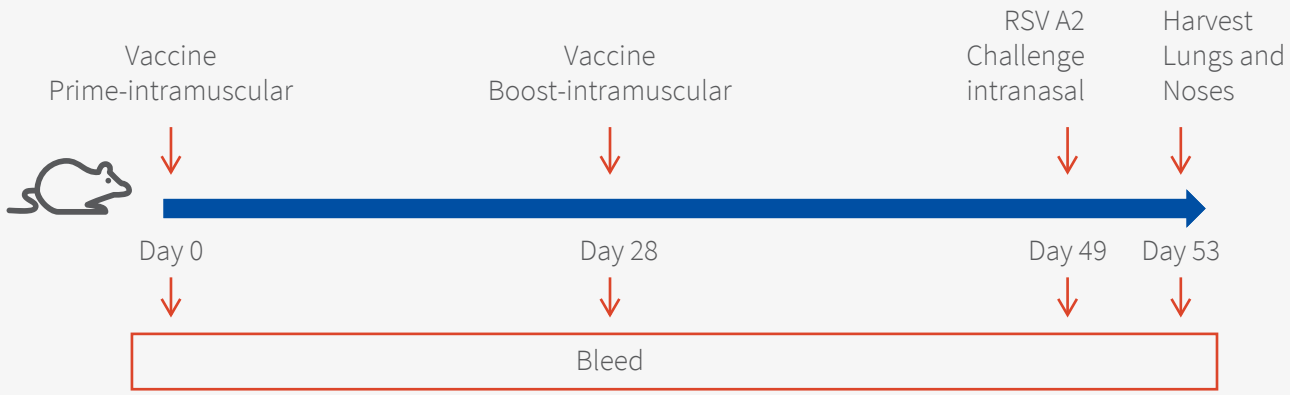
Highlights of RSV preclinical Services at Aragen

- Experts with several years of expertise, delivered over 100 successful RSV studies (30-150 animals/study)
- A wide range of readouts available with regular updates throughout study
- Flexible and customizable assays in RSV models. We have tested small molecules, large molecule, and vaccines for past and existing clients/ partners
- Willing and able to develop models as per client specification with customized studies

Cotton Rat RSV Model for Vaccine development

Salient features

- Useful for both RSV-A and RSV-B strains
- Clinically relevant model used for Synagis® approval
- Model for FI-RSV induced enhanced disease
- USDA regulated



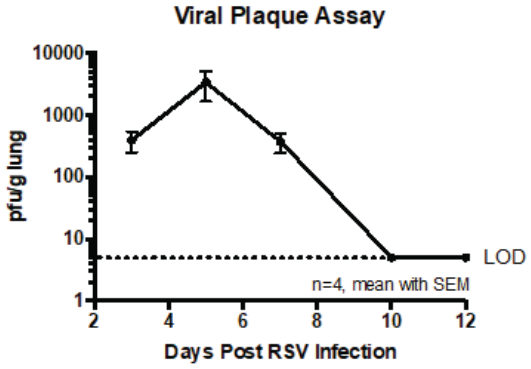
- Mice received from Aragen-approved vendor housed in dedicated room
- Clinical parameters measured
- Weekly body weights measured
- Daily observations for morbidity and ambulatory discomfort
- QCed by operators
- Ex-vivo parameters measured
- Serum processing done
- Lung weights measured
- Lung and nose homogenization with viral plaque assay
- Additional readouts: qPCR, VNA, ELISPOT, Cytokine assays, BAL related assays

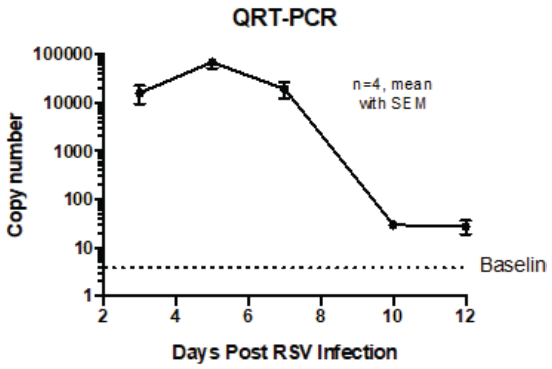
Case Study 1: RSV replication in RSV-A2 infected Cotton Rats

Female Cotton rats 6-8 weeks old were infected with RSV-A2 Long (ATCC: VR-26), intranasally on day 2, observed daily for clinical signs for 12 days. RSV replication was measured routinely by plaque assay and QRT-PCR methods. Ex vivo Assays were also performed.

- RSV titers from lungs and nasal turbinate
- Lung weights and other organ weights, snap frozen or fixed for histology
- qPCR analysis of RSV viral transcripts in lungs or other organs
- Virus neutralization assays, either client specified or in-house protocols
- ELISA serum analysis
- Cytokine analysis on BAL or lung homogenates

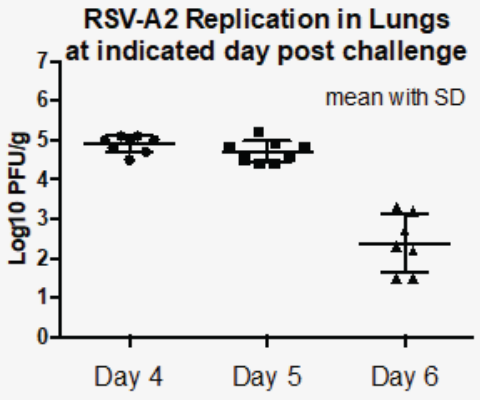
- ELISpot assays for B and T cell analysis
- BAL fluid collection with total leukocyte counts and differentials
- Serum or plasma
- Serum chemistry and whole blood differentials

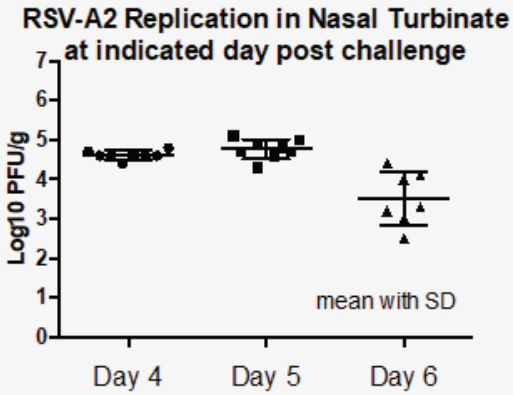




Case Study 2: LUNG AND NASAL TURBINATE TITERS IN RSV-A2 INFECTED COTTON RATS

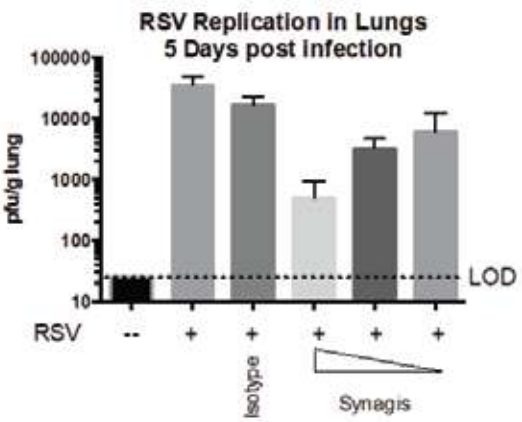
Female Cotton rats: 6-8 weeks old were intranasally infected with RSV-A2 (ATCC VR-1540), observed daily, harvested lungs and noses on day 4, 5 or 6 and viral plaque assay performed in homogenized tissue. Result: Peak RSV replication was observed on 4-5 days post infection.

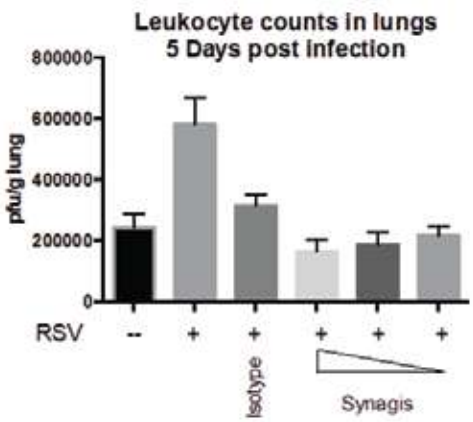




Case Study 3: Effect of SYNAGIS® on RSV replication in Cotton rats

Female Cotton rats 6-8 weeks old were infected with RSV-A2 Long (ATCC: VR-26), intranasally on day 2, observed daily for clinical signs, tissue harvested on day 5 to measure RSV replication status and leukocyte counts. We observed that Synagis® reduced RSV replication and leukocyte count in the lungs.





Case Study 4: Non-GLP Study to Evaluate Prophylactic Efficacy of XXX Antibody Against Respiratory Syncytial Virus (RSV) Strain A2 in Female Cotton Rats

Objective

To evaluate the efficacy of XXX antibody (drug) product in comparison to SYNAGIS (reference drug), in limiting viral replication of RSV (Strain A2) in lungs of female cotton rats. SYNAGIS is a prescription medication that is used to help prevent a serious lung disease caused by RSV in children.

Study Design

Thirty-five cotton rats (approximately 6-8 weeks old) separated into 7 groups (N=5/ group).

Gp1: Test Article (4mg/kg); Gp2: Test Article (2mg/kg); Gp3: Test Article (1mg/kg); Gp4: Test Article (0.5mg/kg); Gp5: Synagis® 4mg/kg; Gp6: Synagis® 2mg/kg; Gp7: PBS

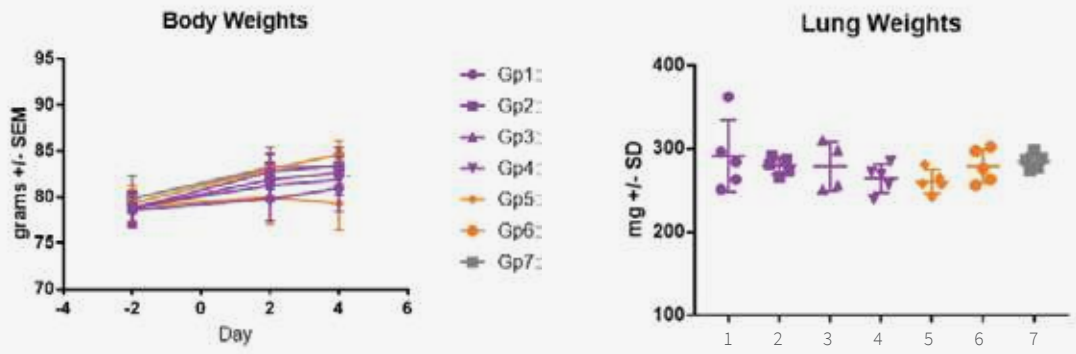
On day -1, rats received a prophylactic intramuscular injection of test article at 4 mg/kg, 2 mg/kg, 1 mg/kg or 0.5 mg/kg or they received a prophylactic intramuscular injection of the control antibody, Synagis® at 4 mg/kg or 2 mg/kg. On Day 0, all animals were inoculated intranasally with 1x105 PFU of RSV strain A2. On day 4, serum, nose and lungs were collected following euthanasia and the viral lung titers were determined by plaque assay.

The test products and Synagis® exhibited dose-dependent antiviral activity in preventing RSV replication in the lungs of cotton rats infected with RSV A2. And treatment with test products (0.5 mg/kg, 1 mg/kg, 2 mg/kg or 4 mg/kg) significantly decreased viral lung titers compared to treatment with PBS ($p<0.001$). Furthermore, the test products decreased viral lung titers on average 16-fold more than the same dose of Synagis® (4 mg/kg group: $p=0.021$; 2mg/kg group: $p<0.001$).

Delivery: Timely completion of the project to the full satisfaction of the Client.

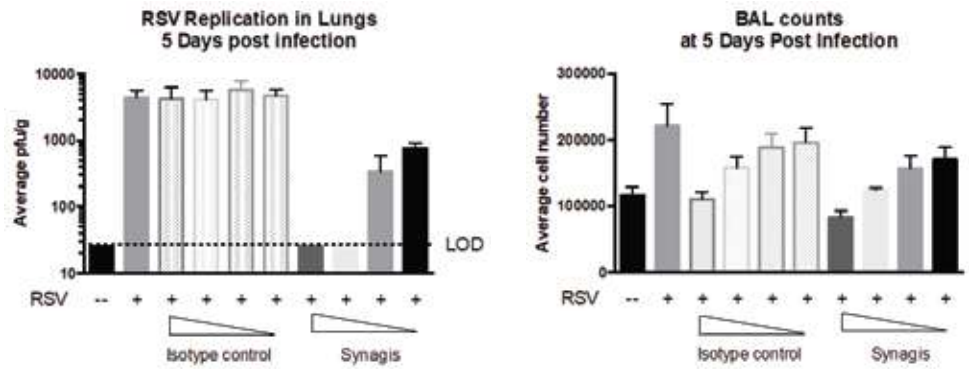
Results

Body weights and lung weights over 6 days are shown in the graphs for all 7 groups.



Case Study 5: Effect of SYNAGIS® on RSV replication

Female BALB/c: 6 weeks old Intranasally injected with RSV strain: RSV-A2 Long (ATCC: VR-26) Left Post SYNAGIS® treatment on day 3, clinical observations done daily until tissue harvest on day 5 (Right)



Bibliography

Note: References 6-15 pertain to Aragen generated case studies.

1. RSV (Respiratory Syncytial Virus) | CDC
2. Preclinical evaluation of bacterially produced RSV-G protein vaccine: Strong protection against RSV challenge in cotton rat model. Sci Rep. 2017;7:42428.
3. Higgins D, Trujillo C, Keech C. Advances in RSV vaccine research and development - A global agenda. Vaccine. 2016 Jun 3;34(26):2870-2875.
4. Cormier SA, You D, Honnegowda S. The use of a neonatal mouse model to study respiratory syncytial virus infections. Expert Rev Anti Infect Ther. 2010;8(12):1371-1380.
5. Nguyen, H et al. "Efficient generation of respiratory syncytial virus (RSV)-neutralizing human MoAbs via human peripheral blood lymphocyte (hu-PBL)-SCID mice and scFv phage display libraries." Clinical and experimental immunology vol. 122,1 (2000): 85-93.
6. ALX-0171 toxicity study in RSV-infected cotton rats: safety and therapeutic potential: Poster presentation at Eurotox 2012
7. ALX-0171 anti-RSV Nanobody is highly potent in vitro and is safe and efficacious following pulmonary administration: Ablynx 20128.
8. Potent High-Affinity Antibodies for Treatment and Prophylaxis of Respiratory Syncytial Virus Derived from B Cells of Infected Patients: Journal Immunology 2009
9. Inhibition of Fatty Acid Synthase in vitro and in vivo Reduces RSV Replication: Poster presentation at ICAAC 2014
10. Preclinical development and safety assessment of the first inhaled NANOBODY® ALX-0171: SOT2013
11. Direct Inhibition of Cellular Fatty Acid Synthase Impairs Replication of Respiratory Syncytial Virus and Other Respiratory Viruses: PLoS One 2015.Potent High-Affinity Antibodies for Treatment and Prophylaxis of Respiratory Syncytial Virus Derived from B Cells of Infected Patients: Journal Immunology 2009
12. Inhibition of Fatty Acid Synthase in vitro and in vivo Reduces RSV Replication: Poster presentation at ICAAC 2014
13. Preclinical development and safety assessment of the first inhaled NANOBODY® ALX-0171: SOT2013
14. Direct Inhibition of Cellular Fatty Acid Synthase Impairs Replication of Respiratory Syncytial Virus and Other Respiratory Viruses: PLoS One 2015

About the Author

Dr. Nagendra has held progressive leadership roles in pre-clinical and clinical oncology research and development. He worked at academic centers like-University of Kansas, Cedars-Sinai Medical Center, Mercer University Medical Center, Vanderbilt University Medical center, Anderson Cancer Institute. He led research teams on brain tumor and breast cancer biology. He had extensively published in peer-reviewed journals and secured US, EU and Japan patents. He directed the Human Tissue banking and biorepository and New Animal Facility. Later he worked in pharmaceutical companies such as Dr. Reddy's Labs, Scintilla BioMarc, PPD/ Thermo Fisher Scientific with focus on in vitro diagnostics, clinical pharmacology and toxicology, medicinal chemistry aspects of clinical drug development. He is presently a Global Senior Director of Scientific Affairs at Aragen Life sciences, USA.