Antiviral Chemistry and Chemotherapy
2020, Vol. 28: 1–12

DOI: 10.1177/2040206620947303
journals.sagepub.com/home/avc
SAGE

Respiratory syntycial virus: Current treatment strategies and vaccine approaches

Elena Margret Thornhill[2], Jessica Salpor[1] and David Verhoeven[2]

Abstract

Respiratory Syncytial Virus is a yearly respiratory virus that causes significant frequencies of morbidities, particularly in the young and elderly populations. However, preventive vaccines and/or treatment therapies are generally lacking, although much attention is now being placed on this virus. Moreover, there are now multiple strategies currently being explored in a race to the first licensed vaccine. While vaccines are being developed, multiple treatment strategies are being explored to attenuate the severity of infection and thus reduce hospitalization rates in vulnerable populations. This review outlines current strategies to prevent or treat this virus in the hopes of reducing significant human morbidity and mortality that occurs yearly with this seasonal virus.

Keywords

Respiratory syncytial virus, vaccine, respiratory infections

Date received: 6 April 2020; accepted: 13 July 2020

Introduction

Respiratory Syncytial Virus (RSV) is a serious respiratory infection that infects almost everyone by the age of two.[1] In fact, RSV accounts for more than 60% of acute respiratory infections in children worldwide.[2] In healthy adults, symptoms are generally not severe, often mimicking a bad cold or, in some cases, that of the flu. However, in vulnerable populations such as infants and the elderly, RSV causes almost 60,000 hospitalizations in children under five, and the hospitalization of 200,000 elderly people each year in the United States[3,4] alone. Children born before full gestation (premies) are particularly prone to severe RSV mediated disease. This virus is adept at evading and countering the immune system and often leads to bronchiolitis, an inflammation, and congestion of the airways,[5] or secondary bacterial infections like pneumonia or more often as acute otitis media.[6,7] The immune systems of the young and the elderly have limitations in preventing the onset of these complications leading to the higher disease severity in these populations.[8,9] Bacterial pneumonia, for which RSV is not often correctly recognized as a significant viral catalyst unlike flu, is attributable to RSV in 20.3% of children aged younger than one year and 10.1% of children aged 1 to 2 years.[7] Thus, development of effective therapeutics or a vaccine would be significant for human health. However, despite many years of trying, no licensed vaccine exists for this virus, and current therapies for reducing viral pathogenesis are limited.

RSV is an enveloped negative-sense single-stranded RNA virus of the family Pneumoviridae and the order Mononegavirales. RSV has ten genes coding for 11 proteins in the following order: Ns1, Ns2, N, P, M, SH, G, F, M2 (–1,–2), and L. To undergo successful transcription, RSV requires its M2-1 protein, a transcription elongation factor, in addition to the N, P, and

[1]Department of Biomedical Sciences, College of Veterinary Medicine, Iowa State University, Ames, USA
[2]Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, USA

Corresponding author:
David Verhoeven, College of Veterinary Medicine, Iowa State University, 1800 Christensen Dr, Ames IA 50011, USA.
Email: davidver@iastate.edu

L proteins. Other members of the Mononegavirales order, such as its closest relatives metapneumovirus and parainfluenza, only require the N, P, and L proteins for this process. While only Ns1 and Ns2 have the primary purpose of suppressing the immune system (through interference of the type 1 interferon response, TLR signaling, and adulteration of normal T helper cell ratios), many of the other genes also interfere with the antiviral response to varying degrees.[10–17] Moreover, the G protein can be produced either in a secreted or non-secreted form, with the former thought to act as a decoy to prevent targeting of the RSV virion by host antibodies.[18] The SH protein is involved with the modulation of TNF-α secretion from macrophages under certain conditions.[19,20] Many of the RSV proteins are of interest in the development of targeted therapies and vaccines. This article will discuss historical and contemporary treatment approaches for RSV as well as current immunogen designs to elicit durable host immunity from vaccination.

RSV modeling limitations and the impact on vaccine research and therapeutics

Although much is being discovered concerning RSV protein pathogenicity, in vitro and in vivo models pose another difficulty in RSV vaccine or therapy development. To test preclinical vaccine candidates, models must be developed and utilized to assess the potential for vaccine enhanced respiratory disease or vaccine-induced immunity.[21] In vitro, RSV replicates in a wide range of cell lines from various tissues and hosts. HEp-2 cell line is most commonly used to grow RSV; however, the purity of this cell line is unclear, so replication in this line may be less suitable for the production of virus used for in vivo applications. The cancerous nature and non-respiratory origin of several cell lines are limitations for translating RSV treatments to humans. To counter this, isolated human airway epithelial cells grown at an air-liquid interface have been used to adapt clinical RSV isolates to cell culture. These cells contain pseudostratified, mucociliary airway epithelium that is similar to the morphologic and phenotypic characteristics of in vivo human cartilaginous airway epithelium.[22] However, these are still cell culture models and limited in the complexity for treating RSV in a human.

In vivo, animal models present an additional challenge. Viruses like bovine RSV and ovine RSV have been identified in their respective species to model disease pathology and immune responses similar to human RSV infections in children.[22] Researchers have been able to utilize these models to understand the pathology and mechanisms of immunity towards pneumovirus infections. This understanding has helped serve as a way to evaluate human RSV vaccine concepts during pre-clinical development.[23] Although beneficial, these models are genetically different from humans in things such as antibody structures (i.e., long CDR3 regions in cow antibodies or fewer antibody-related genes in lambs and cows than humans). Neonatal lambs have been challenged with human RSV and have shown successful disease replication that mirrors human infection. The similarity of size and organization of airway and lymphoid tissue make this model attractive, but lack of cell typing antibodies and genetic sequencing tools, along with the complexity of population maintenance, are pitfalls of human RSV modeling in sheep.[24]

Other non-human primates and small animal mammalian models have also been explored. Chimpanzees are currently a non-human primate model that is permissive to human RSV replication. ARLI (acute lower respiratory tract infection) and SRLI (severe lower respiratory tract infections) have not been induced in this model. The genetic similarity and size of chimpanzees make then this an animal an attractive model, but the ethical burden and economic resources required for the logistical maintenance of small chimpanzee populations inhibits this model's use.[23,24] Other non-human primates like African green monkeys, three species of macaques (rhesus, cynomolgus, and bonnet monkeys), owl monkeys, Cebus monkeys, and baboons have been explored as models for human RSV with varying benefits and limitations. Unlike chimpanzees, these non-human primate species are semi-permissive to human RSV replication, so their viral replication responses were comparatively moderate to low to inoculum levels. Often, clinical signs of disease did not develop or were limited to mild symptoms. Pathology studies were not done on all species models, but those reported showed signs of broncho-interstitial pneumonia, alveolitis, and syncytium of cells. Vaccine-enhanced pathology has been studied in African green monkeys and macaque species, but limited vaccine-enhanced pathology has been explored in the other non-human primate species discussed.[23]

Small animal mammalian models mostly consist of rodent species, including mice, rats, and to lesser degrees ferrets, guinea pigs, Syrian hamsters, and chinchillas.[23] The BALB/c mouse has been the most common animal model for experimental human RSV disease.[24] This mouse model is semi-permissive and shows intermediate susceptibility to human RSV infection. A high, $>10^6$ plaque-forming units (PFU), dose of human RSV is required to produce clinical signs of disease. Unlike clinical signs in humans that focus on ARLI or SRLI, BALB/c mouse disease induced by human RSV is measured as weight loss, ruffled fur,

and hunched posture. Pathological sectioning of airways must be done to measure the pulmonary manifestation of human RSV with studies showing mild to moderate bronchiolitis has been induced.[23] The convenience provided by the vast amount of genetic knowledge and mouse-specific reagents and molecular tools makes this model attractive. However, the innate and adaptive immune response stimulated in BALB/c mice differs significantly from humans and does not provide sufficient antigenic modeling of human disease. The anatomy and size of mice lungs also differ significantly due to fewer bronchioles and less complex airway branching that complicates disease monitoring when compared to humans.[24]

An alternate rodent, the cotton rat, has proven to be a good model. Although semi-permissive, this rat model requires 10^4 PFU to induce ALRI, while peak replication levels are nearly 100 fold higher than in mouse models.[22] Both URI and LRI have been monitored in cotton rat models, and clearance of the virus follows a similar timeline as in humans with clearance happening by day seven post-infection. Pathologic sectioning of the airways has shown mild to proliferative bronchiolitis, sloughed epithelial cells, and patchy atelectasis.[23,24] The cotton rat model has become widely used to evaluate the efficacy of vaccines, antivirals, and neutralizing antibodies like palivizumab.[24] It has also been used to model alveolitis after FI human RSV vaccination, as a model for enhanced respiratory disease (ERD). Although these uses are productive, the extrapolation of vaccine results from this model to show safety in higher mammals should be done with discretion as complete protection stimulated against human RSV in the cotton rat model has failed to show the same efficacy in African green monkeys.[23] Cotton rat care also adds an added difficulty due to their fragile and easily agitated nature. Specialized training is required for their care and handling, but specific immunological reagents are being developed to advance the usage of this model.[24]

Thus, there are a number of limitations of animal models that creates difficulties in testing experimental vaccines. Generally, rodents are used first to rule out whether a vaccine fails to elicit antibodies. These vaccines then need to undergo additional testing in primate models to establish whether they have potential protective efficacy. Only then do vaccines proceed into human clinical trials since vaccine safety and not just efficacy are important before testing in humans. Thus, vaccine develop for RSV can be a very slow process. For therapies, testing candidate treatments that show efficacy in cell culture can also be difficult. Animal models that have divergent replication kinetics can make therapies that target the replication machinery difficult. Limited viral replication or alternative replication sites (i.e., lower versus upper airways) could also lead to misleading efficacy rates using animal models and trying to translate those findings to human clinical trials.

Current treatment standards for RSV

While our understanding of RSV pathogenesis and viral biology has increased over time, prevention of the virus is still lacking with some years, often with severe disease burdens. Treatment for RSV infection is currently limited to supportive care and prophylactic antibody use, with the latter only reserved for preemies. For non-severe infections, treatment often follows that of the common cold, bedrest and ensuring the individual stays hydrated with oral fluids.[2] If the infection is more severe, and the child's oxygen saturation drops below 90%, the patient is often given warm humidified oxygen or intubation with supplemental oxygen.[2] Infants who have a more severe RSV infection are often at risk of aspirating food particles, and can be placed on a feeding tube.[2] Hypertonic saline has also been used in hospitalized patients to improve mucociliary clearance.[2] Pharmacological agents like bronchodilators or corticosteroids have been used to relieve RSV symptoms, but efficacy has not been proven during randomized controlled trials.[25] In elderly populations with chronic lung disease, inhaled and systemic corticosteroids are prescribed to relieve acute exacerbation associated with wheezing and bronchospasm.[26] This practice has not been supported for infants during the first year of life because of safety concerns regarding corticosteroids' effects on rapid lung growth during this developmental time period.[2,27]

In at-risk groups like preemies or infants with compromised immune systems, a prophylactic neutralizing antibody treatment (Palizumab) may be administered.[28] Palizumab, a humanized IgG monoclonal antibody developed by MedImmune, targets the F protein in prefusion formation [29] and has been used since 1998. The antibody targets the RSV F protein on the envelopes of virions and prevents the virus from fusing with a host cell, thus preventing entry and infection.[29] The therapy is currently recommended for preemies born before 35 weeks of gestation or for infants (<2 years old) with chronic lung diseases or heart disease.[2,28] This monoclonal must be given every month intramuscularly or intravenously in preemies[27] and ideally started before the beginning of the RSV season [29] although premies are often treated in the hospital after evidence of infection by the virus. The use of Palizumab is limited to at-risk groups, is expensive ($3,000 per vial), can still allow for breakthrough cases of severe RSV infections,[2,29–31] and is not efficacious for RSV prevention after two years of age.

Attempts to design improved second generation monoclonal therapies have been slow. Medimmune's next-generation candidate Motazumab reduces hospitalization rates due to RSV by 87%, does not clear virus in treated infants, and can exhibit severe side effects.[32] Thus, development of third-generation monoclonal therapies is ongoing to increase the half-life of these antibodies through protein engineering.

Ribavirin, a synthetic nucleoside analog, has broad *in vitro* activity against many RNA and DNA viruses. Approved for severe RSV infection therapy or Hepatitis C infections, ribavirin only provides a modest short-term improvement for RSV. Once supported for routine use in 1993 by the American Academy of Pediatrics Committee on Infectious Diseases, the committee changed its recommendation for the use of Ribavirin to treat RSV in 1996 to 'may be considered'.[33] In immunocompetent patients, RSV infection is asymptomatic for the first 3–5 days post-infection. During those first days, the virus reproduces exponentially and reaches the lungs, causing respiratory distress symptoms after 5–7 days.[34] Administering Ribavirin at this point, post-infection does not have a large effect on the already disappearing RSV viral load, and multiple randomized trials were not able to demonstrate any short- or long-term benefits.[2] Ribavirin's use has further significant drawbacks such as limiting host defenses, prolonged hospitalization due to aerosol administration, risks for potential toxicity, and high cost.[33] Thus, there is certainly a need for additional therapeutics and especially efficacious vaccines to cure or prevent this virus. We outline some of those approaches next.

Vaccines in development

In the 1960s, a formalin-inactivated RSV vaccine was produced and tested in humans but enhanced immunopathology and was withdrawn from further testing. Children given the vaccine had more severe symptomology upon infection, including two deaths,[35] likely from a conformational change in the F vaccine immunogen, creating low-avidity and non-protective antibodies while polarizing the immune response toward Th2 during infection. The non-protective antibodies formed pathogenic immune complexes in the lung, leading to complement activation and lung damage when infected by the virus. This, combined with the skewed Th2 response, triggered an excess of eosinophils, and neutrophils further exacerbated lung damage.[32] Thus, this vaccine set RSV vaccine development back, and all current candidate vaccines are rigorously tested for signs of similar immune profiles.

Correlates of protection

Due to the heterogeneity of the RSV protein landscape and models utilized for vaccine development, the correlates of protection (CoP) against RSV infection and disease have been difficult to determine. Due to the nature of humoral immunity, there may be many inhibitory mechanisms responsible for antigenic neutralization of RSV.[36]RSV-specific nasal IgA, a component of mucosal antigenic memory, may be useful for establishing CoP for infection. One study has shown that IgA more strongly correlates with protection compared to measurements of serum neutralizing antibody in adults.[37] This highlights the importance mucosal immunity may play in RSV protection. The same study also showed that rapidly waning IgA levels caused individuals to be susceptible to RSV reinfection within months. Upon reinfection, IgA producing memory B cells were not significantly mobilized and suggested a vaccine handicap that may need to be overcome through dosage and administration strategies or stimulation of enhanced immunologic memory. Other definitive CoP may be vaccine-type specific. Times-rise in antibody titer could be an indicator of B-cell priming, relevant for live-attenuated vaccines. This vaccine type is targeted towards the naïve pediatric immune system because it generates replication of high amounts of antigenic non-virulent material that stimulates a natural host immune system response.

Standardization of neutralizing assays is a considerable feat, and a recent PATH, WHO, and the National Institute for Biological Standards and Control (NIBSC) exercise examined 12 different neutralizing assays in order to establish standardized neutralizing antibody titers.[36] This regulatory effort led to a new RSV International Standard Antiserum with 1000 IU of RSV subtype A neutralizing activity per vial available through NIBSC.[21] Further standardization of other immunological assays will need to be developed in the future.

Besides humoral immunity, T cell-mediated immune responses could act as a CoP. In cases of LTRI, CD8 T cells are essential for viral clearance.[21,38] Kulkarni et al. [36] suggest that "neutralizing antibodies will likely serve as a CoP in infants and young children, but in older adults, a CoP associated with CTL induced virus clearance might be a better target." Despite this recommendation, measurements of Th1 and Th2 responses have been used as safety measures for most RSV vaccine platforms due to their relationship with ERD in children. High levels of Th2 are indicative that ERD may be induced, and high levels of Th1 are indicative of an appropriate immune response in vulnerable populations. These indications only further support

that establishing CoP will vary depending on vaccine study, target age, and host immune factors.[21,36]

Vaccine strategies

There are currently multiple types of RSV vaccines being pursued, specifically targeting populations of pregnant women, infants, and elderly. Maternal vaccines have typically been targeted at women in their third trimester so that the maternally generated antibodies will be passed on to their offspring and likely protect them from RSV infection after breastfeeding. Many of these vaccines can be seen in PATH,[39] which shows their current status in clinical testing. We outline a select few of these with others shown in Table 1. We illustrate vaccine and therapy targets in relation to the viral life cycle in Figure 1. A vast majority of these vaccines are directed at the F protein, which the virus requires for entry and is fairly well conserved between strains and one that natural infection evokes neutralizing antibodies toward. However, the F protein spontaneously cleaves from a pre-fusion form to a post-fusion form either as a recombinant protein or even on the virus itself. While neutralizing antibodies to both forms can inhibit viral entry, the pre-fusion targeted antibodies are thought to be more protective in vivo. However, no vaccines have yet shown protection, though some have shown a reduction in symptomology. In bovine trials using a pre-fusion F vaccine, complete protection is achieved, although it should be noted that cow's antibodies are quite different from the majority of human antibodies, characterized by their long CDR3 regions that might better reach into active receptor sites that tend to be heavily glycosylated.

Live vaccines. There are a number of live vaccines under development with the advantage that lung mucosal immunization is possible with most of them. Often deleting Ns2 or G protein or using another virus that expresses RSV F protein are the most common live

Table 1. Overview of other RSV vaccine approaches.

Vaccine	Type	Phase	Feature
Codagenix,LID/NAID/NIH (RSV)	Live Attenuated	PreClinical	Uses SAVE (Synthetic Attenuated Virus Engineering) to generate an artificially created RSV strain
LID/NAID/NIH (PIV-3/RSV)	Live Attenuated	PreClinical	Chimeric PIV virus expressing the RSV F protein
Meissa Vaccines (RSV)	Live Attenuated	Phase I Trial	Has Fast Track Designation, increased thermostability, modified G region, and deleted SH region
Artificial Cell Technologies (Peptide microparticle)	Particle Based	Discontinued	Synthetic nanoparticle carrying RSV-G peptide coupled with RSV T-cell target antigens
Fraunhofer (VLP)	Particle Based	PreClinical	Low-energy electron irradiation (LEEI) used to inactivate virus, antigen conservation above 70%
Icosavax (VLP)	Particle Based	PreClinical	Expresses stabilized prefusion F protein
Georgia State University (VLP)	Particle Based	PreClinical	Inactivated detergent-Split RSV exposes epitopes
Sanofi (RSV F Nanoparticle)	Particle Based	PreClinical	Expresses the F protein
Virometix (VLP)	Particle Based	PreClinical	A Phase I trial with V-306 is beginning
University of Massachusetts (VLP)	Particle Based	PreClinical	Viral Like Particle containing the matric of New Castle Virus that expresses RSV G and F ectodomains
Instituto de Salud Carlos III (RSV F)	Subunit	PreClinical	Chimeric RSV F protein containing epitopes from hRSV and hMPV. Proof of concept for chimeric F creation.
University of Georgia (RSV G)	Subunit	PreClinical	Nanocapsule containing G peptides
University of Saskatchewan (RSV F)	Subunit	PreClinical	Codon-optimized F formulated with poly (I:C) and polyphosphazene
BravoVax (Adenovirus)	Recombinant Vectors	PreClinical	Adenovirus expressing the RSV B strain full length and truncated F protein
Vaxart (Adenovirus)	Recombinant Vectors	PreClinical	Expresses the RSV F protein on an adenovirus vector

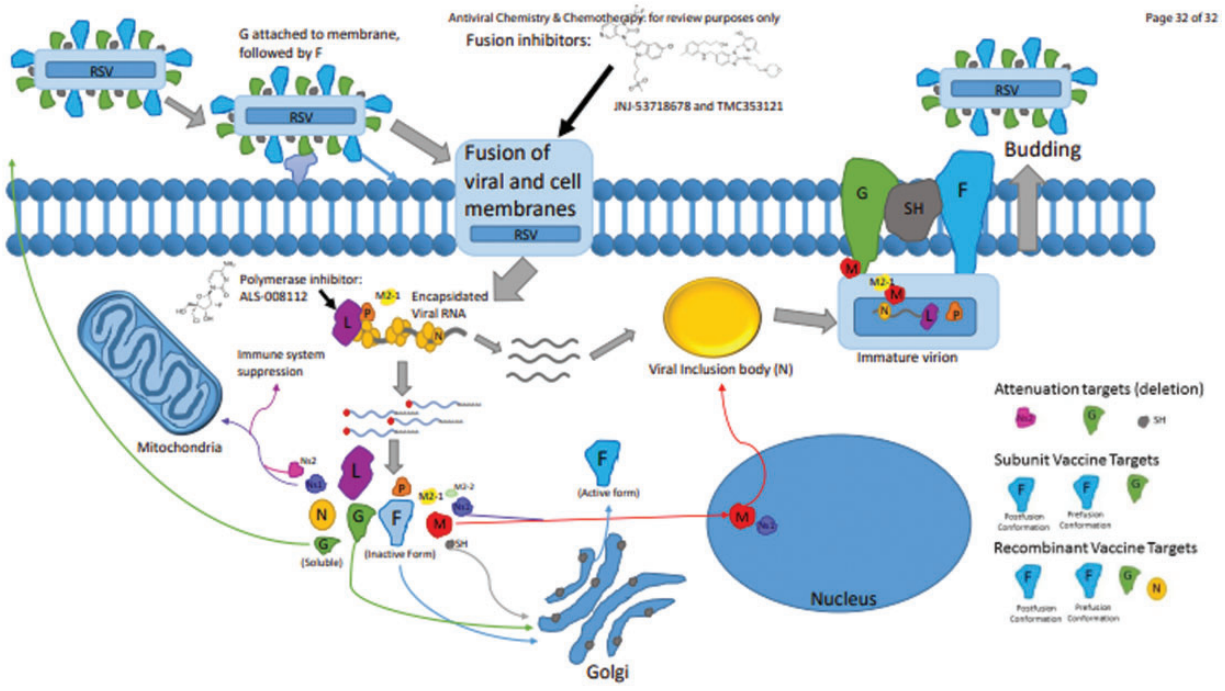

Figure 1. Current vaccine and therapy targets and the location of their inhibition in the replication cycle of RSV are shown in context of the RSV lifecycle. The vast majority of vaccines and therapies target F and its function as an entry protein.

vaccines under development, although cold adaptive RSV is also being explored. RSV replication is attenuated when either of the Ns genes are deleted (Ns2 deletion is less attenuating than Ns1 deletion), and the same is true for deletion of G[14,40] but only *in vivo*. With the G protein, there are recorded cases of clinical isolates obtained from infected individuals lacking the G protein and still being virulent,[41] and thus the efficacy of these vaccines needs to be explored. Intravac is currently testing a G deletion vaccine in phase 1 clinical trials.[42,43] Sanofi is currently testing three NS deletion mutant vaccines in phase 1 and 2 clinical trials. Their ΔNs2/Δ1313/I1314L vaccine was made through reverse genetics with deletions to the Ns2 gene, codon 1313 in the L polymerase, and a substitution of leucine for isoleucine at codon 1314. The 1313 deletion is intended to assist in attenuation and to confer temperature sensitivity to the polymerase, which is stabilized by the 1314 substitution.[44] Their 6120/ΔNs2/1030s vaccine also has a Ns2 deletion and a mutation to the L polymerase like the ΔNs2/Δ1313/I1314L vaccine though the L mutation is at a different nucleotide location it also is intended to confer some temperature sensitivity to the polymerase. This vaccine also contains a deletion in the membrane pore protein SH.[45] St. Jude's RSV vaccine candidate is also in phase 1 trials and is based on expressing the RSV F protein in murine Sendai virus.[39,46] Finally, the Pontifica Universidad Catolica de Chile is testing a mycobacterium bovis Calmette-Guerin (BCG) vaccine that expresses RSV N protein, which in mice decreased viral loads in the lungs and protected from RSV-induced innate immune cell damage.

Another recombinant RSV vaccine candidate from Janssen Pharmaceutical uses adenovirus and is currently being tested during phase two clinical trials (NCT03339713) for efficacy in pediatric and elderly populations. This vaccine uses the gene for the Pre-F conformation as antigenic material. Results from a phase 2 trial in the elderly showed lower RSV infection and reduced disease severity over time.[47,48] A follow-up study is ongoing.[49] The phase two clinical trial (NCT03303625) for the pediatric targeted version of the Janssen Pharmaceutical Adenovirus vaccine candidate is currently vaccinating 60 participants in two age ranges: healthy adults 18–50 years old and RSV-seropositive toddlers 12–24 months old (Janssen Vaccines). GlaxoSmithKline also has an adenovirus-based RSV vaccine, but it differs from Janssen in that it expresses RSV's N and M2-1 proteins in addition to a modified form of F.[50,51]

The other recombinant RSV vaccine candidate, from Bavarian Nordic MVA, uses modified vaccinia Ankara (MVA), a live-attenuated poxvirus derivative as its recombinant vector. This RSV recombinant vaccine candidate utilizes genes for F, G (both RSV subtypes), N, and M2 proteins as its antigenic material.[21] Phase two interim (NCT02873286) results show the

vaccine candidate is well tolerated and induces humoral and T cell responses in older adults after a single vaccination. Planning has begun for the design of a phase three study (Bavarian Nordic). However, the use of even attenuated vaccinia in infants or the elderly may pose an issue as well as if the need for booster vaccines is needed to maintain immunity year after year.

Particle-based vaccines. In contrast, to live attenuated vaccines, a number of groups are developing inactivated, particle, or subunit vaccines. While unlikely to induce similar levels of mucosal immunity as live vaccines, these immunogens would likely have better safety characteristics in infants and the elderly. Moreover, the same issues that have prevented Flumist, an attenuated live flu vaccine, to be approved in children less than two years of age or adults over 55 could similarly restrict live vaccines from the populations. Thus, particle-based vaccines may be preferred. An inactivated RSV vaccine by Blue Willow Biologics uses a nanoemulsion to inject a whole virion retaining RSV's native F antigen structure and has been shown to induce strong Th1 and Th17 associated immunity in mice and cotton rats.[52]

While there are several particle-based vaccines in preclinical trials, Novavax has a RSV F nanoparticle vaccine that entered phase 3 trials several times. This aluminum adjuvanted nanoparticle expresses a modified version of the RSV F protein that exposes the antigenic sites, one being antigenic site two, which Palizumab targets.[53–55] The phase 3 trial is for a maternal immunization vaccine and was tested on 4636 pregnant women who were in their third trimester had no complications and were 18–40 years of age. The women were in either a treatment or placebo group and were vaccinated three months prior to the RSV season. The vaccine was given via intramuscular injection. The primary objective was "Incidence of medically significant RSV LRTI with either hypoxemia ($SpO2 < 95\%$ at sea level or $<92\%$ at altitudes >1800 meters) or tachypnea in infants through 90 days of life with several secondary objectives.[53] Unfortunately, the phase 3 trials failed to meet their primary objective of preventing medically significant RSV LRTI through the first 90 days of life. The vaccine did, however, show a reduction in the amount of "all-cause" LRTI hospitalizations (25%) and hypoxemia (39%).[56] The vaccine appears safe in infants and mothers, and Novavax intends to move forward with additional attempts to license the vaccine [56,57] in the near future.

Subunit vaccines. Another broad vaccine platform being explored is the subunit vaccine type. This vaccine type is targeted towards elderly and maternal populations. Due to previous episodes of ERD elicited in animal models following subunit vaccination, this vaccine platform may be unsuitable for infant populations.[58,59] Subunit vaccines utilize specific purified viral proteins often paired with an adjuvant to elicit immunity. There are currently several vaccine candidates in phase one of clinical trials and one candidate in phase two. Four candidates utilize the F protein as their antigenic material. However, many of these candidate vaccines have been withdrawn due to failure to meet endpoints of protection . One recent late phase vaccine failure in 2016, MEDI-7510 (NCT02508194), a subunit vaccine candidate utilizing a Post-F conformation as its antigenic material, was thought to have failed because the antibodies generated in response lacked appropriate epitope specificity when induced by Post-F. Another company is using the G protein as the antigen in a vaccine though it is still in the early stages.[60,61]

Nucleic acid vaccines. There have been a number of DNA vaccines tested for RSV prevention,[62–67] but they generally have not demonstrated enough efficacy in animal models to advance toward human clinical trials or are continuing to be tested in preclinical models. These vaccines may have suffered from limited antibody development toward F surface proteins and instead favored CD8 T cell memory development. However, most of the CD8 T cells are non-resident memory and would be slow to prevent RSV infection in the lungs. One vaccine that appears to generate good protective immunity in non-human primate models is mRNA vaccine by Merck and Moderna, which expresses the RSV F protein.[68] mRNA vaccines may have more favorable CoP than DNA vaccines, but the long-term protective profile is unknown at this point.

Antiviral therapies in development

Many RSV therapies currently being investigated are small molecule therapeutics that have been shown to inhibit RSV replication, as highlighted further in the text below or Table 2. TMC353121 is an RSV fusion inhibitor that reduces viral replication and shedding in mice and African Green monkeys when administered within 48 hrs.[69–71] TMC353121 doesn't destroy the virus; it only inhibits its entry and reduces inflammatory cytokine levels caused by RSV. This effect is dose-dependent and requires a lower dosage that is administered for palizumab treatment to be effective.[70,71]

JNJ-53718678 is like TMC353121 in that they are both RSV F fusion inhibitors, but they differ in that JNJ-53718678 binds to a pocket in the prefusion form of F and prevents it from cleaving into its active post fusion conformation. JNJ-53718678 has specific activity towards RSV F and inhibits established RSV infection in rodent and neonatal lamb

Table 2. Overview of other therapies in development.

Therapy	Target	Purpose
MitoQ	mtROS (mitochondria reactive oxygen species)	Reduce disease severity from RSV induced mtROS generation
siRNA	Any RSV gene/mRNA	Silence target gene/mRNA to inhibit virus replication
VEGF (Vascular Endothelial Growth Factor)	Unknown	Reduce disease severity, possibly through recruitment of macrophages or modifications on epithelial cells
KI (Potassium Iodide)	Generation of hypoiodous acid	Reduction of disease severity and viral replication through activation of the Duox/LPO system
Recombinant human CC10	Unknown	Reduction of RSV M37 pneumonia
ALX-0171	F protein	Inhibits RSV cell entry by binding to F

models.[72]JNJ-53718678 is being tested in phase 2 clinical trials. In a challenge study of participants aged 18–45 who were mostly men, JNJ-53718678 reduced peak viral load, severity of clinical symptoms, and duration of viral shedding. No concerning adverse effects were reported. The authors of the study acknowledge its small sample size and plan to follow up with a larger study.[73] Currently, a phase 2 clinical trial is recruiting participants aged 28 days to 3 years who have been hospitalized or are receiving outpatient care due to a RSV infection. The goals of this trial are to test the safety and efficacy of JNJ-53718678 to reduce viral load, and disease severity in RSV infected individuals.[74]

ALS-008176 is the bioavailable product of ALS-008112, which is a cystidine nucleoside analog. ALS-008176 enters the respiratory tract and has a half-life of 29 hrs. It has been tested as a RSV inhibitory molecule and inhibits RSV replication intracellularly. A phase 2 trial was conducted in 2014 by Alios Biopharma Inc to determine efficacy and pharmodynamics in an RSV challenge model. The trial was conducted in adult participants 18–50 years of age. ALS-008176 was shown to be safe and to reduce viral loads in this challenge study. There is also a phase 1 trial of infants who were hospitalized due to an RSV infection, though results for this study do not appear to have been published at this time.[75–79]

Another therapy approach highlighted on the PATH Snapshot is the immune-prophylaxis/combination platform focused on developing mAb for passive immunity targeting the F protein. Pharmaceutical companies MedImmune and Sanofi have joined during the development of this prophylaxis and indicated that pricing would emulate vaccine markets.[21]

Conclusion

RSV is a dangerous respiratory pathogen that causes thousands of hospitalizations and deaths each year. Science has tried to combat RSV infection since the '60s, and yet little progress has been made. Care is mostly supportive, and the prophylactic antibody Palizumab is restricted in use and is expensive. Vaccine development for RSV has been hampered by the failed formalin-inactivated vaccine, which led to increased RSV pathology and killed two toddlers. While there are many vaccine candidates in the works, few have made it to phase 3 testing, and none have been market approved. The many approaches to vaccine development from live attenuated to new nucleic acid vaccines mean that there are many lessons to be learned each day that may lead to a functional RSV vaccine. Many vaccine candidates are focused on the RSV F protein, and yet none have succeeded. New therapy development for RSV treatment is limited, and few pass the hurdle of positive results in human trials. More research is needed into the regulatory mechanisms and pathogenesis of RSV so that new targets can be identified.

RSV vaccine development has expanded in response to the significant global disease burden. Although the FI vaccine failure of 1966 hindered vaccine development, progress has been made towards achieving a viable human RSV vaccine. As the understanding of the structure and pathogenicity of RSV proteins is enhanced, vaccine developers can narrow their target genetic material to more adequately stimulate a protective immune response balancing humoral and cellular immunity. Once these targets are further developed, the RSV vaccine field will need to continue to grow the understanding of in vitro and in vivo models to adequately test developing RSV vaccines. CoP can then be established to act as a standard for future RSV vaccine development. Current vaccine progress and failures that result during clinical trials help broaden the scientific understanding of immunity against RSV, and many candidates currently in development have shown promising results.

Although there is a significant amount of information that evades vaccine/therapy developers today, emerging knowledge and innovation will improve

current points of development and allow researchers to maximize the effectiveness of their drug/vaccine candidates. Moving forward, it appears based on the understanding of protein structure and pathogenicity that the RSV vaccine development field is moving towards targeting the F surface protein as opposed to the G surface protein. While G protein may elicit a strong antibody response, targeting F is supported by superior F protein conservation between RSV strains. Specificity for the F protein has also emerged with the Pre-F conformation being targeted as opposed to the widely used Post-F conformation. This shift should elicit new information and has been supported through the success of recent Pre-F conformation targeted candidates in the vaccine PATH Snapshot. However, some pre-clinical vaccines are still targeting the G protein in addition to F. Further defining CoP in the future will help standardize the immune responses necessary for generating complete immunity, especially in infants and the elderly whose immune systems are limited compared to adults. Development of a viable vaccine is crucial to combatting worldwide RSV infection, but better therapeutics would certainly help as we continue to wait for an efficacious vaccine to be developed. A more thorough understanding of RSV pathogenesis, regulation, and lifecycle will lead to better model generation and foster the identification of additional viral targets for next-generation therapies or vaccines. The history of RSV vaccine development is full of disappointments, but development continues onward, looking to the future. With new techniques and technologies arising every day, the lessons of the past contribute to a future where a viable RSV vaccine may appear on the horizon.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iDs

Elena Margret Thornhill https://orcid.org/0000-0002-4159-2642
Jessica Salpor https://orcid.org/0000-0001-5473-5473
David Verhoeven https://orcid.org/0000-0002-0766-4606

References

1. Glezen WP, Taber LH, Frank AL, et al. Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child* 1986; 140: 543–546.
2. Piedimonte G and Perez MK. Respiratory syncytial virus infection and bronchiolitis. *Pediatr Rev* 2014; 35: 519–530.
3. Falsey AR and Walsh EE. Respiratory syncytial virus infection in elderly adults. *Drugs Aging* 2005; 22: 577–587.
4. Rose EB, Wheatley A, Langley G, et al. Respiratory syncytial virus seasonality – United States, 2014-2017. *MMWR Morb Mortal Wkly Rep* 2018; 67: 71–76.
5. Meissner HC. Viral bronchiolitis in children. *N Engl J Med* 2016; 374: 62–72.
6. Heikkinen T, Thint M and Chonmaitree T. Prevalence of various respiratory viruses in the middle ear during acute otitis media. *N Engl J Med* 1999; 340: 260–264.
7. Weinberger DM, Klugman KP, Steiner CA, et al. Association between respiratory syncytial virus activity and pneumococcal disease in infants: a time series analysis of US hospitalization data. *PLoS Med* 2015; 12: e1001776.
8. Verhoeven D. Influence of immunological maturity on respiratory syncytial virus-induced morbidity in young children. *Viral Immunol* 2019; 32: 76–83.
9. Fulton RB and Varga SM. Effects of aging on the adaptive immune response to respiratory virus infections. *Aging Health* 2009; 5: 775.
10. Lo MS, Brazas RM and Holtzman MJ. Respiratory syncytial virus nonstructural proteins NS1 and NS2 mediate inhibition of Stat2 expression and alpha/beta interferon responsiveness. *J Virol* 2005; 79: 9315–9319.
11. Elliott J, Lynch OT, Suessmuth Y, et al. Respiratory syncytial virus NS1 protein degrades STAT2 by using the Elongin-Cullin E3 ligase. *J Virol* 2007; 81: 3428–3436.
12. Ling Z, Tran KC and Teng MN. Human respiratory syncytial virus nonstructural protein NS2 antagonizes the activation of beta interferon transcription by interacting with RIG-I. *J Virol* 2009; 83: 3734–3742.
13. Teng MN and Collins PL. Altered growth characteristics of recombinant respiratory syncytial viruses which do not produce NS2 protein. *J Virol* 1999; 73: 466–473.
14. Jin H, Zhou H, Cheng X, et al. Recombinant respiratory syncytial viruses with deletions in the NS1, NS2, SH, and M2-2 genes are attenuated in vitro and in vivo. *Virology* 2000; 273: 210–218.
15. Bossert B and Conzelmann KK. Respiratory syncytial virus (RSV) nonstructural (NS) proteins as host range determinants: a chimeric bovine RSV with NS genes from human RSV is attenuated in interferon-competent bovine cells. *J Virol* 2002; 76: 4287–4293.
16. Sun Y and Lopez CB. The innate immune response to RSV: Advances in our understanding of critical viral and host factors. *Vaccine* 2017; 35: 481–488.
17. Verhoeven D, Xu Q and Pichichero ME. Differential impact of respiratory syncytial virus and parainfluenza virus on the frequency of acute otitis media is explained by lower adaptive and innate immune responses in otitis-prone children. *Clin Infect Dis* 2014; 59: 376–383.
18. Bukreyev A, Yang L, Fricke J, et al. The secreted form of respiratory syncytial virus G glycoprotein helps the virus evade antibody-mediated restriction of replication by

acting as an antigen decoy and through effects on Fc receptor-bearing leukocytes. *J Virol* 2008; 82: 12191–12204.
19. Fuentes S, Tran KC, Luthra P, et al. Function of the respiratory syncytial virus small hydrophobic protein. *J Virol* 2007; 81: 8361–8366.
20. Murawski MR, Bowen GN, Cerny AM, et al. Respiratory syncytial virus activates innate immunity through toll-like receptor 2. *JVI* 2009; 83: 1492–1500.
21. Mazur NI, Higgins D, Nunes MC, Respiratory Syncytial Virus Network (ReSViNET) Foundation, et al. The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates. *Lancet Infect Dis* 2018; 18: e295–e311.
22. Heylen E, Neyts J and Jochmans D. Drug candidates and model systems in respiratory syncytial virus antiviral drug discovery. *Biochem Pharmacol* 2017; 127: 1–12.
23. Taylor G. Animal models of respiratory syncytial virus infection. *Vaccine* 2017; 35: 469–480.
24. Bem RA, Domachowske JB and Rosenberg HF. Animal models of human respiratory syncytial virus disease. *Am J Physiol Lung Cell Mol Physiol* 2011; 301: L148–56.
25. Higgins D, Trujillo C and Keech C. Advances in RSV vaccine research and development – a global agenda. *Vaccine* 2016; 34: 2870–2875.
26. Branche AR and Falsey AR. Respiratory syncytial virus infection in older adults: an under-recognized problem. *Drugs Aging* 2015; 32: 261–269.
27. Turner TL, Kopp BT, Paul G, et al. Respiratory syncytial virus: current and emerging treatment options. *Clinicoecon Outcomes Res* 2014; 6: 217–225.
28. American Academy of Pediatrics Committee on Infectious D and American Academy of Pediatrics Bronchiolitis Guidelines C. Updated guidance for palivizumab prophylaxis among infants and young children at increased risk of hospitalization for respiratory syncytial virus infection. *Pediatrics* 2014; 134: 415–420.
29. MedImmune. "SYNAGIS® (Palivizumab)." *SYNAGIS (Palivizumab) for Prevention of Severe RSV Disease*, www.synagis.com/what-is-synagis.html#HowSynagisgiven (2017, accessed 2 February 2020).
30. Wegzyn C, Toh LK, Notario G, et al. Safety and effectiveness of palivizumab in children at high risk of serious disease due to respiratory syncytial virus infection: a systematic review. *Infect Dis Ther* 2014; 3: 133–158.
31. Banerji A, Panzov V, Young M, et al. The real-life effectiveness of palivizumab for reducing hospital admissions for respiratory syncytial virus in infants residing in Nunavut. *Can Respir J* 2014; 21: 185–189.
32. Jares Baglivo S and Polack FP. The long road to protect infants against severe RSV lower respiratory tract illness. *F1000Res* 2019; 8: 610.
33. Rezaee F, Linfield DT, Harford TJ, et al. Ongoing developments in RSV prophylaxis: a clinician's analysis. *Curr Opin Virol* 2017; 24: 70–78.
34. Mohapatra SS and Lockey RF. Respiratory syncytial virus infection: from biology to therapy: a perspective. *World Allergy Organ J* 2008; 1: 21–28.
35. Dudas RA and Karron RA. Respiratory syncytial virus vaccines. *Clin Microbiol Rev* 1998; 11: 430–439.
36. Kulkarni PS, Hurwitz JL, Simoes EAF, et al. Establishing correlates of protection for vaccine development: considerations for the respiratory syncytial virus vaccine field. *Viral Immunol* 2018; 31: 195–203.
37. Habibi MS, Jozwik A, Makris S, Mechanisms of Severe Acute Influenza Consortium Investigators, et al. Impaired antibody-mediated protection and defective IgA B-Cell memory in experimental infection of adults with respiratory syncytial virus. *Am J Respir Crit Care Med* 2015; 191: 1040–1049.
38. Graham BS. Vaccine development for respiratory syncytial virus. *Curr Opin Virol* 2017; 23: 107–112.
39. PATH. RSV Vaccine and mAb Snapshot. In: RSV-snapshot-2019_08_28_High%20Resolution_PDF.pdf, (ed.). VACCINE RESOURCE LIBRARY: PATH, 2019.
40. Techaarpornkul S, Barretto N and Peeples ME. Functional analysis of recombinant respiratory syncytial virus deletion mutants lacking the small hydrophobic and/or attachment glycoprotein gene. *J Virol* 2001; 75: 6825–6834.
41. Venter M, van Niekerk S, Rakgantso A, et al. Identification of deletion mutant respiratory syncytial virus strains lacking most of the G protein in immunocompromised children with pneumonia in South Africa. *J Virol* 2011; 85: 8453–8457.
42. Thomassen YE, Van der Welle J, Van der Kooi S, et al. A live attenuated RSV vaccine, process development studies" in "Vaccine Technology VI. ECI Digital Archives: Vaccine Technology VI", Laura Palomares, UNAM, Mexico Manon Cox, Protein Sciences Corporation, USA Tarit Mukhopadhyay, University College London, UK Nathalie Garçon, BIOASTER Technology Research Institute, FR Eds, ECI Symposium Series, 2016.
43. Visser DI. ITV1501. 2018-04-24 ed. Netherlands Trial Register: NL58147.000.17; LG 15-004; ITV1501; CHDR1542, 2018.
44. Karron RA, Luongo C, Mateo JS, et al. Safety and immunogenicity of the respiratory syncytial virus vaccine RSV/DeltaNS2/Delta1313/I1314L in RSV-seronegative children. *J Infect Dis* 2019;■■.
45. Luongo C, Winter CC, Collins PL and Buchholz UJ. Respiratory syncytial virus modified by deletions of the NS2 gene and amino acid S1313 of the L polymerase protein is a temperature-sensitive, live-attenuated vaccine candidate that is phenotypically stable at physiological temperature. *J Virol* 2013; 87: 1985–1996.
46. Stockwell M and Sheffield M. St. Jude Children's Research Hospital signs agreement to commercialize RSV vaccine, https://www.stjude.org/media-resources/news-releases/2017-medicine-science-news/st-jude-signs-agreement-to-commercialize-rsv-vaccine.html (2017, accessed 2 February 2020).
47. DeVincenzo J, Gymnopoulou E, De Paepe E, ED, et al. 902. A randomized, double-blind, placebo-controlled study to evaluate the efficacy of a single immunization of Ad26.RSV.preF against RSV infection in a viral challenge model in healthy adults. *Open Forum Infectious Diseases* 2019; 6: S27–S28.

48. Trial JVPBVC. An exploratory study to evaluate the prophylactic efficacy of a single immunization of Ad26.RSV.preF against respiratory syncytial virus infection in a virus challenge model in healthy 18 to 50 year-old adults, https://clinicaltrials.gov. : U.S National Library of Medicine 2017. 2019.
49. Trial JVPBVC. A study to evaluate the safety and immunogenicity for regimen selection of Ad26.RSV.preF and/or RSV preF protein combinations followed by expanded safety evaluation in adults aged 60 years and older. *A Randomized, Double-blind, Placebo-controlled Phase 1/2a Study for Safety and Immunogenicity Evaluations for Regimen Selection of Ad26RSVpreF and/or RSV preF Protein Combinations Followed by Expanded Safety Evaluation in Adults Aged 60 Years and* Older, https://clinicaltrials.gov. : U.S National Library of Medicine., 2018–2019.
50. Cicconi P, Jones C, Sarkar E, et al. First-in-human randomized study to assess the safety and immunogenicity of an investigational respiratory syncytial virus (RSV) vaccine based on ChAd155 viral vector expressing RSV viral proteins F, N and M2-1 in healthy adults. *Clin Infect Dis* 2020; 70: 2073–2081. DOI: 10.1093/cid/ciz653.
51. Trials GC. RSV Investigational Vaccine in RSV-seropositive Infants Aged 12 to 23 Months. *A study to evaluate safety, reactogenicity and immunogenicity of GSK Biologicals' RSV investigational vaccine based on viral proteins encoded by Chimpanzee-derived adenovector (ChAd155-RSV) (GSK3389245A) in RSV-seropositive Infants,* https://clinicaltrials.gov. : U.S National Libray of Medicine, 2016–2020.
52. O'Konek JJ, Makidon PE, Landers JJ, et al. Intranasal nanoemulsion-based inactivated respiratory syncytial virus vaccines protect against viral challenge in cotton rats. *Hum Vaccin Immunother* 2015; 11: 2904–2912.
53. Chau LA, Bluestone JA and Madrenas J. Dissociation of intracellular signaling pathways in response to partial agonist ligands of the T cell receptor. *J Exp Med* 1998; 187: 1699–1709.
54. Thomas DN. A study to determine the safety and efficacy of the RSV F vaccine to protect infants via maternal immunization. *A phase 3, randomized, observer-blind, placebo-controlled study to determine the immunogenicity and safety of a respiratory syncytial virus (RSV) F nanoparticle vaccine with aluminum in healthy third-trimester pregnant women; and safety and efficacy of maternally transferred antibodies in preventing RSV disease in their infants,* https://ClinicalTrials.gov. : U.S National Library of Medicine, 2019.
55. Novavax. Clinical Stage Pipeline – Novavax – Creating Tomorrow's Vaccines Today. Novavax.com, https://novavax.com/page/11/clinical-stage-pipeline.html. ■■: Novavax, 2019.
56. Brown S and Cohen A. Novavax announces topline results from phase 3 PrepareTM Trial of ResVax^{TM} for prevention of RSV disease in infants via maternal immunization, https://ir.novavax.com/news-releases/news-release-details/novavax-announces-topline-results-phase-3-preparetm-trial. -: Novavax, 2019.
57. Keown A. Novavax RSV vaccine fails to hit primary endpoint in phase III Trial, https://www.biospace.com/article/novavax-rsv-vaccine-fails-to-hit-primary-endpoint-in-phase-iii-trial/. : BioSpace, 2019.
58. Murphy BR, Sotnikov AV, Lawrence LA, et al. Enhanced pulmonary histopathology is observed in cotton rats immunized with formalin-inactivated respiratory syncytial virus (RSV) or purified F glycoprotein and challenged with RSV 3-6 months after immunization. *Vaccine* 1990; 8: 497–502.
59. Connors M, Collins PL, Firestone CY, et al. Cotton rats previously immunized with a chimeric RSV FG glycoprotein develop enhanced pulmonary pathology when infected with RSV, a phenomenon not encountered following immunization with vaccinia–RSV recombinants or RSV. *Vaccine* 1992; 10: 475–484.
60. Zhang S, Zhao G, Su C, et al. Neonatal priming and infancy boosting with a novel respiratory syncytial virus vaccine induces protective immune responses without concomitant respiratory disease upon RSV challenge. *Hum Vaccin Immunother* 2019; 16: 1–9. DOI: 10.1080/21645515.2019.1671134.
61. Li C, Zhou X, Zhong Y, et al. A recombinant G protein plus cyclosporine A-based respiratory syncytial virus vaccine elicits humoral and regulatory T cell responses against infection without Vaccine-Enhanced disease. *J Immunol* 2016; 196: 1721–1731.
62. Hamad ME, Amer HM, Farrag MA, et al. Naked DNA immunization with full-length attachment gene of human respiratory syncytial virus induces safe and protective immune response. *Acta Virol* 2018; 62: 137–146.
63. Kinnear E, Lambert L, McDonald JU, et al. Airway T cells protect against RSV infection in the absence of antibody. *Mucosal Immunol* 2018; 11: 249–256.
64. Kwon YM, Hwang HS, Lee YT, et al. Respiratory syncytial virus fusion protein-encoding DNA vaccine is less effective in conferring protection against inflammatory disease than a virus-like particle platform. *Immune Netw* 2019; 19: e18.
65. Ma Y, Jiao YY, Yu YZ, et al. A Built-In CpG adjuvant in RSV F protein DNA vaccine drives a Th1 polarized and enhanced protective immune response. *Viruses* 2018; 10: 38.
66. Smith TRF, Schultheis K and Broderick KE. Nucleic acid-based vaccines targeting respiratory syncytial virus: delivering the goods. *Hum Vaccin Immunother* 2017; 13: 2626–2629.
67. Smith TRF, Schultheis K, Morrow MP, et al. Development of an intradermal DNA vaccine delivery strategy to achieve single-dose immunity against respiratory syncytial virus. *Vaccine* 2017; 35: 2840–2847.
68. Espeseth AS, Cejas PJ, Citron MP, et al. Modified mRNA/lipid nanoparticle-based vaccines expressing respiratory syncytial virus F protein variants are immunogenic and protective in rodent models of RSV infection. *NPJ Vaccines* 2020; 5: 16.
69. Watterson D, Robinson J, Chappell KJ, et al. A generic screening platform for inhibitors of virus induced cell

fusion using cellular electrical impedance. *Sci Rep* 2016; 6: 22791.
70. Olszewska W, Ispas G, Schnoeller C, et al. Antiviral and lung protective activity of a novel respiratory syncytial virus fusion inhibitor in a mouse model. *Eur Respir J* 2011; 38: 401–408.
71. Ispas G, Koul A, Verbeeck J, et al. Antiviral activity of TMC353121, a respiratory syncytial virus (RSV) fusion inhibitor, in a Non-Human primate model. *PLoS One* 2015; 10: e0126959.
72. Roymans D, Alnajjar SS, Battles MB, et al. Therapeutic efficacy of a respiratory syncytial virus fusion inhibitor. *Nat Commun* 2017; 8: 167.
73. Stevens M, Rusch S, DeVincenzo J, et al. Antiviral activity of oral JNJ-53718678 in healthy adult volunteers challenged with respiratory syncytial virus: a placebo-controlled study. *J Infect Dis* 2018; 218: 748–756.
74. Janssen Research & Development LCTStudy to evaluate safety and antiviral activity of doses of JNJ-53718678 in children (>=28 days to <=3 years) with respiratory syncytial virus infection. *A phase 2, double-blind, placebo-controlled study to evaluate the antiviral activity, clinical outcomes, safety, tolerability, and pharmacokinetic/pharmacodynamic relationships of different doses of JNJ-53718678 in children >=28 days and <=3 years of age with acute respiratory tract infection due to respiratory syncytial virus infection*, https://ClinicalTrials.gov. : U.S National Library of Medicine, 2020.
75. DeVincenzo JP, McClure MW, Symons JA, et al. Activity of oral ALS-008176 in a respiratory syncytial virus challenge study. *N Engl J Med* 2015; 373: 2048–2058.
76. Fathi H. A Phase 2a Study to Evaluate ALS-008176 in the Virus Challenge Model. *A Randomised, Phase 2a, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Pharmacokinetics and Antiviral Activity of Multiple Doses of Orally Administered ALS-008176 Against Respiratory Syncytial Virus Infection in the Virus Challenge Model*, https://ClinicalTrials.gov. : U.S National Library of Medicine 2014.
77. DeVincenzo JP, McClure MW and Fry J. ALS-008176 for respiratory syncytial virus infection. *N Engl J Med* 2016; 374: 1391–1392.
78. McClure M. A study of ALS-008176 in infants hospitalized with RSV. *A randomized, double-blind, placebo-controlled, 2-Part study of orally administered ALS-008176 to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of single ascending dosing and multiple ascending dosing in infants hospitalized with respiratory syncytial virus (RSV) infection*, https://ClinicalTrials.gov. : U.S National Library of Medicine, 2018.
79. Arasaratnam R. ALS-008176 for respiratory syncytial virus infection. *N Engl J Med* 2016; 374: 1391.