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Vaccines Against Respiratory Syncytial Virus: The Time Has Finally Come

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Abstract

Respiratory syncytial virus causes a significant public health burden, particularly in very young infants and the frail elderly. The legacy of enhanced RSV disease (ERD) from a whole formalin-inactivated RSV vaccine, and the complex biology of the virus and the neonate have delayed the development of effective vaccines. However, new insights into factors associated with ERD and breakthroughs in understanding the antigenic structure of the fusion (F) glycoprotein have increased optimism that vaccine development is possible. This has led to investment of time and resources by industry, regulatory authorities, governments, and nonprofit organizations to develop the infrastructure needed to make the advanced clinical development of RSV vaccine candidates a reality.

Keywords

RSV; immunization; vaccination; structure-based vaccine design; fusion; neutralization; vaccine-enhanced illness; bronchiolitis; pneumonia; infants; elderly; Th2; eosinophilia; subunit vaccine; vaccine vector; wheezing; asthma; protein conformation; epitope

Epidemiology

Respiratory syncytial virus (RSV) is the most common cause of hospitalization in children under 5 years of age (1). In developing countries RSV also causes substantial mortality in children under 1 year of age (2). All children are infected by the age of 3 and people are repeatedly infected throughout life (3). In otherwise healthy children over 5 years of age and in adults, RSV typically causes an upper respiratory syndrome sometimes complicated by sinusitis and otitis media (4, 5). In individuals with T cell deficiencies like Severe Combined Immunodeficiency (SCID) or following allogenic bone marrow transplantation or lung transplantation, RSV can cause a life-threatening progressive pneumonia (6, 7). In addition, RSV infection in the frail elderly is associated with excess mortality at frequencies comparable to influenza virus infection (8). Infections tend to be seasonal in temperate climates, but in tropical climates can be detected throughout the year (9).

Approximately 20 per 1000 infants less than six months of age are hospitalized with severe RSV illness, and in the institutionalized elderly about 1–2 per 1000 (1, 8, 10, 11). Hospitalized children have a higher frequency of wheezing during childhood than their counterparts with milder disease. This predisposition to wheezing subsides during adolescence (12, 13). In children prophylactically treated with palivizumab (a neutralizing monoclonal antibody specific for antigenic site II on the RSV fusion glycoprotein) the frequency of subsequent wheezing is diminished (14). Conversely, there is also genetic, clinical, and experimental evidence that a pre-existing tendency towards allergic inflammation is associated with more severe disease (15, 16).

Pathology

RSV infects ciliated epithelium in the upper and lower respiratory tract. Bronchiolar epithelium is especially susceptible to infection, and type I pneumocytes in the alveoli are also commonly infected resulting in high frequency (~20%) of children with hypoxemia, even in those without other significant symptoms. In children with severe RSV disease, it is thought that a major feature of pathology is airway obstruction. The few autopsy studies of fatal RSV infection show that small airways can be obstructed by sloughed epithelium and inflammatory cells combined with mucus and fibrin (17). The airways can also become hyper-reactive contributing to the signs of wheezing and retractions characteristic of infants with severe disease. RSV is one of the first pathogens encountered by young infants and in premature infants who are especially susceptible to severe disease, the impact of RSV on the developing lung and airways is not well understood. One of the important consequences of having a successful vaccine for RSV would be the opportunity to ask whether diminishing the severity of RSV disease in early childhood would reduce the frequency of childhood asthma and airway hypersensitivity.

Goals of vaccination

The primary clinical goal for an RSV vaccine is to prevent severe lower respiratory tract disease in young infants. Endpoints would include the prevention of hospitalization or medically attended lower respiratory tract infection (MALRI) in industrialized countries, prevention of mortality and hospitalization or MALRI in developing countries, and if possible, development of a clinical severity index as a continuous variable for disease severity. Secondary goals are to: 1) prevent medically attended lower respiratory tract infection in young children, 2) prevent hospitalization and mortality in the elderly, and 3) reduce childhood wheezing, otitis media, and overall morbidity associated with RSV infection in children and adults. To achieve these goals RSV vaccines have been considered for use in five main populations including 1) pregnant women, 2) infants <6 months of age, 3) infants and children >6 months to 2 years, 4) young (2–5 years old) and school-age children, and 5) individuals >60 years of age (Figure 1). The critical distinction between target populations is whether the subject is RSV antigen naïve and vaccination will be the first inductive priming event or the subject has already experienced natural infection and vaccination will be boosting pre-existing immunity. This will influence which vaccine approach is selected for a given population.

While protecting infants prior to the peak of hospitalization at 2 months of age by direct vaccination would be ideal, there are several factors that make this challenging including presence of maternal antibody, lack of significant capacity for somatic mutation and affinity maturation of antibody, Th2-biased immune response patterns. In addition, there are more idiosyncratic events like apnea in neonates, which complicates the interpretation of adverse events temporally related to vaccination. Immunization of pregnant women is proposed as a way of protecting neonates by boosting maternal antibody that is acquired by the infant transplacentally. The main objective is to delay the time of first RSV infection until the child is at least 6 months old when airways are more fully developed and have larger diameters to reduce the risk of obstruction from mucus, inflammatory debris, and airway hypersensitivity. Children between 6 and 24 months still have significant morbidity from RSV and would directly benefit from a preventive vaccine. It would also allow the vaccination event to be the first exposure to RSV antigen in many cases, providing the opportunity to induce more effective priming than natural infection. Immunization of young and school-age children would be done in the presence of pre-existing immunity from prior natural infection. Therefore, the likelihood of adverse events would be lower, but the direct benefit to the individual would also be lower, since by age 5 most people have achieved a level of immunity that prevents serious lower airway disease unless immunity is compromised by disease or aging. Immunization of this population would be primarily to reduce transmission to neonates and the elderly, based on RSV transmission dynamics studies (18, 19) showing that most neonatal infections come from older siblings, and the precedent in influenza showing that immunization of school-age children is more cost-effective than immunizing the elderly (20). Boosting pre-existing immunity in the elderly may reduce RSV-related disease, but immunity and pathogenesis in this population is complex, making the definition of clinical endpoints short of hospitalization difficult. Typically viral shedding is limited in this population making pathogen-specific diagnosis more challenging, and disease is often manifest as multi-organ failure rather than confined to the respiratory tract. Deficiencies in both T cell- and antibody-mediated immunity may contribute to the higher susceptibility to disease, and may require alternative antigen designs, formulations, and delivery approaches than younger populations. Better definitions of severe lower respiratory tract disease in young infants and the elderly would facilitate the use of more discriminating clinical endpoints in future vaccine trials (21).

Virology

RSV is a pneumovirus in the Paramyxoviridae family. It has a single-stranded, negative-sense RNA genome of about 15 kilobases with 10 genes separated by stop/start sequences that encode 11 known proteins. There are two major subtypes of RSV that are distinguished largely by variation in the G glycoprotein (22). The subtypes tend to alternate in dominance from year to year, but co-circulate and are not exclusive in any given season. While there is genetic variation between strains characteristic of any RNA virus, it is not extreme and does not seem to explain the susceptibility to reinfection. The first two genes at the 5’ end of the genome are NS1 and NS2 (non-structural) that are primarily devoted to evading the induction and effector functions of type I interferons (IFN). The genetic capital invested in this function suggests it is a critical element of the RSV life cycle. RSV is a pleomorphic

enveloped virus about 100–200 nanometers in diameter. The virus enters and buds from the apical surface of polarized epithelial cells. The replication cycle is entirely cytoplasmic. During infection, RhoA is activated and produces filopodia in which the virus assembles emerging from the cell initially as filamentous particles that can be up to 10 microns in length (23). The filamentous projections from cells can mediate syncytium formation with adjacent cells or produce budding particles. As the matrix protein that underlies the viral envelope fragments the particles become pleomorphic and then round.

Surface proteins

There are three proteins in the lipid envelope exposed on the surface of virions including SH (small hydrophobic), G, and F (fusion) (24). SH is a 64–65 amino acid long pentameric ion channel analogous to the M2 protein of influenza virus. G is a heavily glycosylated 298 amino acid long type II integral membrane protein with a molecular weight of about 90 kilodaltons. It major features include a high serine/threonine content of close to 30% resulting in a high level of O-glycosylation, and about 10% proline residues, giving it a chemical composition resembling mucins. The F glycoprotein is a type I integral membrane protein that is 574 amino acids long and mediates viral entry as a class I fusion machine. It has 25 amino acid signal peptide and 2 endoproteolytic cleavage sites resulting in F2 (26–109) and F1 (137–574) subcomponents and a liberated 27 amino acid glycopeptide of unknown function. F2 has 2 N-linked glycans at aa27 and aa70 and F1 has a single N-linked glycan at aa500, while the 27aa peptide has 2 or 3 N-linked glycosylation sites. The native F is a trimer of heterodimers with the F2 and F1 subcomponents connected by 2 disulfide bonds. F1 has a cysteine-rich region between the 2 heptad repeats that form a series of loops in the assembled molecule.

Most of the neutralizing activity in human serum is directed against the F glycoprotein (25, 26). F has several antigenic sites associated with neutralizing monoclonal antibodies (mAbs) (27–33). To date, all these antibodies work through fusion inhibition, meaning that they do not prevent attachment and can prevent virus entry when added after virus has attached to the cell. There is one known antigenic site associated with neutralization on the G glycoprotein near the cysteine noose in the central conserved domain (34–36). MAbs to this site can prevent virus attachment, particularly when evaluated on primary human airway epithelium (37). SH is not required for virus entry and antibodies to SH do not neutralize RSV. However, similar to antibodies specific for the M2 protein of influenza, anti-SH antibodies can reduce viral load and protect against illness in animal models using antibody Fc-mediated immune functions like ADCC (38).

Correlates of immunity

The basis for frequent RSV reinfection is not known. The fact that there is relatively little genetic variation suggests that it is based on other mechanisms of immune avoidance. One possibility is that the induction of immunity during the initial priming event is ineffective and permanently alters the ability to achieve sustained immunity. The initial exposure often occurs in very young children in the presence of maternal antibody and in hosts with a tendency towards lower IFN activity. These factors could result in a suboptimal specificity

and function of B and T cell precursors. In this scenario, it is possible that vaccination as the primary RSV antigen exposure for most infants could significantly alter lifetime RSV immunity and reduce the frequency of reinfection. Alternatively, RSV could be so prevalent and contagious that infection of the superficial epithelium in the upper airways can occur because the gradient of antibody between serum and mucosal secretions in the upper airway is steep, and local mucosal responses are not sustained. Therefore, finding immune correlates of protection in serum may be challenging. It is also possible that there are more nuanced antigenic differences between subtypes and between strains from year to year that are not yet understood.

Neutralizing antibody has long been regarded as a correlate of protection against severe RSV disease based on maternal-infant pairs (39); passive protection studies in animal models (40); and clinical studies with passively administered high titer polyclonal serum (41) or monoclonal antibodies specific for antigenic site II on the F glycoprotein (42, 43). However, the correlation of neutralizing activity and protection from infection has not been as clear. There are trends or marginally significant differences in pre-existing serum NT activity in naturally infected elderly patients (44) or experimentally infected young adults (45, 46). There has been an association between pre-existing RSV-specific nasal antibody titers and susceptibility to infection (44, 45, 47), but there is not yet an analysis of epitope-specific antibody responses and susceptibility, and the role of ADCC and other Fc-mediated immune functions on antibody are not established in humans. Mapping all the antibody epitopes on RSV surface proteins, and using new reagents and serological assay platforms to define epitope-specific antibody functions, may provide more clarity on the basis for antibody-mediated protection in the future.

RSV-specific T cells have been associated with both viral clearance and immunopathology. One study showed a paucity of CD8 T cells in lung tissue in children with fatal infection (48). Particularly in persons with Severe Combined Immunodeficiency (SCID), allogenic bone marrow and lung transplant recipients, RSV can cause a fatal pneumonia with widespread syncytia formation and epithelial destruction (6, 7, 49–51). In one patient with SCID and life-threatening RSV infection, passively administered neutralizing mAb did not impact viral load, but a syngeneic bone marrow transplantation and expansion of CD8 T cells was temporally correlated with a drop in viral load and a worsening in respiratory function (7). It has also been shown that HIV-infected infants can shed RSV for months (52), although the same type of lethal cytopathic process does not occur in this type of T cell deficiency. In murine models it has been shown that CD8 T cells are required for normal viral clearance, but are also positively correlate with disease severity in this setting where a large virus inoculum is required to establish infection (53). In humans, pre-existing CD8 T cell memory is likely to be useful in rapidly clearing virus-infected cells, as suggested by human challenge studies where pre-existing intraepithelial (tissue-resident) CD8 T cells are negatively correlated with disease severity (54). Overall, the weight of evidence suggests vaccine approaches that can induce CD8 T cell responses in antigen-naïve infants would have value for virus clearance and for producing IFN-gamma to promote a Th1-biased CD4 T cell response. In persons with pre-existing immunity, particularly in the elderly where there is some evidence to suggest waning CD8 T cell immunity (55, 56), boosting CD8 T cell responses may also have value.

Vaccine-enhanced disease

A vaccine-enhanced disease syndrome was associated with immunizing antigen-naïve infants with whole formalin-inactivated RSV (FI-RSV) formulated in alum (57–60). Although the exact mechanism underlying the FI-RSV vaccine-enhanced RSV disease (ERD) is not known, there are two major immunological phenomena associated with this syndrome based on evaluation of pathology from the original cases (61, 62) and extensive studies in mice, cotton rats, nonhuman primates (NHP), and cattle (63–66). First, the original vaccine induced antibody responses measured by complement fixation or ELISA, but induced poor functional antibody responses measured by neutralizing (67) or fusion-inhibition assays (68). This was associated with evidence of immune complex formation and complement deposition in small airways of the affected infants (69). Secondly, studies in animal models have consistently shown that immunizing antigen-naïve animals with FI-RSV intramuscularly followed by airway challenge with RSV results in a Th2-biased CD4 T cell response and alveolitis (66, 70, 71). This is consistent with the finding of eosinophils in the lungs of infants who died (60). A similar disease syndrome has been demonstrated in NHP models of measles virus attempting to reproduce the atypical pneumonia caused by measles in recipients of the whole-inactivated measles virus vaccine when neutralizing antibody titers waned (72). The FI-RSV ERD presents a difficult safety concern for vaccines intended for antigen-naïve infants. The major difficulty is the lack of adequate animal models to insure that the FI-RSV ERD will not happen. The rodent and even the NHP models are semi-permissive for RSV and therefore too easy to protect. To demonstrate the Th2-biased T cell response and alveolitis pathology, vanishingly low doses of vaccine and/or long intervals between vaccination and challenge are needed to preserve susceptibility to infection. Without active virus replication in lung, the pathological effects cannot be evaluated. The bovine model using bovine RSV probably recapitulates the pathogenesis of natural RSV infection better than the animal models for human RSV, but still requires a large intra-tracheal inoculum for consistent infection and disease severity is highly variable (64). This calls into question whether the risk of vaccine-induced ERD can ever be excluded solely by results from currently available animal models. However, the collective experience evaluating this syndrome does provide some useful guidance for regulators. There is abundant evidence that live-attenuated virus vaccines are not associated with this syndrome whether given mucosally or parenterally. Therefore, vaccines based on live-attenuated, live chimeric, replication-defective vectors, or nucleic acid delivery that express native proteins and induce CD8 T cells and Th1-biased responses should be safe in antigennaïve infants. Also, there is significant evidence that once memory responses have been established by live virus infection, FI-RSV immunization does not result in ERD. Therefore, only vaccines that induce strong functional antibody responses relative to total binding antibody, and those that induce Th1-biased CD4 T cell responses should be considered for direct immunization of antigen-naïve infants. For persons with pre-existing actively-induced RSV immunity, boosting with any vaccine platform should be relatively safe from ERD, but induction of functional antibody responses in proportion to binding antibody responses would be preferred.

Vaccine approaches

Since the FI-RSV vaccine episode, only live-attenuated or chimeric live vector vaccine approaches given intranasally have been advanced in antigen-naïve infants. However, over the last few years, there has been a large surge in the number and variety of RSV candidate vaccines (Table 1). Live-attenuated and chimeric live vectors have not been associated with ERD, and have generally been safe and well tolerated (73). Some of the recent designs have demonstrated higher levels of immunogenicity despite greater attenuation and are still being advanced (74–76). An earlier study showed that delivery of live virus intramuscularly was also safe in this age group, but poor immunogenicity curtailed development (77). Directly immunizing antigen-naïve infants is challenging because of pre-existing maternal antibody and the idiosyncratic events that may occur when infecting the airway of young infants, and currently efforts are focused on infants older than 6 months of age. As noted above, it is thought that gene-based replication-defective approaches using viral vectors (like adenovirus) (78, 79) or nucleic acid (80) would also be safe in this target population because antigen presentation will recapitulate live virus infection with induction of CD8 T cell responses as well as antibody and CD4 T cell responses. These types of approaches are currently in early phase clinical testing in adults.

Vaccine approaches that rely on phagocytosis and obligate MHC class II presentation pathways like subunit proteins or protein-based nanoparticles are currently only being proposed for immunization of antigen-experienced individuals, particularly pregnant women and the elderly. The approaches are designed to primarily induce antibody-mediated protection, and are primarily based on presenting the F glycoprotein. However, some approaches focus on ADCC responses that can be induced against SH (38) or anti-G antibodies that may be able to protect through neutralizing virus in vivo (37) or through preventing some of the immunomodulatory effects of the secreted G (81, 82).

Delivery of multiple RSV antigens through virus-like particles (83), virosomes (84), and other complex formulations (85) including modern adjuvants will engage antigen processing machinery and activate innate immunity in a variety of ways. Therefore, the antigen content and formulation will need to be considered on a product-by-product basis. They may or may not induce protective CD8 T cell responses in addition to antibody-mediated immunity.

F structure and implications for vaccine development

Solving the crystal structure of the F glycoprotein trimer in its pre-triggered (prefusion) conformation is a recent breakthrough that has energized RSV vaccine development (29, 86, 87). The prefusion F (pre-F) surfaces are targeted by antibodies from multiple distinct binding competition groups of which five are currently published (27, 28). Only two of these sites are present on the shared surface that remains on the postfusion F (post-F) trimer, which is the end-product of a massive molecular rearrangement, the process designed to mediate membrane fusion and allow entry of the viral nucleocapsid into the host cell. Unlike other class I fusion proteins like the hemagglutinin (HA) of influenza, the pre-F conformation is very unstable. Soluble trimers of HA can be expressed and the molecule remains in the pre-triggered state until pH is lowered. In contrast, RSV F expressed as a

soluble protein spontaneously rearranges into the highly stable post-F, 6-helix antiparallel bundle form. This rearrangement or “flipping” occurs even in the virus membrane (88), and may be part of the viral strategy for evading neutralization. The post-F form is about 16 nm tall, while the pre-F form is only about 11 nm tall. When the virus is assembled in RhoA-induced filopodia and emerges from the infected cell as long filamentous structures, the matrix layer is highly ordered (89), and in those structures, the F trimer is more often in the pre-F conformation (88). However, when virus buds and becomes asymmetric and then round, the matrix layer becomes fragmented and the F tends to flip into the post-F conformation. Therefore, most transmitted virus has an abundance of post-F on its surface that may shield access to the untriggered pre-F. The major implications for vaccine development are: 1) post-F is not a fusion-competent molecule, so antibodies targeting post-F will not neutralize virus, and 2) the neutralization epitopes specific for pre-F are much more neutralization-sensitive than epitopes shared with post-F (27–29, 62, 90). Therefore, using pre-F (the functional form of the molecule) as the vaccine antigen will *induce* significantly more neutralizing activity than post-F antigens as a primary immunogen in antigen-naïve children. Also, because in pre-immune individuals virtually all neutralizing activity is attributable to antibody that recognizes pre-F surfaces (25), it is expected that preserving those features in an immunogen will *boost* neutralizing activity in older children and adults better than post-F.

Guided by the crystal structure of pre-F, stabilizing mutations have been identified that allow pre-F to be produced as a soluble protein (91, 92). F antigens that preserve the pre-F surfaces have superior immunogenic properties than F antigens in the post-F conformation whether presented as subunit proteins (91, 92) or expressed from a vaccine vector (75). The pre-F molecule also has significantly different antigenic properties than post-F because of its unique surfaces, and because it is the functional form of the molecule. Therefore, using pre-F as a reagent in serological assays instead of post-F will allow the measurement of different antibody specificities and functions (25). The insights provided by the stabilized pre-F trimer will improve our understanding of the immune correlates of protection and improve the efficacy of vaccines designed to induce antibody-mediated protection.

Conclusions

RSV is a major cause of morbidity and mortality and is a high priority for vaccine development. Despite the fact that humans are frequently reinfected by RSV despite a lack of significant genetic variation and the legacy of FI-RSV ERD, there is optimism that a vaccine solution for preventing severe disease is possible. This is based on new insights into virus morphology, atomic-level structure of the F glycoprotein in the pre-F and post-F conformational states, mechanisms of virus neutralization, serological responses to natural infection, methods of virus attenuation, and immunological factors associated with FI-RSV ERD. These events have resulted in new reagents, ideas, and an accelerated pace of basic research into the virology, immunology, and pathogenesis of RSV. In addition, the possibility of a vaccine intervention has in part motivated more detailed studies of epidemiology, transmission dynamics, and clinical assessment of disease severity that are providing the information needed for clinical trial design and to establish efficacy endpoints. Importantly, there is now significant involvement of regulatory authorities, and investment

by both large and small pharmaceutical companies, to create the clinical development pathways and manufacturing approaches needed to achieve licensed vaccine products.

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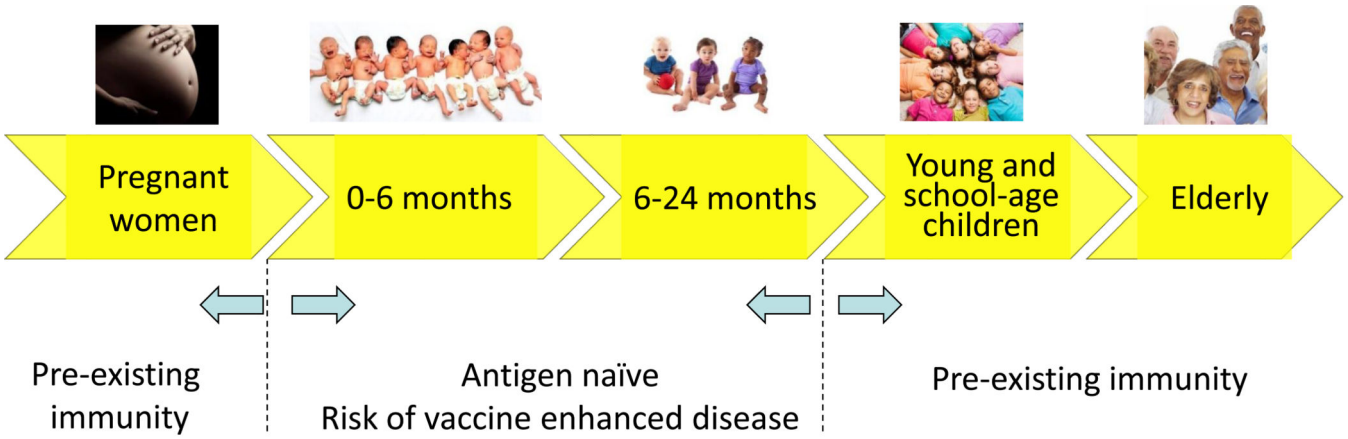

Figure 1.

Potential target populations for RSV vaccines include 1) pregnant women, 2) infants <6 months of age, 3) infants and children >6 months to 2 years, 4) young (2–5 years old) and school-age children, and 5) individuals >60 years of age. Children less than 2 years of age and the elderly would derive the most direct benefit from an effective vaccine. Since all children are infected early in life, anyone over 2 years of age is likely to have experienced natural infection, so vaccination of older children and adults is designed to boost pre-existing immunity. Vaccination in children under 2 years of age could be the primary immunization event.

Table 1

RSV vaccine approaches in development*

Live-attenuated RSV
Deletion of M2-2 (delta-M2-2)
Modification or deletion of NS1 and/or NS2
Codon deoptimization
Selected cold-adapted or temperature sensitive mutations
Deletion of G (delta-G)
Live chimeric vectors
Chimeric parainfluenza viruses – expressing F and/or G
Sendai virus – expressing F
BCG – expressing N
Replication-defective vectors
Various recombinant adenovirus vectors expressing F or F/M2-1/N
Recombinant alphavirus vectors expressing F
Recombinant MVA expressing F and/or G or F/M2-1/N
Nucleic acid delivery
mRNA
DNA
Particle-based vaccines
Membraned virus-like particles (VLP) based on RSV, Newcastle Disease Virus, or Influenza
Bacterium-like particles displaying RSV F
RNA bacteriophage displaying RSV peptides
Alfalfa mosaic virus VLP displaying RSV G peptide
Multilayer polypeptide nanofilms displaying RSV peptides
Protein subunits
Stabilized prefusion F trimer
Postfusion F either as single trimer or an aggregate rosetted on fusion peptide
SH delivered as a pentameric complex
Various versions of G glycoprotein
Various peptide formulations
Whole-inactivated RSV
Virosomes
Oil-in-water emulsion

*Adapted from: http://sites.path.org/vaccinedevelopment/files/2015/07/RSV-snapshot-July2015.pdf and reference 21.