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Review

The journey to a respiratory syncytial virus vaccine

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Key Messages

- Respiratory syncytial virus (RSV) is a major global health problem associated with significant morbidity and mortality in low- and middle-income countries.
- There are several vaccine candidates in the pipeline directed toward different target populations: infants younger than 6 months, children older than 6 months to 2- to 5-year-old children, and the elderly.
- To protect the young infant from severe RSV infection, a combined strategy using passive and active immunization with maternal vaccination and high-potency, extended half-life monoclonal antibodies may be needed.
- Vaccines will have the opportunity to decrease not only the burden of acute RSV disease but also the long-term respiratory morbidity associated with this infection.

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ABSTRACT

Objective: The high burden associated with respiratory syncytial virus (RSV) has made the development of RSV vaccine(s) a global health high priority. This review summarizes the journey to an RSV vaccine, the different strategies and challenges associated with the development of preventive strategies for RSV, and the diverse products that are undergoing clinical testing.
Data Sources: Studies on RSV biology, immunology, epidemiology, and monoclonal antibodies (mAbs) and vaccines were searched using MEDLINE. We also searched PATH.org and ClinicalTrials.gov for updated information regarding the status of RSV vaccines and mAbs undergoing clinical trials.
Study Selections: We selected relevant studies conducted in infants and young children, pregnant women, and elderly population for the prevention of RSV infection.
Results: Identification of a safe and immunogenic vaccine has been an important but elusive initiative for more than 60 years for different reasons, including the legacy of formalin-inactivated vaccine, our limited understanding of the immune response to RSV and how it relates to clinical disease severity, or the need for different end points according to the different vaccine platforms. Nevertheless, there are currently 39 vaccines and mAbs under development and 19 undergoing clinical trials.

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Conclusion: Over the past decade, there have been significant advances in our knowledge of RSV molecular and structural biology and in understanding the human immune response to RSV. Despite the barriers, there are several promising mAbs and RSV vaccines undergoing clinical trials that hope to offer protection to the most vulnerable populations.

Introduction

Respiratory syncytial virus (RSV) is one of the great threats to child health and is associated with considerable short-term and long-term morbidity.[1-4] In infants and toddlers, RSV is the leading cause of viral lower respiratory tract infection (LRTI, including bronchiolitis and pneumonia) worldwide. Globally, it is estimated that RSV causes 33 new million episodes of acute LRTI in children younger than 5 years, resulting in approximately 3.2 million hospitalizations per year and approximately 120,000 deaths annually.[5] In resource-limited countries, RSV represents the second most common cause of infant mortality.[6]

In adults, the few studies published to date suggest that RSV carries a significant burden, especially in elderly where it is associated with substantial morbidity and mortality. In this patient population, RSV is responsible for approximately 1.5 million episodes of LRTI and mortality rates that range from 4% to 10%, depending on the patient characteristics, with those with underlying cardiac or pulmonary diseases carrying the highest mortality rates.[7-12] In addition, RSV is a major pathogen for immunocompromised individuals[13] and has been associated with the development of persistent wheezing and asthma.[14,15]

Respiratory syncytial virus is ubiquitous, with relatively homogeneous distribution worldwide, and invariably and predictively causes yearly outbreaks. By their first birthday, nearly 70% of infants have been infected with RSV at least once, and essentially all children are infected with this virus within the first 2 years of life.[16] The increasingly recognized burden associated with RSV has made the development of RSV vaccine(s) a global health high priority. The World Health Organization has developed a research and development roadmap to facilitate the development and implementation of vaccines and monoclonal antibodies (mAbs) in low- and middle-income countries and estimates that RSV vaccination will be available in the next 5 to 10 years.[17] This review summarizes the history and journey of RSV vaccines, the different strategies and challenges associated with the development of RSV vaccines and mAbs, and the diverse products that are undergoing clinical testing.

RSV Structure

Respiratory syncytial virus is an orthopneumovirus that belongs to the recently created Pneumoviridiae family. Human RSV exists as 2 antigenic subgroups, A and B, which can cocirculate during the same season and exhibit genome-wide sequence divergence. The nonsegmented, single-stranded, negative-sense genome contains 10 genes (15,222 nucleotides) that encode 11 proteins. Of those, 3 are nonstructural proteins (NS1, NS2, and M2-2), and 8 are structural proteins (Fig 1).

Of the 8 structural proteins, 3 are in the surface viral membrane: the small hydrophobic (SH), the attachment (G), and the fusion (F) glycoproteins, and 5 are internal proteins (N, P, M, M2-1, and L). The SH protein is not required for initiating virus infection, whereas the F and G proteins are crucial for the infectivity and pathogenesis of the virus. The attachment (G) protein targets the ciliated cells of the airways and mediates adherence of the virus to the host cells.[18-21] The fusion (F) protein initiates viral penetration by fusing viral and cellular membranes and late in the infection causes infected cells to fuse, inducing the production of the characteristic syncytia.[22,23]

RSV G and F carry the antigenic determinants that elicit the production of neutralizing antibodies by the host.[24,25] However, RSV F is the preferred target for vaccine, and neutralizing mAb development because it plays an essential role in host cell viral entry, is highly conserved among RSV A and B subtypes, and carries several antigenic sites that elicit the production of high-potency neutralizing antibodies. In fact, 90% or more of neutralizing antibodies are directed against this protein.[26] The G protein is mostly covered by glycans, leaving only its central domain available for neutralizing antibody binding. Except for this domain, which is involved in receptor antibody binding,[27] G is not well conserved. It is recognized by few neutralizing antibodies, which has reduced its value as a vaccine target.

Our understanding of the RSV F protein in its 2 conformations, prefusion (pre-F) and postfusion (post-F), has revolutionized the field of RSV biology.[28] Pre-F is the active form of the F protein on the virion surface; it is metastable, meaning that is triggered easily and unpredictably to refold to its post-F conformation, whereas post-F is extremely stable and cannot return to its functional pre-F form. Briefly, once a virion is bound to a target cell via its G protein, pre-F is triggered and refolds in a dramatic structural rearrangement that links and fuses the virion and host cell membranes, becoming post-F. During the rearrangement, the final post-F loses its top neutralization-sensitive antigenic sites, sites Ø and V (Fig 2). Antibodies that bind to pre-F are more efficient at neutralizing RSV than those shared by both pre-F and post-F. As examples, antibodies against site Ø bind significantly more efficiently to pre-F, than palivizumab binds to site II, present in both pre-F and post-F, and antibodies against antigenic site I, also shared between pre-F and post-F, show weak or no neutralization.[26]

RSV uses different mechanisms for immune evasion, which will need to be circumvented by an effective and protective RSV vaccine. In addition to the protein F—losing fundamental antigenic sites on

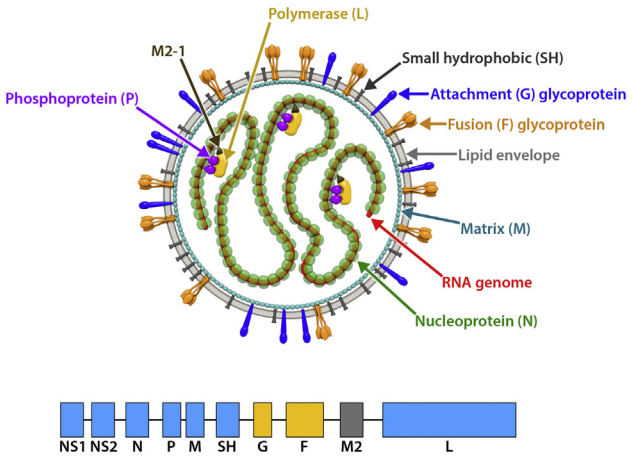

Figure 1. The respiratory syncytial virus (RSV) virion. It is an enveloped, negative-sense, single-stranded RNA virus with a genome that contains 10 genes (15,222 nucleotides) that encode 11 proteins. Of the 3 transmembrane surface glycoproteins, the adhesion (G) and fusion (F) proteins are crucial for the infectivity and pathogenesis of the virus and carry the antigenic determinants that elicit the production of neutralizing antibodies by the host. The F glycoprotein is present in the host in 2 forms: in a metastable prefusion confirmation state (pre-F) and after the virus fuses with the cell (post-F).

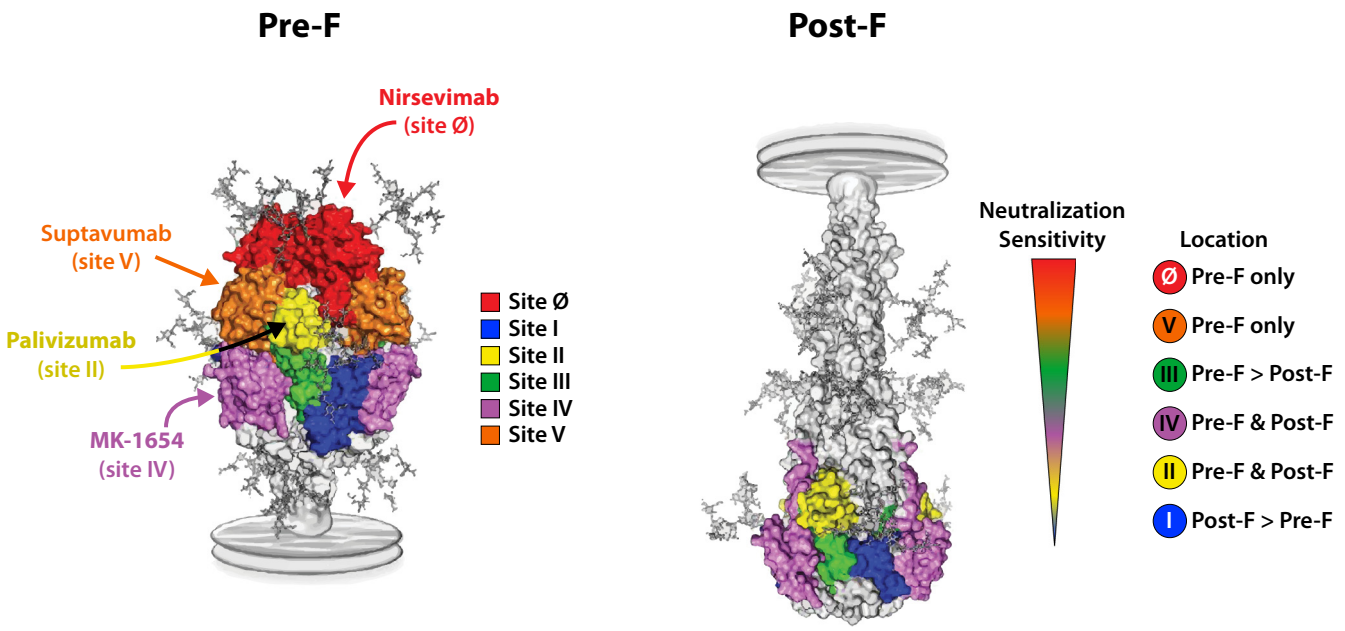

Figure 2. Antigenic sites of the respiratory syncytial virus fusion (F) protein and monoclonal antibodies (mAbs). The conformation of the pre-F and post-F proteins and the specific antigenic side are included in the left panel (pre-F) and right panel (post-F), respectively. Of the 5 major neutralizing sites (Ø, II, III, IV, and V) present on the pre-F surface, sites Ø and V are the most neutralization sensitive. Niservimab and suptamuvab are pre-F–specific mAbs that bind to antigenic sites Ø and V, respectively, whereas palivizumab and MK-1654 bind to sites II and IV, respectively, which are present in the pre-F and post-F conformations. The triangle illustrates the most to least neutralization sensitive epitopes. Modified from Graham et al.[30]

rearrangement, the interferon-inhibiting activity mediated by the viral nonstructural proteins is key. NS1 and NS2 inhibit the induction and effector functions of type I interferon, which are both critical components of the innate immune response and also favor B-cell activation.[29] In addition, a soluble form of G is released from infected cells and can act as a decoy, evading immune responses and altering immune cell migration.

Nevertheless, other viral antigens are also being used as targets of the immune response through different mechanisms, such as antibody-dependent cell-mediated cytotoxicity against SH- or T-cell–mediated immunity against N or M or any of the other internal proteins. These mechanisms may complement the ongoing efforts for vaccine development using RSV F (pre-F more than post-F) and are included in some of the vaccines that are in clinical development.[30]

Challenges Associated With the Development of RSV Vaccines

In 1956, a new cytopathogenic virus, chimpanzee coryza virus, was identified. However, a serosurvey found antibodies to this virus in humans, not chimpanzees.[31] What later came to be known as RSV was first isolated from infants with severe lower respiratory tract illness by Robert Chanock in 1957.[32] The infant disease burden associated with RSV has been recognized for many years; however, identification of a safe and immunogenic vaccine has been an important but elusive goal for more than 60 years. The major challenges associated with the development of an RSV vaccine for infants are listed below. Of those, concerns with safety and our limited understanding of the immune response to RSV and how it relates to clinical disease severity represent 2 major hurdles.

Inefficient Protective Natural Immunity

RSV is a substantial contributor to lower respiratory tract disease throughout life, and reinfections are common: immunity is neither complete nor long-lasting.[16,33] Although infants of young age can generate humoral and cellular immune responses after infection (and vaccination), the efficacy of subsequent responses is partially influenced by the first response. As such, in infants, primary infections are more severe, but milder reinfections occur frequently during the second (approximately 80%) and third years (46%-65%) of age.[33] These findings, together with the observation that RSV-immune adult volunteers could be repeatedly infected with a single viral strain, reveal the inadequacy of the immune response to RSV. The mechanisms interfering with the induction of long-lasting immunity are not well understood. Data suggest that cellular and viral factors influence the activation of innate immune responses, which plays a critical role in the development of adaptive immunity.[34,35] Nevertheless, an ideal effective RSV vaccine should induce improved and more durable B- and T-cell memory responses than natural infection.

Legacy of the Formalin-Inactivated Vaccine

The development of RSV vaccines was hindered for many years because of safety concerns arising from the first RSV candidate vaccine trial in the 1960s.[36] The vaccine virus was produced in cell culture, inactivated with formalin, and mixed with alum as an adjuvant. Newer data have shown that the vaccine expressed mostly the post-F conformation of the F protein.[37] The vaccine was well tolerated, but on natural exposure to RSV, many of the naive infants who had been vaccinated experienced enhanced RSV disease (ERD): 80% of vaccine recipients required hospitalization and 2 died.[38] Older children who had likely been primed by a previous RSV infection did not experience ERD. Several possibilities have been advanced as explanations for this disappointing outcome, including the production of an excess of non-neutralizing antibodies, complement deposition in the lungs, a skewed T_H2 immune response, and impaired T-cell responses.[39] Moreover, ERD is of concern in the naive infant population but not in the older RSV seropositive children or in adults. The potential for ERD is a fundamental aspect that is considered in the development of inactivated and protein vaccines in seronegative children, and

Table 1
Disease Burden, Morbidity, and Mortality Across the Different Target Populations for RSV Vaccine Development

Population	Disease burden	Clinical characteristics	Mortality	Diagnosis	Remarks
Pregnant women	HICs: AR 10%-13% (second and third trimesters)[47,73]; LMICs[a]: AR 2%-4% (second and third trimesters)[46,74,75]	URTI (40%-90%); fever (<30%), headache (approximately 40%), LRTI (approximately 50%), PNA (approximately 5%)	None	PCR and serologic testing	Underrecognized, offspring generally not affected, few studies
Children <1 y	AR: 50%-70%[16]; first cause of hospitalization (approximately 120,000/y in United States; 3%)[4]; 600,000 outpatient visits[50,76]	Bronchiolitis/PNA (70%-85%),[2] apnea in <1 mo, URTI and AOM (30%-60%), fever (approximately 50%)	LMICs: second cause of mortality[6]; HICs: healthy infants <1%[77]; premature infants approximately 2%[78]	PCR	Primary infections more severe,[a] risk factor for severe disease: young age and prematurity, CLD, and CHD
Children <5 y	First cause of LRTI/PNA,[5,79,80] 33 million episodes; LRTI/y,[5] 3.2 million hospital admissions and 2 million outpatient visits (approximately 4%)[2]	Bronchiolitis (20%-30%), PNA (10%-40%), URTI (30%), wheezing or asthma (20%-60%)	60,000-120,000 deaths/y[5]	PCR	Most common cause of PNA in LMICs[79] and HICs[80]
Older children and adults	AR in those aged 17-33 y: 11%-14%[81,82]; AR in those aged 18-65-y: 7%-25%[83,84]	URTI (75%-95%), fever (40%), LRTI (25%-50%)	1.4%-2.5% (in 50-64 yr old)[7]	PCR and serologic testing	Most infections symptomatic, frequent absenteeism >40%, few studies
Older adults (>65 y)	AR: 3%-10%,[8-10] 1.5 million episodes, LRTIs/y[7] >250,000 admissions/y, outpatient visits (18%)	URTI (20%-60%), fever (50%), LRTI (40%-80%), complications (20%-30%)[1,12,84]	14,000 deaths/y; 4%-10%[8,9,11,12]	PCR and serologic testing	Older age and comorbidities risk factors for severe disease and mortality, incomplete data from LMICs

Abbreviations: AOM, acute otitis media; AR, attack rate; CHD, congential heart disease; CLD, chronic lung disease; HICs, high-income countries; LMICs, low- to middle-income countries; LRTI, lower respiratory tract infection (increased work of breathing and wheezing); PCR, polymerase chain reaction; PNA, pneumonia; RSV, respiratory syncytial virus; URTI, upper respiratory tract infection (cough, rhinorrhea, and sore throat).
[a]Fever is an inclusion criterion (as part of influenza-like illness definition in studies conducted in LMICs), possibly underestimating disease burden.

strategies to assess safety risks in relation to the different vaccine platforms in the infant population are required.

Target Populations

Epidemiologic and clinical studies suggest that there are 3 main target populations that will benefit from RSV vaccines and might require different approaches: young naive infants (aged <4-6 months), children older than 6 months, and elderly people older than 65 years.[40] Table 1 summarizes the burden of RSV disease and the morbidity and mortality across different age groups as potential target populations for RSV vaccine development. Vaccination of older children (aged 2-5 years) may also limit transmission because older siblings frequently introduce RSV into the household.[41] In addition, children and adults undergoing hematopoietic stem cell or lung transplantation may benefit from RSV vaccination beforehand because once these individuals acquire RSV, the risk of progression to LRTI, lung rejection, or death is high.[42,43] Nevertheless, each vaccine target population has its own nuances and characteristics (Fig 3).

Infants younger than 4 to 6 months

Infants younger than 4 to 6 months have an immature and developing immune system characterized by low expression of interferon, abundance of regulatory T cells with tolerogenic reactivity, and a limited B-cell repertoire owing to inefficient generation of somatic hypermutations.[44] All these factors are associated with a poor response to foreign antigens, interfering with efficient antigen presentation and the optimal generation of high-affinity maturation antibodies. In addition, infants are born with circulating IgG antibodies derived from their mother, which slowly disappear, with a half-life of approximately 25 days. The presence of maternal antibodies may interfere with vaccine immunogenicity. Young infants represent the main target population because the peak of severe RSV disease is in the first 3 months of life. The protection afforded by immunoprophylaxis has fueled interest in vaccinating pregnant women, with the goal of protecting the young infant from RSV through passive transfer of maternal antibodies. A number of RSV maternal vaccines are currently in clinical development. Regardless, passive immunoprophylaxis with mAbs will still be needed and complement the maternal vaccination strategy, especially in premature infants, because most of the IgG transfer occurs in the last trimester of gestation.

The main goal of *maternal vaccination* is to boost neutralizing antibody titers against RSV and thereby transplacental antibody transfer, conferring protection to the infant during the first months of life. Active transplacental antibody transfer, begins at approximately 28 to 30 weeks of gestation; thus, vaccination should be timed so optimal antibody responses are achieved at the time of delivery. Nevertheless, the optimal timing for vaccination during the second or third trimester and the durability of protection in the infant needs to be defined. In addition, in low- to middle-income countries, the high prevalence of hypergammaglobulinemia, associated with HIV or malaria, that impairs transplacental antibody transfer indicates the need for high maternal antibody titers for effective transfer in this population. Nonetheless, RSV antibody transfer through breastfeeding (IgG more than IgA) may complement the maternal vaccination strategy.[45]

Vaccinating pregnant women could be questioned if it exclusively benefits the infant and not the mother. The limited data available in regard to the incidence of RSV infection in pregnant women are mostly derived from influenza surveillance studies, which likely underestimate the real incidence of RSV.[46] More recent data derived from the United States, however, have shown that the RSV attack rates in pregnant women during the second or third trimester are at least approximately 10% to 13%, suggesting

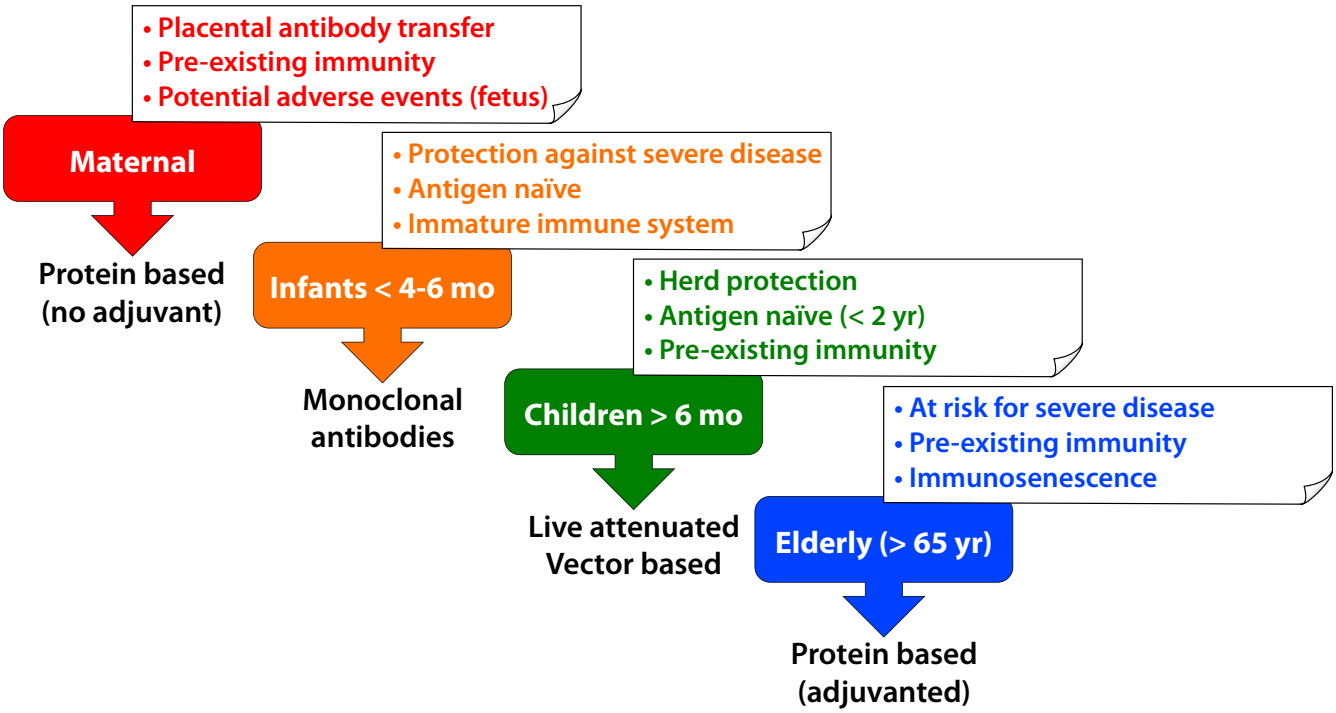

Figure 3. Target populations and respiratory syncytial virus vaccine types. There are different vaccine strategies according to the main target populations. The 4 main target populations are color coded. The nuances and characteristics of each target population are included in the adjacent balloon and the preferred vaccine strategy underneath each target population.

that vaccination could also benefit the mother.[47] The concerns regarding adverse fetal outcomes, such as miscarriage, small for gestational age, or congenital anomalies, are relatively low because this would not be the first time the mother's immune system encounters RSV antigens, and the safety profile of other vaccines used in pregnancy, such as Tdap or influenza, is excellent. A number of RSV maternal vaccines, most of them nonadjuvanted, are currently in clinical development (Table 2).[48]

Older infants and children younger than 2 years

Although the peak of severe RSV disease is within the first 3 to 4 months of life, RSV also causes significant disease in older infants and toddlers. Globally, more than half of hospitalizations for acute RSV LRTIs and RSV-related deaths occur in children older than 6 months. In the United States, RSV is responsible for a substantial number of hospital admission and outpatients visits in children aged 6 to 24 months, highlighting the importance of developing strategies for the prevention of RSV in these children.[5,49,50] On the basis of the experience of the formalin-inactivated RSV vaccine, within this age group those who are naive at the time of vaccination might be at risk of ERD with protein vaccines. This target population would likely benefit most from live-attenuated vaccines (LAVs) or vectored vaccines.

Elderly

In the elderly RSV is responsible for 3% to 10% cases of LRTI, more than 250,000 annual admissions, and approximately 14,000 deaths each in year in the United States (Table 1).[7,51] The immunosenescence of adults older than 65 years and the presence of additional comorbidities may compromise vaccine responses and the ability to assess efficacy. This population might benefit most from adjuvanted vaccines because studies have found that boosting and maintaining sufficiently high titers of neutralizing antibodies can confer protection from severe disease.[52] In addition, LAV will likely not be effective in this age group because preexisting immunity would possibly prevent adequate viral replication and therefore immunogenicity.

Clinical End Points

The main goal of an RSV vaccine is a reduction of disease severity; however, the lack of a standard definition of severe disease and/or precise markers to assess clinical disease severity in infants has represented for years an important barrier for vaccine development. This problem is related to our limited understanding of the immune response to RSV and how it relates to clinical disease severity.[53-58]

Clinical end points that define a successful vaccine might be different, depending on the target population. Hospitalization has been traditionally considered as a main indicator of severity; however, other end points that capture the inpatient and outpatient burden of the disease should be considered, such as a reduction in medically significant visits for RSV infection.[59] Another goal would include the prevention of post-RSV respiratory morbidity (ie, recurrent wheezing and asthma). Developing composite end points that include a combination of viral factors, clinical parameters, and fast turnaround point-of-care biomarkers could help with patient classification and the standardization of definitions in a more precise manner.[57] In addition, long-term follow-up is advisable because studies suggest that interventions aimed at reducing the acute burden of RSV disease may also reduce recurrent wheezing and asthma.[60]

Immune Correlates of Protection

Defining the immune correlates of protection for RSV vaccines requires a better understanding of disease pathogenesis, immunity, and transmission dynamics. Achieving sterilizing immunity, although attractive, might not be possible or even desirable because RSV reexposures in the upper airway may be sufficient to boost the immune response, limiting progression of the infection to the lower respiratory tract and/or RSV transmission. Serum neutralizing antibodies (IgG against pre-F, post-F, and G) have

Table 2
RSV Vaccines in Clinical Development

Vaccine type (manufacturer)	Viral target	Target population	Administration route	Clinical development	Advantages	Challenges
PROTEIN VACCINES						
Particle based						
RSV F nanoparticle (Novavax, Gaithersburg, Maryland)	Prefusogenic	Maternal, elderly, pediatric	Systemic	Phase 3, phase 2, phase 1	Safe, immunogenic	Post-F based? Risk of ERD, antibody durability
Subunit						
DS-Cav1 (NIH/NIAID, Bethesda, Maryland)	Pre-F	Maternal and elderly	Systemic	Phase 1	Induce high-affinity neutralizing antibody, facilitate cross-priming, safe	Factors that affect transplacental transfer, instability of pre-F, antibody durability, no protection for premature infants
GSK RSV F (GlaxoSmithKline, Brentford, United Kingdom)	Pre-F	Maternal and elderly	Systemic	Phase 1		
DPX-RSV (Immunovaccine, Dartmouth, Canada, and VIB, Flander, Belgium)	SH	Elderly	Systemic	Phase 1		
RSV-F (Janssen, Beerse, Belgium)	Pre-F	Elderly	Systemic	Phase 1		
RSV-F (Pfizer, New York, New York)	Pre-F	Maternal and elderly	Systemic	Phase 2		
RSV-G (Advaccine Biotech, Beijing, China)	G	Pediatric and elderly	Systemic	Phase 1		
LIVE VACCINES						
Vector based						
AdV26 RSV (Janssen)	Pre-F	Pediatric and elderly	Systemic	Phase 2	Not attenuated, low risk of ERD, no interference with maternal antibodies	Potential for developing antivector immunity
ChAdV155-RSV (GlaxoSmithKline)	Pre-F, N, M2-1	Pediatric	Systemic	Phase 2		
VXA-RSV (AdV5) (Vaxart, South San Francisco, California)	Post-F	Elderly	Mucosal and systemic	Phase 1		
MVA-BN RSV (Bavarian Nordic, Kvistgaard, Denmark)	Post-F, GA/GB, N, M2	Elderly	Systemic	Phase 2		
Live-attenuated/chimeric						
rBCG/N-hRSV (Universidad de Chile, Santiago, Chile)	N	Newborn	Systemic	Phase 1	Predominant T_H1 immune responses	
RSV/ΔG (Intravac)	Lacks G	Pediatric	Mucosal	Phase 1	Low risk of ERD, intranasal delivery, replication in presence of maternal antibody, broad stimulation of immune responses	Balance of attenuation/ immunogenicity, reverse to wild type, stability for mass production
RSV ΔNS2 Δ1313/1314L	Pre-F/post-F	Pediatric	Mucosal and systemic	Phase 1		
RSV 276						
RSV 6120/ΔNS2/1030_S (Sanofi Pasteur, Lyon, France, and NIH)						
SeV/RSV (St Jude Hospital, Atlanta, Georgia)	F	Pediatric	Mucosal	Phase 1		

Abbreviations: Adv, adenovirus; ERD, enhanced RSV disease; F, fusion; G, attachment; MVA, modified vaccinia Ankara virus; ND, not disclosed; NIAID, National Institute of Allergy and Infectious Diseases; NIH, National Institutes of Health; post-F, postfusion; pre-F, prefusion; RSV, respiratory syncytial virus; SeV, Sendai virus; SH, small hydrophobic.

Table 3
Monoclonal Antibodies for the Prevention of RSV Infection in Infants

Antibody (manufacturer)	Viral targets	Antibody characteristics	In vitro/in vivo potency	Development phase	Target populations	Comments
RespiGam (RSV IVIG) (Medimmune Inc, Gaithersburg, Maryland)	Polyclonal	High titers of IgG with RSV neutralizing activity	Six-fold higher neutralizing activity than regular IVIG,[85] 10-fold more potent than regular IVIG at reducing RSV titers in cotton rats	Discontinued (1997)	High-risk infants	41% reduction in RSV hospitalizations in premature in CLD,[86] monthly dosing during RSV season (approximately 5 doses), fluid overload, long-lasting intravenous infusions, interference with live vaccines
Palivizumab[87] (MEDI-493) (Medimmune Inc)	Antigenic site II (pre-F and post-F)	Humanized mAb, half-life of approximately 28 d	50- to 100-fold more potent than RSV-IVIG,[87] >99% RSV neutralization in cotton rats	Phase IV marketed (1998)	High-risk infants (premature <29 wk GA, CLD <32 wk; CHD <12 mo)[88]	55% reduction in RSV hospitalizations,[62] monthly dosing during RSV season (approximately 5 doses)
Nirsevimab (MEDI-8897) (MedImmune and Sanofi Pasteur, Lyon, France)	Antigenic site Ø (pre-F)	Human mAb (YTE technology), half-life of 63-73 d[89]	>50-fold more potent than palivizumab in vitro,[90] 9-fold than palivizumab at reducing viral loads in cotton rats	Phase IIb/III (ongoing)	Premature and full-term infants	70% reduction in MALRI, 78% reduction in RSV hospitalizations,[91] 1 dose required
MK-1654 (Merck & Co Inc, Kenilworth, New Jersey)	Antigenic site IV (pre-F and post-F)	Human mAb (YTE technology), half-life of 70-85 d[92]	>50 more potent than palivizumab in vitro,[92] upper airway protection vs palivizumab in cotton rats	Phase I/Iia (ongoing)	Premature and full-term infants	Undergoing clinical evaluation, 1 dose required
Suptavumab (REGN-2222) (Regeneron Pharmaceuticals Inc, Tarrytown, New York)	Antigenic site V (pre-F)	Human mAb, extended half-life, half-life of 32-35 d	40-fold more potent than palivizumab	Discontinued (2017)	Premature infants	Drug manufacturing discontinued because of RSV mutants (RSV B) resistant to the mAb after phase 3 studies, 1-2 doses required

Abbreviations: CLD, chronic lung disease; IVIG, intravenous immunoglobulin; mAb, monoclonal antibody; MALRI, medically attended lower respiratory tract infection; post-F, postfusion; pre-F, prefusion; RSV, respiratory syncytial virus; YTE, 3-amino acid substitution to extend the mAb half-life.

classically represented the main surrogate of protection, as shown by the effectiveness of immunoprophylaxis using polyclonal immunoglobulin with high titers of anti-RSV neutralizing antibody (Respigam)[61] or an anti-F mAb (palivizumab) in high-risk infants[62] or the protection conferred by maternal antibodies.[63,64] Ratios of serum antibody titers and neutralization may be important; however, a precise correlate of immunity or a standardized protective threshold has not been defined yet.

Systems biology approaches are helping to define the optimal correlates of protection, which are complex and depend on multiple factors rather than a single cut-off value in antibody assays. Immune end points will need to be adjusted to each vaccine type and target population because focus on a single correlate may lead to missing additional and relevant vaccine effects. Other correlates of protection may include F epitope–specific antibodies, mucosal IgA, interferon responses, antibody-dependent cell-mediated cytotoxicity, and cell-mediated immunity because vaccine-induced $CD4^+$ T cells (including T follicular helper cells and T-regulatory cells) will be needed for optimal induction and duration of antibody responses. In addition, a balanced T_H1/T_H2 immune response, indicated by a high IgG2a/IgG1 ratio, is desirable.

Other Factors

RSV epidemiology

The increasing number of RSV interventions undergoing clinical testing emphasize the need for accurate information about RSV epidemiology, including temporal and geographic patterns of RSV circulation in inpatients and outpatients across different age groups, and RSV-associated mortality.[65] Implementing specific-RSV surveillance platforms, which include a respiratory-symptom–based definition different from the traditional influenza-like-illness definition that includes fever, and a robust multiplex-PCR–based surveillance that detects a number of respiratory viruses, could help to document the global burden of the disease and assess the impact of interventions in such burden. They could also help to monitor the development of scape mutants, or the contribution of other respiratory viruses that cause RSV-like illnesses.

Animal models

The lack of an ideal animal model has also slowed down RSV vaccine development. Respiratory syncytial virus is not a natural pathogen for rodents, and African green monkeys are semipermissive to the infection. Conducting studies in other nonhuman primates has been eliminated for humane treatment reasons. More recently, human challenge models of RSV infection have been developed.[66] These models, although useful as proof of concept, have some limitations. For obvious reasons, infected volunteers are young healthy adults, which limits the generalizability of the results to the main vaccine target populations: young naive infants and elderly people with immunosenescence. In addition, in these models, upper respiratory tract infection but not LRTI is induced, limiting the assessment of the effect of vaccines on disease severity

Vaccine Strategies

In recent years, there has been an explosion of passive and active immunization strategies for RSV moving through the drug discovery pipeline.[67] Different vaccine strategies are being explored for preventing severe RSV infection in the main target populations: protein vaccines that use stabilized pre-F protein subunits or viruslike particles, live vaccines that included attenuated RSV strains, or virus vectors that express RSV proteins. Age and whether patients are naive (or have been previously exposed to RSV) will influence the use of each vaccine type (Fig 3). There are 38 vaccine candidates under development (https://path.org/resources/rsv-vaccine-and-mab-snapshot/); of those, 19 are undergoing clinical trials (Table 2). The most effective approach to protect young infants and children from severe RSV infection may be a combined strategy using passive and active immunization: maternal vaccination with stabilized pre-F or viruslike particles that contain the F protein or mAb against pre-F administered before the RSV season starts followed by pediatric active immunization with a live vaccine, either attenuated RSV or a virus vector that expresses the pre-F protein (or other proteins in some cases). The ideal vaccine candidate should be able to prevent severe disease and also limit transmission.

Protein Vaccines

Particle based

The recombinant adjuvanted RSV prefusogenic nanoparticle vaccine is the most advanced vaccine in clinical development. Results from a phase 3 clinical trial that enrolled 4636 pregnant women in the third trimester demonstrated a decrease in RSV hospitalizations in the offspring; however, the study did not meet the primary end point defined as prevention of medically significant RSV LRTI. Overall, the day 90 vaccine efficacy was 39.4%, with a 97.5% CI that ranged from −1.0% to 63.7%. However, in South Africa, the vaccine had a significant protective effect (vaccine efficacy, 57.0%; 95% CI, 32.7%-72.5%). The factors associated with these striking differences are not well understood and have stimulated ample discussions and follow-up studies. Particle-based vaccines are designed also to target children older than 6 months to 5 years of age and elderly.

Subunit vaccines

Subunit vaccines consist of purified, adjuvanted proteins that until 2013, when the structure of RSV pre-F was solved, used mostly post-F combined with other viral antigens (G or M) unsuccessfully. Current subunit vaccines use stabilized pre-F as the main antigen with promising results because most of the RSV neutralizing activity in sera targets exclusively pre-F antigenic sites.[64] Stabilized pre-F vaccines are mainly being tested in pregnant women or elderly rather than in naive young children because of the risk of ERD. Other subunit vaccines in clinical or preclinical stages are using SH or G as the main vaccine antigens.

Live Vaccines

Vector based

There are 4 vector-based vaccines in clinical development. The first 3 use different adenoviruses as vectors (adenoviruses 5, 26, and 155), whereas the other vaccine uses a modified vaccinia Ankara virus. These vaccines induce a robust innate immune response that boosts both T-cell responses and B cells, enhancing neutralizing antibody production. Two of these vaccines are intended for use in pediatric seronegative patients. All the vectored vaccines express RSV F (pre-F more than post-F, depending on the vaccine candidate), and 2 of them also express other viral antigens, such as N, M2, and G proteins.

Live-attenuated Vaccines

Live-attenuated vaccines represent an attractive alternative for older infants (older than 4-6 months) and young children because they provide active immunization that mimics natural infection without causing ERD. These vaccines have been at the forefront of development since the 1960s, following the failure of the formalin-inactivated RSV vaccine. The main challenge for the development of LAV has been balancing attenuation with immunogenicity, particularly given the young age of the target population. Live-attenuated vaccines are administered intranasally, and some are temperature

sensitive, replicating in the upper but not in the lower respiratory tract, despite the presence of maternal antibodies and are able to elicit broad innate, humoral, and cellular responses. Importantly, these vaccines have not been associated with ERD and thus are considered safer in this population. The use of reverse genetics has made possible to incorporate different mutations in the viral genome, making LAV immunogenic and sufficiently attenuated and, except for rhinorrhea, not associated with significant adverse events.[68-70] There are 6 intranasal LAVs undergoing phase 1 clinical trials; 4 are using attenuated RSV, 1 is using Sendai virus as a backbone expressing RSV F, and the last one is a chimeric vaccine using Bacille Calmette Guerin. The Bacille Calmette Guerin/RSV vaccine is the only LAV intended to be administered systemically (subdermal) and to newborns.

mAbs

mAbs are also being evaluated for the prevention of RSV LRTI in young infants (Fig 2).[71] They have advanced faster and more successfully than RSV vaccines because of their enhanced efficacy while potentially improving patient adherence because fewer doses will be required and ultimately reducing costs. However, there is the possibility of developing escape mutations that may alter the susceptibility of natural RSV strains that lack the epitope for the mAb. This possibility emphasizes the need to conduct surveillance studies before, during, and after clinical trials with mAbs to assess the effect of these potential novel mutations on the immune protection associated with the mAb. Table 3 summarizes the main mAbs that are being evaluated in clinical trials.

Nirsevimab

Nirsevimab (MEDI-8897) is a highly potent human neutralizing IgG_{1K} that targets the pre-F–specific antigenic site Ø. Its half-life is extended because of 3 amino acid substitutions in the F_C region of the IgG heavy chain (YTE technology). A phase 2b clinical trial in healthy, 29- to 34-week gestation, preterm infants was completed and met the primary end point. The trial found a 70% reduction in the incidence of medically attended RSV LRTIs and a 78% reduction in RSV-related hospitalizations. Phase 2/3 clinical trials are now ongoing in palivizumab-eligible patients, and phase 3 trials are ongoing in late preterm and full-term infants. The long-term goal of nirsevimab is to provide passive immunization for the prevention of RSV LRTI to all infants (preterm and full term) entering their first RSV season using a fixed, single intramuscular dose.

MK-1654

MK-1654 (Merck & Co Inc, Kenilworth, New Jersey) is also an extended half-life mAb currently undergoing phase 1 clinical trials. It is directed against antigenic site IV, which is present in both the pre-F and post-F forms.

Suptavumab

Suptavumab (REGN-2222; Regeneron Pharmaceuticals Inc, Tarrytown, New York) is a human monoclonal IgG1 antibody that targets antigenic site V of RSV pre-F protein. In in vitro and animal models, suptavumab was 40 times more potent than palivizumab at neutralizing RSV. Nevertheless, during the execution of the phase 3, randomized, placebo-controlled trial that included premature infants at 35 week of gestation or less, RSV B was the predominant circulating strain and carried 2 point mutations that conferred resistance to the mAb.[71,72] The study did not meet the primary end point (medically attended RSV infections), and its clinical development has been interrupted.

Conclusion

During the past decade, there have been significant advances in our knowledge of RSV molecular and structural biology and in the understanding of the human immune response to RSV. In addition, the increasing interest of academic, industry, and international entities, such as the World Health Organization and the Bill & Melinda Gates Foundation, is helping to rapidly move this field forward, promoting the implementation of surveillance platforms and standardization of clinical definitions, assays, and surrogate markers of protection. Despite the barriers, there are several opportunities for RSV vaccine development to protect the most vulnerable populations. In addition, several lessons from previous vaccine failures have helped in the design of novel and informed effective strategies. Furthermore, the development of newer cost-effective and long-acting mAbs represent a promising advance for the prevention of RSV in young infants.

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