Advances in RSV vaccine research and development – A global agenda

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A B S T R A C T

Respiratory syncytial virus (RSV) is an important cause of viral lower respiratory tract illness in infants and children globally. It is responsible for one-third of deaths resulting from acute lower respiratory infection (ALRI) in the first year of life [1]. RSV, which is transmitted by direct and indirect contact with nasal or oral secretions, causes repeat infections throughout life and significant disease in pediatric and elderly populations [2,3]. The pathogen is an enveloped, non-segmented, single-stranded, negative-sense RNA pneumovirus belonging to the family Paramyxoviridae. The viral genome consists of 10 genes encoding 11 proteins. The fusion (F) and attachment (G) surface glycoproteins are most important in their ability to induce neutralizing antibodies [4]. The virus circulates seasonally in temperate regions, usually between the late fall and early spring, and lasts three to four months in a community (although timing varies between years and regions and within communities). In tropical regions, the seasonal relationship is less defined with virus detectable year round in some locations.

The annual global burden of RSV is estimated to be 33.8 million new episodes of ALRI in children less than five years old, 3.4 million hospital admissions, and in 2010, 253,000 deaths, with most of the fatalities occurring in developing countries [1,5]. The lack of supportive care contributes to the increased severity and mortality in such settings. The RSV mortality rate is difficult to accurately assess in many countries due to the unavailability and infrequent use of diagnostics. Under-reporting of cases and deaths may also occur because they never come in contact with the local medical system. The RSV Global Estimates Network is in the process of updating morbidity and mortality data, with results expected in 2016 [6]. The epidemiology and burden of RSV disease points to several target populations for RSV vaccines: (1) infants younger than six months of age, who are at highest risk of severe disease; (2) children six months of age and older to prevent disease not only in these children but to help decrease transmission to younger children and the elderly; (3) pregnant women to decrease their transmissibility and to protect newborns by placental antibody transfer; and (4) the elderly, who are also at risk for severe disease [7]. RSV is also a significant concern for other high-risk members of the community, such as pre-term infants, the immunocompromised, and those with pulmonary or congenital heart disease.

Several RSV diagnostic methods are in use today (i.e., cell culture, nucleic acid amplification, and immunofluorescence assays) and are quite heterogeneous in format, specimen preparation, and assay readout. There is only one type of point-of-care diagnostic in use – a rapid antigen detection platform based on immunochromatography. It is generally easy-to-use and provides results within 15–30 min. This assay tends to have moderate sensitivity but high specificity, and yields only qualitative results. However, as infants have higher RSV viral loads, these tests may be a useful option for specific studies (e.g., evaluating vaccine effectiveness in infants). More complex microbiological and molecular laboratory-based
platforms have higher sensitivity and specificity, but these tests are more technologically challenging and time consuming, requiring experienced laboratory personnel. Molecular methods are being increasingly adopted, however, because they allow for the detection of an array of pathogens, which is useful since many ALRIs are clinically indistinguishable.

While vaccines are among the most cost-effective health interventions for infectious diseases, there are none yet available for RSV. Treatment is usually reserved for patients with severe ALRI and primarily consists of supportive care including supplemental oxygen and mechanical ventilation, if needed. Bronchodilators, corticosteroids, and ribavirin have failed to show clear benefit in randomized controlled trials and are not currently recommended for use in many countries. Immunoprophylaxis with the neutralizing monoclonal antibody (mAb) palivizumab is used to a limited extent in the United States and some other high- and middle-income countries to prevent RSV disease in extremely premature infants or those with congenital heart disease. The high cost and requirement for monthly dosing precludes its use in resource-constrained settings.

1. Feasibility for vaccine development

While there is currently no licensed vaccine for RSV, several observations support the feasibility for RSV vaccine development:

- Primary RSV infection occurs in most infants within the first two years of life, with virtually all children infected by three years of age. Infections recur throughout life but, as natural immunity increases, the disease becomes less severe so that older children and healthy younger adults typically experience a mild upper respiratory illness. Preventing RSV–associated ALRI in the youngest populations, therefore, may be an achievable goal.
- Older adults are at risk for more severe RSV disease. The reason may be multifactorial and could be attributable to underlying cardiac or pulmonary disease and/or immunosenescence [2].
- The ability of RSV-specific functional antibodies to neutralize viral infection has been demonstrated in vitro. Protection has been demonstrated in numerous preclinical models (i.e., mouse, cotton rat, guinea pig, calf, and non-human primate) [9]. Furthermore, prophylactic administration of monoclonal or polyclonal antibodies reduces the incidence of severe RSV disease in children [9]. Although serum-neutralizing antibody clearly protect against RSV–associated ALRI, other types of immunity (e.g., mucosal antibody cell-mediated immunity) may also contribute and be induced by certain vaccines (e.g., DNA, vector).
- A reduced incidence of RSV ALRI during the first months of life correlates with higher concentrations of RSV-specific maternal antibody [10].
- Live-attenuated and inactivated virus vaccines have been successfully developed for influenza, a more mutable and antigenically variable respiratory virus than RSV, though both share some virologic and clinical similarities.
- Recent progress defining the RSV pre-F protein structure, and identification of several neutralizing epitopes found only on pre-F, improve the likelihood for making a potent vaccine.

2. Constraints on RSV vaccine development

The history of RSV vaccine development is notable for the vaccine-enhanced illness that occurred after a formalin-inactivated RSV (Fl-RSV) vaccine was administered to seronegative infants in the 1960s [11–14]. The severe lung inflammation, worsened disease, and deaths that occurred in vaccines raised concerns that other non-replicating RSV vaccines might also predispose infants and RSV-naïve children to aberrant immune responses. However, the immunization of older children with the Fl-RSV vaccine did not result in enhanced disease as prior infection had primed for a non-lethal response. Proposed mechanisms for disease enhancement include the induction of high titers of non-neutralizing antibodies and Th2-biased cellular immune profile. These types of immunologic responses can lead to immune complex deposition and complement activation and allergic inflammation [8,15–17]. Enhanced respiratory disease (ERD) has been recapitulated in several animal models, including mice, cotton rats, bovine calves, and African green monkeys [18–20]. The legacy of ERD led to hesitancy from vaccine developers, clinical investigators, and regulators for use of RSV vaccines requiring MHC class II processing in RSV-naïve infants. Consequently, only live-attenuated vaccines have been tested for active infant immunization for the last four decades.

2.1. General approaches to vaccine development for low- and middle-income country markets

A growing number of RSV vaccine candidates, across multiple platforms, has emerged as of late.

- Live-attenuated RSV vaccines to protect pediatric populations from RSV disease have been in development for decades and do not appear to cause enhanced disease in RSV-naïve infants. Recent approaches include engineered viruses that use knowledge of RSV gene function to create ‘knock-out’ viruses that are attenuated but still immunogenic, such as the M2-2 deletion mutant that favors transcription over replication of the genome, leading to more protein production, but limited virus production. Naturally attenuated chimeric viruses combining genes from RSV-related viruses such as Sendai, parainfluenza virus, and bovine RSV are also in development.
- Protein-based vaccine approaches (including whole-inactivated virus, subunit antigens that associate to form aggregate particles, and non-particle based subunit antigens) have been developed for protecting elderly populations from severe disease and are often formulated with an adjuvant. Particles can display viral proteins, peptides, or neutralizing epitopes with increased density to enhance B cell receptor binding. Particle and protein–subunit vaccines are also being developed for immunization during pregnancy to boost pre-existing immunity to increase transplacental transfer of RSV-specific antibody to infants. Maternal RSV vaccine will likely be more acceptable either as unadjuvanted formulations or adjuvanted only with alum given their history of safe use in this population.
- Replication competent and deficient alphavirus, adenovirus, and modified vaccinia virus Ankara (MVA) vectors encoding RSV surface antigens (including replication-competent and -deficient variations) are being developed for use in infant and pediatric populations. These vectors are intended to express surface proteins in their authentic conformation and are processed by MHC class I and class II pathways, thus eliciting robust humoral and cellular immunity.
- Nucleic acid vaccines using either plasmid DNA or messenger RNA encoding RSV antigens are being targeted to protect both pediatric and elderly populations. Combination approaches with DNA and protein are in early development as well and could induce both cellular and humoral immunity.
- A modified version of the D25 mAb, which is specific for the neutralizing epitope in antigenic site Ø on the pre-F conformation of RSV F protein is being developed for passive prophylaxis in pediatric populations. Genetic modifications that increase potency and half-life may provide protection for an entire RSV season with just a single dose.
A reduction in the incidence and severity of RSV-related ALRI in children younger than five years of age through vaccination in low- and middle-income countries (LMICs) would directly work toward reaching the fourth Millennium Development Goal of reducing child mortality [5]. To achieve this goal, increased awareness and data on RSV disease burden in LMICs is needed, particularly to inform policy makers, regulators, and societies on the potential benefits of vaccine development. In recognition of differing risk and immune profiles, vaccine development will likely have to follow a two-pronged approach that divides the target population into two age groups – younger and older than six months of age.

The incidence of severe RSV disease is highest in infants younger than six months of age [10,21]. The need for immediate protection and the difficulty of achieving protective efficacy via active immunization in this age group has made maternal immunization and infant passive prophylaxis a priority strategy for protecting young infants. A goal of passive immunoprophylaxis is to provide four to six months of protection with a single dose antibody. A maternal immunization approach would be intended to protect infants for the first two to six months of life. Either of these strategies could be followed with active infant immunization later in life as maternal/passive antibody titers wane.

Numerous live-attenuated and chimeric virus RSV vaccine approaches are being developed. Achieving a proper balance between attenuation (safety) and immunogenicity, as well as genetic stability, has been difficult, although recent advances with recombinantly engineered RSV suggest that this may be feasible [22]. A live vaccine approach targeted to older infants and young children could ultimately complement a maternal/passive immunization approach by protecting these older populations and decreasing transmission of the virus. This approach would bypass the challenge of adequately attenuating live candidates for newborns, and the optimal time for active immunization will depend on the duration of protection afforded by passive immunization.

To reduce the burden of childhood pneumonia, there is strong consensus that focus should be placed on children in their first six months of life, when the risk of severe RSV-associated respiratory disease is highest. To better protect these infants, maternal/passive immunization has become a greater priority. Protection of preterm infants with palivizumab and motavizumab has already been demonstrated and likely will show the same for the next generation of RSV F mAbs in development. A maternal vaccination strategy will be just as important, but, like mAbs, will be limited to the very young and will not protect children beyond four to six months of age. In children with respiratory co-morbidities (e.g., asthma, congenital heart disease, bronchopulmonary dysplasia, cystic fibrosis), an effective RSV vaccine could have significant impact on morbidity. There are reports that up to 50 percent of children who suffer severe RSV bronchiolitis are subsequently diagnosed with asthma. RSV may precipitate the development of asthma or simply be worse in those who are predisposed to asthma [23]. As the mechanism is not understood, the benefit of an RSV vaccine for asthma outcomes is not clear. It is also not well understood how much an RSV vaccine will impact the incidence of secondary respiratory bacterial infection [24].

### 2.2. Technical and regulatory assessment

Overall, 16 RSV candidates are currently advancing through Phase 1 to Phase 3 clinical trials. The regulatory pathways for RSV vaccines are defined by the following requirements: (1) to establish evidence of protection against RSV disease affecting the very youngest age group; (2) to provide additional ERD safety assurances for an infant vaccine; (3) to generate safety information about the different technologies used (i.e., live, non-replicating, vectored, subunit antigen, extended half-life mAb); and (4) to chart a relatively new pathway if pursuing a maternal indication, which requires immunizing one group and measuring outcomes in another. The World Health Organization (WHO) Consultation on Respiratory Syncytial Virus Vaccine Development held March 23–24, 2015 concluded that safety, immunogenicity, and efficacy data in preclinical models (including those that exhibit enhanced disease immunopathology such as the mice, rat, cotton rat, bovine, and baboon models) should be reviewed when considering the advancement of a vaccine candidate into the clinic [25]. In general, safety and immunogenicity studies should first be performed in healthy adults. Early trials that involve seronegative infants should occur in a setting with appropriate facilities for the management of adverse events [6].

A maternal immunization pathway could start with healthy non-pregnant women of child bearing age, advancing to pregnant women with safety follow-up in both mother and infant. The pathway could be accelerated by integrating high-income country and LMIC clinical development, as immunization during pregnancy is more widely accepted and adopted in the developing world. RSV mAb trials may be able to more easily recruit participants given the effectiveness of palivizumab, the existence of an established clinical and regulatory pathway, and the fact that pregnant women will not have to be immunized. However, if the intervention is intended for all, not just children at high risk of severe disease, requirements for showing the mAb is safe and free of any developmental or off-target effects will be high. Pathways for pediatric vaccines using a live-attenuated approach could likewise follow established pre-existing pathways. Development pathways for active infant vaccination using novel vectored approaches could involve age de-escalation safety studies. Since no animal model can absolutely rule out the risk of ERD, advancing vaccine development for vectored vaccines from a seropositive toddler to seronegative infant population involves some level of risk. An NIH/FDA RSV vaccine workshop held June 1–2, 2015 concluded studies in seropositive children would not provide any assurance of safety of subunit or inactivated vaccines for RSV-naïve children, such that there is no clear development path forward for such endeavors. For all clinical development strategies, assessing safety and immunogenicity during co-administration with representative routine vaccines appropriate for the targeted recipients would be necessary.

A commercial RSV IgG ELISA assay from IBL International evaluates immunogenicity of F and G proteins, but does not assess antibody function. Numerous neutralization assay formats have been developed to measure functional antibodies against both RSV A and B subtypes. Harmonization across formats using an international antibody standard (IS) could facilitate the comparison and prioritization of RSV vaccine candidates. A recent survey study across 12 divergent neutralization assay formats testing a common sample panel demonstrated feasibility for harmonization of output by use of a standard. Plans for establishing this IS are being developed by WHO and the National Institute for Biological Standards and Control (NIBSC). A binding competition assay is being used to measure antibodies able to compete with palivizumab for binding to the RSV F protein [26]. The palivizumab competitive antibody (PCA) assay shows promise as a means to characterize antibody responses to the RSV F site II neutralizing epitope, one of many neutralizing epitopes on the F protein, but does not ensure that the competing antibodies are neutralizing. In passive prophylaxis studies with RSV-IGIV in rodents and infants, high titers of serum neutralizing antibody correlated with protection of the lower respiratory tract [27–29]. Correlates of protection against severe disease in young infants may differ by type of vaccine used and will need to be evaluated in the context of efficacy trials. Regulatory alignment on measurements of disease severity and definitions are important to allow for the advancement of vaccine development programs. Another key component of Phase 3 trials, consensus
case definitions/severity scoring systems, are being drafted and discussed [6]. These systems include clinical features that are considered objective, easily standardized, generalizable to multiple global settings, and based on generally accepted markers of disease severity. Endpoints for licensure should include safety and reduction of severe disease, but also assess impact on mild or moderate disease. Once licensure is obtained, advancing to WHO prequalification rapidly is critical because the most severe disease occurs in the countries with greater resource constraints. Therefore, the definition of endpoints relevant to LMIC populations and efficacy data from these settings should be planned for in efficacy trials.

3. Status of vaccine R&D activities

The success of passive immunization with an RSV F mAb (palivizumab and motavizumab) provides the rationale for developing a vaccine that elicits functional antibody responses. While antibodies to F protein are cross-reactive across RSV A and B subtypes, antibodies to G protein are much less so. However, antibodies to the neutralizing site in the central conserved region of G should work on both subtypes, while antibodies binding the heavily glycosylated regions of G do not neutralize and would not be cross-reactive. The efficacy of palivizumab and motavizumab, which bind to antigenic site II on the RSV F protein, has led many developers to focus on RSV F as a primary immunogen. As of December 2015, 60 RSV vaccine candidates are in preclinical or clinical development and encompass five broad platforms (Table 1). Live-attenuated approaches targeting pediatric populations have been in development for decades, spearheaded by the US National Institute of Allergy and Infectious Diseases and MedImmune. These were the primary candidates in clinical testing for many years, but recently there has been a significant surge in RSV vaccine candidates using other platforms. While the majority of the vaccine candidates under development are still in the preclinical stage, 16 candidates are now in clinical development. Several of these utilize an RSV F protein-based approach. Novavax recently initiated late stage development of both elderly and maternal immunization vaccine candidates (Phase 3), and advanced a pediatric candidate into the clinic (Phase 1). GlaxoSmithKline (GSK, Phase 2), GSK (legacy Novartis, Phase 1), and MedImmune (Phase 2) are also testing RSV F candidates for use in maternal immunization and/or immunization of the elderly. GSK (Phase 1) is testing an adenvirus prime/boost candidate for use in children beyond the neonatal period. Janssen Pharmaceutical (Phase 1) and Bavarian Nordic (Phase 1) advanced to the clinic in 2015 with their own adenvirus

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<tr>
<th>Vaccine platform</th>
<th>RSV vaccine candidates</th>
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<tbody>
<tr>
<td>Live-attenuated and live-vectorized</td>
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<tr>
<td>Protein-based</td>
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<tr>
<td>Whole-inactivated</td>
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<td>Particle-based</td>
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<td>Subunit antigens</td>
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<td>Nucleic acid</td>
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<tr>
<td>Gene-based vectors</td>
<td>11</td>
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<tr>
<td>Combination and immunoprophylaxis</td>
<td>3</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>60</strong></td>
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Table 1: RSV vaccine candidate numbers in research and development per vaccine platform.

**Fig. 1.** RSV vaccine candidates in research and development.
and MVA vaccine products respectively. Preclinical vaccine developers include pharmaceutical companies, government agencies, academic institutions, and biotechnology organizations targeting infant, child, and elderly populations (Fig. 1).

Recent advances in understanding RSV F protein structure and instability could inform vaccine development [36]. RSV F is present on the viral surface in two states: a metastable pre-F and stable post-F form. The newly characterized antigenic site Ω has been shown to elicit antibodies more potent than palivizumab in preclinical studies [31]. In addition, there are at least two other sites exclusive to pre-F (AM14 and MPE8) that are more neutralization sensitive than site Ω [32]. The stabilization of pre-F and its subsequent demonstration of potent immunogenicity has enabled testing of this protein as a vaccine candidate. Several other RSV F protein-based candidates with or without alum adjuvant are currently being developed for maternal immunization. Novavax is advancing a rosetted post-F for their elderly, maternal immunization, and pediatric vaccine candidates. GSK’s 2015 acquisition of Novartis Vaccines has united five RSV vaccine candidates using three technologies (pre- and post-F subunit protein, nucleic acid, and gene-based vector) into a single organizational portfolio. MedImmune is advancing a post-F subunit candidate vaccine for the elderly population (Phase 2), and is testing an extended half-life RSV F mAb directed against the newly identified antigenic site Ω to protect infants through their first RSV season. These two enhanced features may make protecting newborns through their first RSV season with a single dose possible, which could provide an alternative to maternal immunization for high-, middle-, and low-income countries.

The success of a maternal immunization strategy will require access to and acceptability of vaccination in pregnant women. Platforms exist for vaccine delivery to pregnant women that leverage the likelihood that, even in the least-developed countries, the majority of women will have some antenatal care [6]. The successful global Maternal and Neonatal Tetanus Elimination Initiative, the recent recommendation by WHO that pregnant women be the highest priority group for influenza vaccine, and the recent recommendation for maternal immunization to protect infants from pertussis by the US Advisory Committee on Immunization Practices all provide important precedents for the acceptance and justification of a maternal immunization approach.

4. Likelihood for funding

Since the focus of RSV vaccine development in LMICs will be on protecting infants and children younger than five years of age, the vaccines will be in line with the priorities set by Gavi, the Vaccine Alliance. While some RSV burden data are available, additional information is needed to inform evidence-based decisions – particularly mortality, morbidity, and cost of illness data from LMICs. In addition, methods to increase the accuracy of infant mortality data in countries where an appreciable number of home deaths result in underreporting would facilitate case building for RSV vaccines.

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