A History of Respiratory Syncytial Viral Vaccine Development… What is next?

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Abstract

Although the disease burden of Respiratory Syncytial Virus (RSV) is significant, no human vaccine exists. Historical clinical trial complications in the 1960s and the lack of understanding concerning RSV viral correlates of protection (CoP) and viable in vitro and in vivo models have delayed RSV vaccine development. The World Health Organization (WHO), PATH, the Bill & Melinda Gates Foundation, and the Respiratory Syncytial Virus Network have been dedicated to RSV research efforts. The new vaccine and monoclonal antibody (mAb) candidates, built upon emerging knowledge, show potential for human vaccine viability by 2025 according to the WHO's Product Development for Vaccines Advisory Committee (Mazur et al., 2018; Shi et al., 2017). This review will focus on challenging aspects of RSV vaccine development and aim to explore current vaccine approaches in clinical development to discuss which show potential for human vaccine viability.

1. Introduction

Human RSV is a negative-sense, enveloped, nonsegmented, single-stranded- RNA virus of the Paramyxoviridae family, genus *Pneumovirus* (Higgins, Trujillo, & Keech, 2016; Piedimonte & Perez, 2014). A major cause of respiratory disease in pediatric and elderly populations, the first episode of RSV infection commonly occurs early during the first two years of life and presents as an acute lower respiratory infection (ALRI) with congestion, cough, and possible upper respiratory nasal irritation (Mazur et al., 2018; Piedimonte & Perez, 2014; Piedimonte, 2015). In high-risk RSV populations such as: young infants (0-6 months), older infants and young children (2 months and older), and older adults (65 years or older), severe lower respiratory infection (SLRI) can develop due to defects in the populations' immune systems triggering bronchiolitis, pneumonia, and episodes of apnea (Mazur et al., 2018; Mohapatra & Lockey, 2008; Piedimonte, 2015). RSV can also have long-term adverse effects on pediatric patients like impairment of learning and memory storage and potentiation of asthma throughout childhood following wheezing caused by RSV (Espinoza et al., 2013; Rezaee, Linfield, Harford & Piedimonte, 2017).

Unfortunately, many complications exist with developing viable vaccine candidates. The pediatric assumed naïve and elderly weakened immune statuses complicate the understanding of which immune responses are necessary for complete RSV immunity. RSV naturally generates incomplete immunity, so subsequent infections are currently probable, despite the presence of significant antibody titers to immunogenic proteins (Piedimonte & Perez, 2014). Subsequent infection generally elicits mild upper respiratory infection (MURI) in immunocompetent populations with sneezing, coughing, and rhinorrhea (Higgins et al., 2016). The virus is passed between individuals via physical contact with respiratory secretions from the nasopharyngeal or

conjunctival mucosa of infected individuals. It has been shown that RSV can survive, "on hard surfaces for up to six hours, on rubber gloves for 90 minutes, and on skin for 20 minutes," (Piedimonte & Perez, 2014). Air sampling data has shown that RSV is rarely detectable, and transmission via aerosolization is uncommon (Piedimonte & Perez, 2014).

1.1 Disease Burden

Globally it was estimated in 2015 that RSV was responsible for 28% of all ALRI episodes and 13-22% of all ALRI mortalities in children less than five years old. These estimates, as shown in Figure 1., equate to an estimated 33·1 million episodes of RSV-ALRI and 118,200-149,400 RSV-ALRI deaths in young children in 2015. Thirty million of these RSV-ALRI cases occurred in children in low-income and middle-income countries; however, these estimates may not reflect the complete RSV burden due to under-reporting of cases and deaths that may have occurred due to inadequate healthcare access, diagnostic capacity, and biologic handicaps (Shi et al., 2017; Geoghegan et al., 2017; Higgins et al., 2016). Annually, in the United States, RSV is responsible for an estimated 125,000 pediatric hospitalizations and 250 infant deaths (Piedimonte & Perez, 2014). While RSV has a substantial effect on children, elderly populations also suffer as it is estimated that annually in the United States 11,000 elderly individuals die as a result of illnesses related to RSV infection. Elderly adults most at risk include those who are community-dwelling, in long-term care, or who frequent adult day care settings. Other high-risk adult groups include immunocompromised patients and those with cardiopulmonary disease (Branche & Falsey, 2015; Falsey, Hennessey, Formica, Cox, & Walsh, 2005).

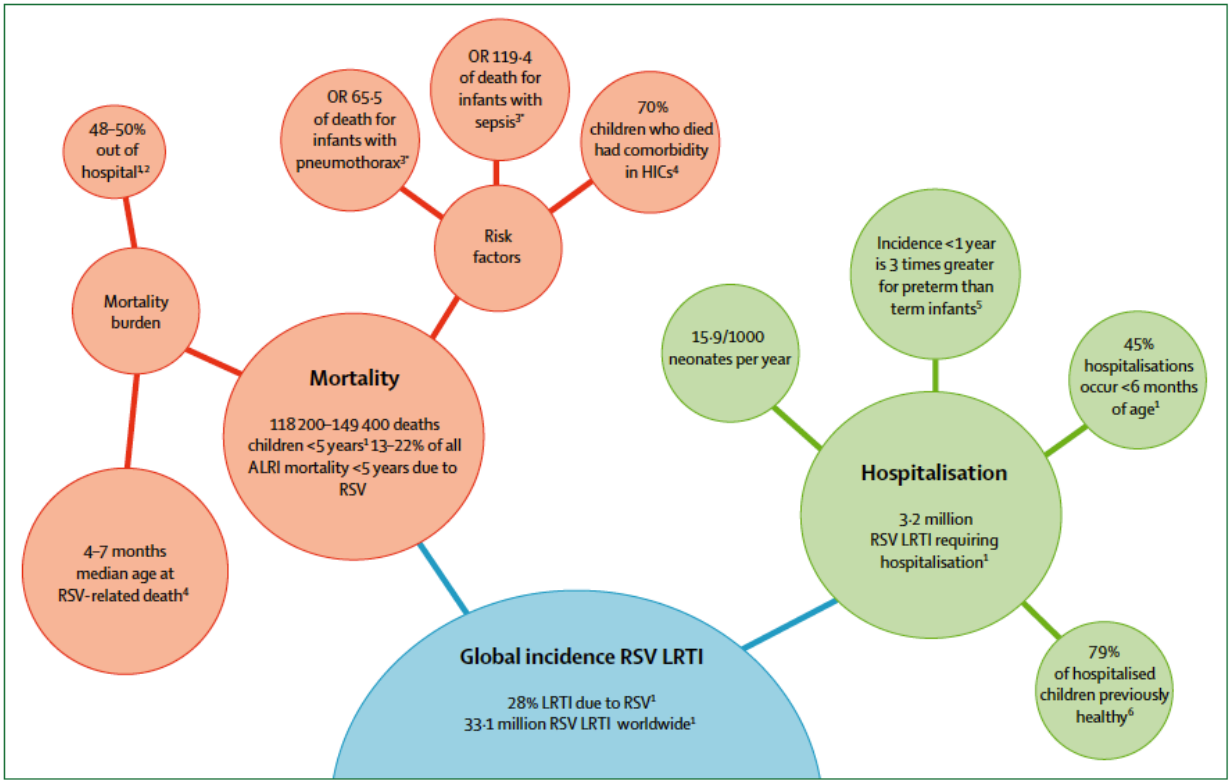

Figure 1: **Global burden of RSV in children under 5 years of age[1-6]**
Incidence is shown worldwide for children less than 5 years of age unless otherwise stated. The hospital admission rate of 15·9 hospital admissions per 1000 neonates per year is in developing countries. The RSV ALRI hospital admission rate of 15·9 among neonates is reported per 1000 individuals per year in developing countries. OR=odds ratio. LRTI=lower respiratory tract infection. RSV=respiratory syncytial virus. HIC=high-income country. *Compared with children who survived RSV hospitalisation and were mechanically ventilated. (Mazur et al., 2018)

1.2 RSV Treatment

Currently, there are no treatment options to cure RSV. Most RSV care is palliative, and focuses on opening distressed airways with supplemental oxygen and mechanical ventilation if necessary (Higgins et al., 2016). Proper hydration is paramount, so intravenous fluids have been utilized in hospital settings (Branche & Falsey, 2015). Hypertonic saline has also been used for hospitalized patients to improve mucociliary clearance (Piedimonte & Perez, 2014). Pharmacological agents like bronchodilators or corticosteroids have been used to relieve RSV symptoms, but efficacy has not been proven during randomized controlled trials (Higgins et al., 2016). In elderly populations with chronic lung disease inhaled and systemic corticosteroids are prescribed to relieve acute exacerbation associated with wheezing and bronchospasm (Branche & Falsey, 2015). This practice has not been supported for infants during the first year of life because of safety concerns regarding corticosteroids' effects on rapid lung growth during this developmental period (Piedimonte & Perez, 2014).

Ribavirin, a synthetic nucleoside analog, has broad in vitro activity against many RNA and DNA viruses (Piedimonte & Perez, 2014). Approved for severe RSV infection therapy, it only provides a modest short-term improvement of respiratory infections. Once supported for routine use in 1993 by the American Academy of Pediatrics Committee on Infectious Diseases, the committee changed its recommendation for the use of Ribavirin to treat RSV in 1996 to "may be considered" (Rezaee et al., 2017). In immunocompetent patients, RSV infection is asymptomatic for the first three to five days post-infection. During those first days, the virus, "reproduces exponentially and reaches the lungs," where it begins causing respiratory distress symptoms after five to seven days (Piedimonte, 2015). Administering Ribavirin at this point post-infection does not have a large effect on the already disappearing RSV viral load, and multiple randomized trials were not able to demonstrate any short- or long-term benefits (Piedimonte & Perez, 2014). Ribavirin's use has since been restricted to patients with T cell immunodeficiency due to limited host defenses, prolonged hospitalization due to aerosol administration, risks for potential toxicity, and high cost (Rezaee et al., 2017). One passive immunity neutralizing mAb, Palivizumab, is available for RSV prevention in high-risk infants aged less than two years but lacks data for treatment in children or adults (Branche & Falsey, 2015). This study gap may be due to the tremendous financial burden multiple dosing studies would assume since Palivizumab can cost $8000 or more per dose (Higgins et al., 2016; Branche & Falsey, 2015).

2. RSV Vaccine Development Difficulties to Overcome

2.1 Historical Caution

One of the most detrimental factors that has inhibited Human RSV vaccine development is the catastrophic vaccine failure of a formalin-inactivated (FI) RSV vaccine from 1966. During one study, 80% of infants given the vaccine developed severe bronchiolitis or pneumonia after wild-type infection, compared to only 5% for the placebo group. Ultimately two of the vaccinated infants died (Kim et al., 1969). This disease response following vaccination caused alarm that other non-replicating RSV vaccines may cause RSV-naïve children to experience high titers of non-neutralizing antibodies and Th2, MHC II cellular processing that can lead to immune complex deposition, complement activation, and allergic inflammation. This harmful immune response became known as enhanced respiratory disease (ERD), and the specific etiology remains unknown (Higgins et al., 2016; Rezaee et al., 2017; Acosta, Caballero, & Polack, 2015). Current vaccine candidates must avoid inducing ERD to progress through development.

2.2 Protein Structure & Pathogenicity

Another difficulty in developing a Human RSV vaccine has been establishing an understanding of the pathogenicity of different RSV proteins and their abilities to elicit an immune response in order to establish CoP. Learning which protein structures elicit the strongest immunological memory will help inform future vaccine development. A and B strains exist and each consist of 10 genes that encode 11 proteins as shown in Figure 2. The strains circulate alternatively every 1-2 years, and it is thought that a more severe disease pattern may be characteristic of RSV strain A (Dudas & Karron, 1998; Lee & Chang, 2017). Two of the most important proteins that stimulate neutralizing antibodies are the F (fusion) and G (attachment) transmembrane surface proteins. G protein's amino acid sequence is not highly conserved, and this variation is what causes division between strains A and B. G protein only shares 53% similarity between strains (Lee & Chang, 2017). Little is also known about the surface structure of G protein, but based on its location within a host, G protein can change structure from, "an oligomer on the surface of RSV particles," to a, "monomer when secreted from infected cells in soluble form" (Mazur et al., 2018). The G protein monomer soluble form, "can act as a decoy that helps the virus to evade the host antibody response" (Mazur et al., 2018; Escribano-Romero et al., 2004; Bukreyev et al., 2008). Although G protein induces a robust neutralizing antibody response, it is not commonly targeted for vaccine development due to antigenic and genetic variability (Mazur et al., 2018; Lee & Chang, 2017).

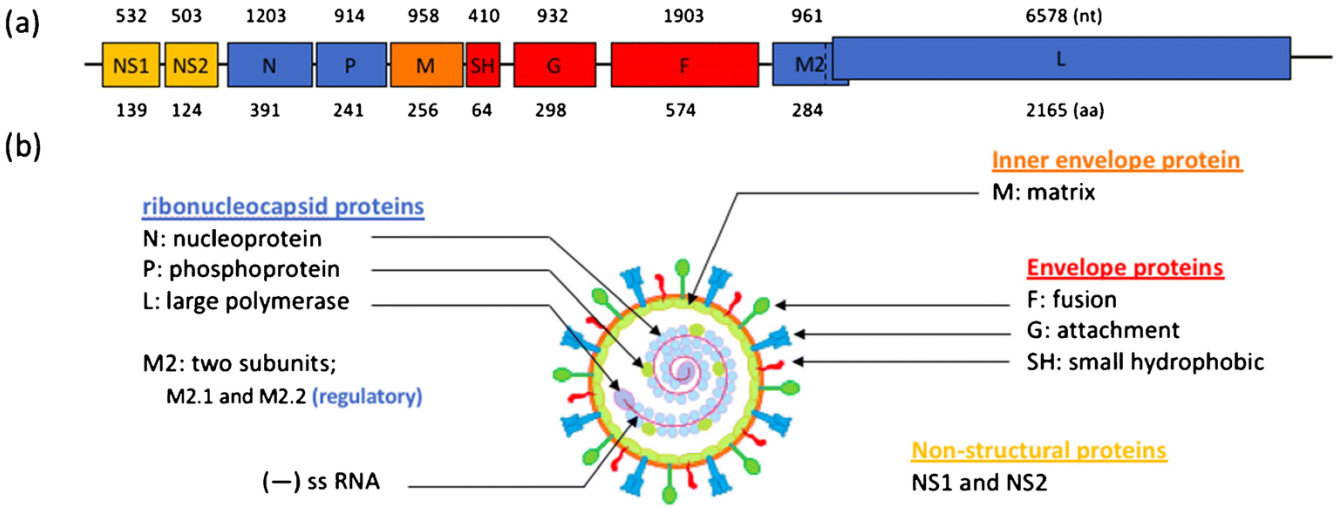

Figure 2. RSV genome and proteins. a Map of negative-sense RNA genome where nt and aa indicate nucleotides and amino acid lengths, respectively. b Mapping of 11 proteins on RSV virion and their corresponding classes (Taleb, Asmaa, Khalid, & Hadi, 2018).

The F protein is more commonly targeted than G protein during vaccine development due to its high conservation (90%) between A and B strains (Taleb et al., 2018). It is also responsible for the syncytia for which RSV is named. Upon fusion of the host and vial plasma membranes, the F protein causes the aggregation of multinucleated cells allowing for transmission of the virus between host cells (Piedimonte & Perez, 2014). F protein exists in two variant conformations, Pre- and Post- F. Post-F had been isolated for many years, and in 2013 Pre-F was discovered in a metastable state. Under natural conditions, its viability lasts for a fraction of a second before turning into stable Post-F (Higgins et al., 2016; Taleb et al., 2018). The main differences between

the F protein variants are the surface antigenic sites located on the protein. Both conformations share sites I, II, III, and IV, but Pre-F also presents sites Ø and V. Ø and V sites have shown high neutralizing activity, and Ø has shown in pre-clinical studies to, "elicit antibodies more potent than palivizumab" (Higgins et al., 2016; Mazur et al., 2018). Since the most neutralization-sensitive sites are exclusive to the Pre-F conformation, many new vaccine candidates have targeted Pre-F. Shared sites II, III, and IV have also shown medium to high neutralization potential, but site III stimulates a stronger antibody response when isolated on the Pre-F conformation (Graham, 2017). Historically site II has been an important antigenic target due to its conservation between RSV strains A and B (Rezaee et al., 2017).

Most of the RSV proteins have been explored as possible antigenic targets, but few apart from F and G are being utilized currently in vaccine development. The third transmembrane surface protein, SH, is small and hydrophobic with transmembrane and extracellular domains thought to possibly aid in fusion (Mohapatra & Lockey, 2008; Mazur et al., 2018; Rezaee et al., 2017). The extracellular domain has been shown to inhibit cell apoptosis via inhibition of the TNF-α pathway, and this action may prove essential for induction of antibody dependent cell-mediated cytotoxicity. Other non-membrane RSV proteins such as N, NS2, M2-1, M2-2, and M may be essential for vaccine development to induce potent T-cell responses. The M and N proteins are structural in their functions. M protein is associated with the viral membrane and gives virions their filamentous shape. N protein is responsible for encapsidation of the RNA genome. The other possible antigenic proteins are nonstructural and focus on viral function and survival. NS2 inhibits the host interferon response and promotes epithelial cell shedding. M2-1 and M2-2 are specific to pneumoviridae viruses and are vital for viral transcription (Rezaee et al., 2017; Mazur et al., 2018).

2.3 RSV Modeling

Although much is being discovered concerning RSV protein pathogenicity, in vitro and in vivo models pose another difficulty in RSV vaccine development. In order to test preclinical vaccine candidates, models must be developed and utilized to assess the potential for ERD and vaccine immunity (Mazur et al., 2018). In vitro, RSV replicates in a wide range of cell lines from various tissues and hosts. The HEp-2 cell line is most commonly used to grow RSV. The purity of this cell line is unclear, so replication in this line may be less suitable for the production of virus used for vaccine applications. The cancerous nature and non-respiratory origin of several cell lines are limitations for RSV replication. Isolated human airway epithelial cells grown at an air-liquid interface have been used to adapt clinical RSV isolates to cell culture. The product cells contain, "pseudostratified, mucociliary airway epithelium that displays similar morphologic and phenotypic characteristics of the in vivo human cartilaginous airway epithelium" (Heylen, Neyts, & Jochmans, 2017).

In vivo animal models present an additional challenge. Viruses like bovine RSV and ovine RSV have been identified in their respective species to model disease pathology and immune responses that are similar to human RSV infections in children (Heylen et al., 2017). Researchers have been able to utilize these models to understand the pathology and mechanisms of immunity towards pneumovirus infections. This has helped serve as a way to evaluate human RSV vaccine concepts during pre-clinical development (Taylor, 2017). Although beneficial, these models are genetically different from human RSV. Neonatal lambs have been challenged with Human RSV

and have shown successful disease replication. The similarity of size and organization of airway and lymphoid tissue make this model attractive, but lack of antibodies and genetic sequencing tools along with the complexity of population maintenance, are pitfalls of human RSV modeling in sheep (Bem, Domachowske, & Rosenberg, 2011).

Other non-human primates and small animal mammalian models have also been explored. Chimpanzees are currently the only non-human primate model that is permissive to human RSV replication and infection. ARLI and SRLI have not been induced in this model, but MURI has been monitored in chimpanzees. The genetic similarity and size of chimpanzees make then attractive models, but the ethical burden and economic resources required for the logistical maintenance of small chimpanzee populations inhibits this model's use (Bem et al., 2011; Taylor, 2017). Other non-human primates like African green monkeys, three species of macaques (rhesus, cynomolgus, and bonnet monkeys), owl monkeys, Cebus monkeys, and baboons have been explored as models for human RSV with varying benefits and limitations. Unlike chimpanzees these non-human primate species are semi-permissive to human RSV replication, so their viral replication responses were comparatively moderate to low to inoculum levels. Often clinical signs of disease did not develop or were limited to MURI symptoms. Pathology studies were not done on all species models, but those reported showed signs of broncho-interstitial pneumonia, alveolitis, and syncytium of cells. Vaccine-enhanced pathology has been studied in African green monkeys and macaque species, but limited vaccine-enhanced pathology has been explored in the other non-human primate species discussed (Taylor, 2017).

Small animal mammalian models mostly consist of rodent species including mice, rats, and to lesser degrees ferrets, guinea pigs, Syrian hamsters, and chinchillas (Taylor, 2017). The BALB/c mouse has been the most common animal model for experimental human RSV disease (Bem et al., 2011). This mouse model is semi-permissive and shows intermediate susceptibility to human RSV infection. A high, $>10^6$ plaque-forming units (PFU), dose of human RSV is required to produce clinical signs of disease. Unlike clinical signs in humans that focus on ARLI, SRLI, or MURI, BALB/c mouse disease induced by human RSV is measured as weight loss, ruffled fur, and hunched posture. Pathological sectioning of airways must be done to measure the pulmonary manifestation of human RSV with studies showing mild to moderate bronchiolitis has been induced (Taylor, 2017). The convenience provided by the vast amount of genetic knowledge and mouse-specific reagents and molecular tools makes this model attractive. However, the innate and adaptive immune response stimulated in BALB/c mice differs significantly from humans and does not provide sufficient antigenic modeling of human disease. The anatomy and size of mice lungs also differ significantly due to fewer bronchioles and less complex airway branching and complicates disease monitoring when compared to humans (Bem et al., 2011).

An alternate rodent model, the cotton rat, has proven to be a superior model. Although semi-permissive, this rat model requires a 10^4 PFU dose of human RSV to induce ALRI while peak replication levels are nearly 100 fold higher than in mouse models (Heylen et al., 2017). Both URI and LRI have been monitored in cotton rat models, and clearance of the virus follows a similar timeline as in humans with clearance happening by day seven post-infection. Pathologic sectioning of the airways has shown mild to proliferative bronchiolitis, sloughed epithelial cells, and patchy atelectasis (Taylor, 2017; Bem et al., 2011). The cotton rat model has become widely used to evaluate the efficacy of vaccines, antivirals, and neutralizing antibodies like palivizumab

(Bem et al., 2011). It has also been used to model alveolitis after FI human RSV vaccination, as a model for ERD. Although these uses are productive, the extrapolation of vaccine results from this model to show safety in higher mammals should be done with discretion as complete protection stimulated against human RSV in the cotton rat model has failed to show the same efficacy in African green monkeys (Taylor, 2017). Cotton rat care also adds an added difficulty due to their fragile and easily agitated nature. Specialized training is required for their care and handling, but specific immunological reagents are being developed to advance the usage of this model (Bem et al., 2011).

2.4 Correlates of Protection

Due to the heterogeneity of the RSV protein landscape and models utilized for vaccine development, the CoP against RSV infection and disease have been difficult to determine. As researchers develop new vaccine approaches, the endpoints of their studies vary based on multiple regulatory points. The sizeable historical use of mAbs to protect high-risk patients via passive immunity has established a plethora of data concerning correlations between neutralizing antibodies and disease prevention and could act as a cornerstone for establishing RSV correlates of prevention from disease. This type of passive immunity has been analyzed in maternal sera, cord blood, infant sera, and by passive antibody transfer studies (Kulkarni, Hurwitz, Simões, & Piedra, 2018; Jorquera, Oakley, & Tripp, 2013).

Due to the nature of humoral immunity, there may be many inhibitory mechanisms responsible for antigenic neutralization of RSV (Kulkarni et al., 2018). RSV-specific nasal IgA, a component of mucosal antigenic memory, may be useful for establishing CoP for infection. One study has shown that IgA more strongly correlates with protection compared to measurements of serum neutralizing antibody in adults (Habibi et al., 2015). This highlights the importance mucosal immunity may play in RSV protection. The Habibi et al. (2015) study also showed rapidly waning IgA levels caused individuals to be susceptible to RSV reinfection within months. Upon reinfection, IgA memory B cells were not significantly mobilized and suggested a hardship vaccine development will need to overcome through dosage and administration strategies or stimulation of enhanced immunologic memory.

Antibodies targeted towards specific RSV proteins, as previously discussed, may offer systemic immunity. Neutralizing assays will need to be developed in response to the specificity of vaccine antibody type, but no standardization or threshold exists despite the frequent use of neutralizing activity of serum as an endpoint for vaccine trials. An ELISA to neutralization response ratio representing the, “ratio of times-increase in RSV-binding antibodies to times-increase in RSV-neutralizing antibodies,” of less than one may be a significant correlate of protection (Mazur et al., 2018). Other definitive CoP may be vaccine-type specific. Times-rise in antibody titer could be an indicator of B-cell priming, relevant for live-attenuated vaccines (Mazur et al., 2018). This vaccine type is targeted towards the naïve pediatric immune system because it generates replication of high amounts of antigenic non-virulent material that stimulates a natural host immune system response.

Standardization of neutralizing assays is a considerable feat, and a recent PATH, WHO, and the National Institute for Biological Standards and Control (NIBSC) exercise examined 12 different neutralizing assays in order to establish standardized neutralizing antibody titers (Kulkarni et al.,

2018). This regulatory effort led to a new RSV International Standard Antiserum with 1000 IU of RSV subtype A neutralizing activity per vial available through NIBSC (Mazur et al., 2018). Further standardization of other immunological assays will need to be developed in the future.

Besides humoral immunity, T cell-mediated immune responses could act as a CoP. In cases of LRI, CD8 T cells are essential for viral clearance, and as discussed with the FI vaccine candidate from 1966, Th2, MHC II- biased immune responses are indicative of ERD across human and animal models of disease (Graham, 2017; Mazur et al., 2018). Kulkarni et al. (2018) suggest that, "neutralizing antibodies will likely serve as a CoP in infants and young children but in older adults, a CoP associated with virus clearance might be a better target." Despite this recommendation, measurements of Th1 and Th2 responses have been used as safety measures for most RSV vaccine platforms due to their relationship with ERD in children. High levels of Th2 are indicative that ERD may be induced, and high levels of Th1 are indicative of an appropriate immune response in vulnerable populations. These indications only further support that establishing CoP will vary depending on vaccine study, target age, and host immune factors (Mazur et al., 2018; Kulkarni et al., 2018).

3. RSV Vaccine and mAb Candidates

3.1 Historical Perspective

Many variables affect vaccine development as discussed previously. New knowledge and vaccine failures help further RSV vaccine development, and PATH creates a monthly RSV Vaccine and mAb Snapshot to organize current RSV vaccine efforts. For four decades only live-attenuated vaccines were tested for active infant immunization due to fear of ERD, but due to the overwhelming necessity for an RSV vaccine globally, RSV vaccine development has increased, and other vaccine types have been explored (Higgins et al., 2016). In December 2015, 60 vaccine and mAb candidates were in varying stages of development and represented six broad vaccine platforms and one immune-prophylaxis platform. In October 2018, 45 vaccine and mAb candidates were in varying stages of development, and this change represents failures and advancements through different stages of clinical development. As shown in Table 1., 31 of the vaccine and mAb candidates are shared between December 2015 and October 2018 PATH Snapshots. In the following section, examples representing the various vaccine platforms will be discussed.

Vaccines are targeted for use in three populations: pediatric, elderly, and maternal. Pediatric and elderly populations are targeted due to their severe and immense disease burden. Pregnant women have been targeted as potential RSV vaccine recipients due to the passive immunity they afford their children during utero and possible breastfeeding after birth (Blanco et al., 2017). Development of vaccines requires multiple resources, and this continuation of candidate development shows the potential vaccine candidates must have before entering clinical development and trials.

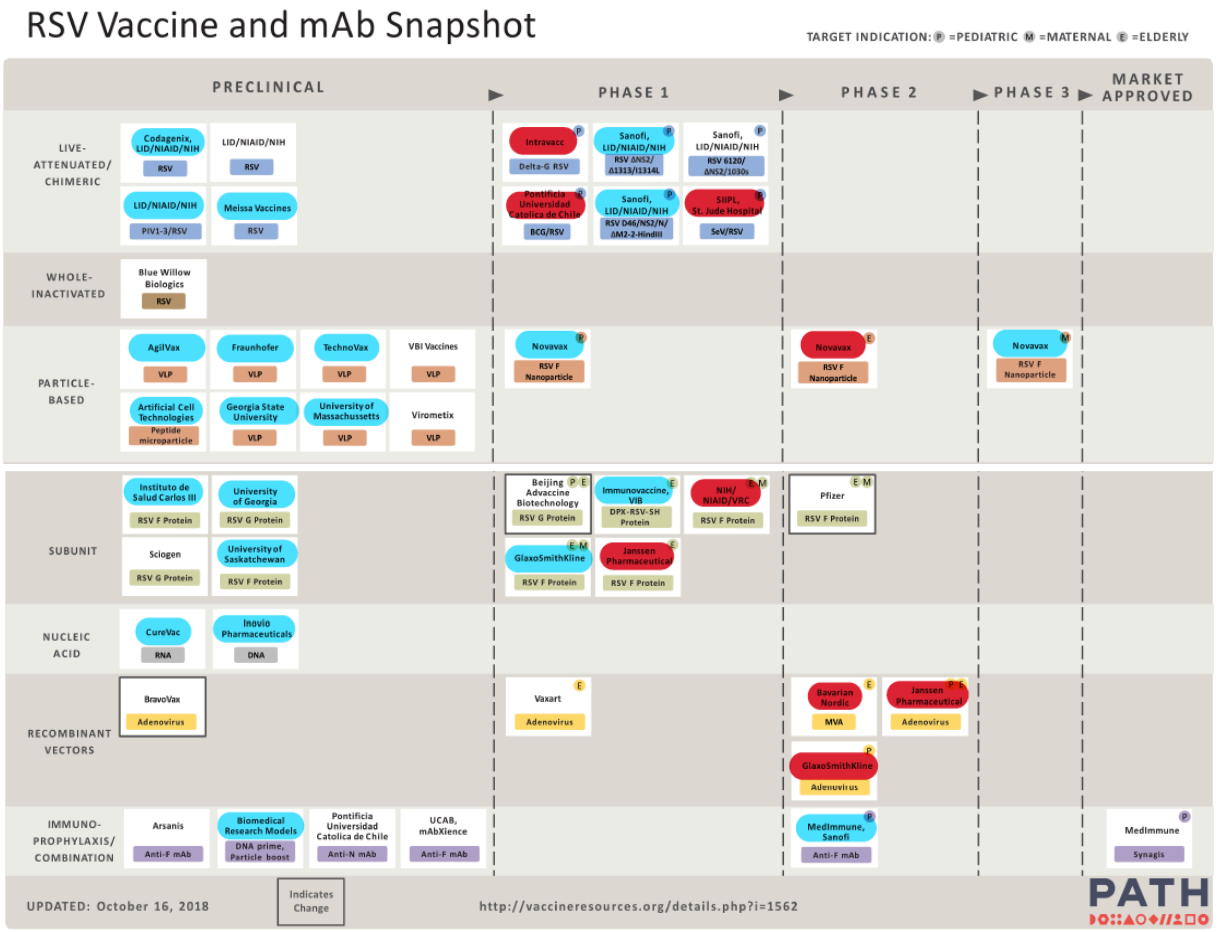

Table 1. PATH RSV Vaccine and mAb Snapshot October 2018. (PATH, 2018)

Highlighting represents shared candidates between the December 2015 PATH Snapshot and updated version (PATH, 2015). Blue highlighting represents vaccines that remain in the same phase of development between snapshots, and red shows movement through clinical development.

3.2 Live Attenuated/ Chimeric Vaccine Candidates

Current live-attenuated vaccines have focused on genetically altering RSV genes to achieve attenuation. Traditional vaccine attenuation techniques like the passage in sub-optimal replication cell types or temperatures have historically failed RSV development (Karron et al., 2015). The balance of achieving high levels of attenuation while preserving immunogenicity is the inherent challenge for this vaccine type, and through the advancements in RSV protein knowledge, researchers have been able to utilize modern reverse genetic systems to target and alter RSV proteins to achieve attenuation (Collins & Melero, 2011). This approach allows researchers more attenuation stability than relying on mutagen strains. This approach is primarily targeted towards infant populations due to their assumed RSV-naïve status. Live-attenuated vaccines generally elicit humoral and cell-mediated immune responses, and a study of seven live-attenuated vaccine candidates in seronegative children showed safe profiles for children as young as one month old (Wright et al., 2007). Currently, four live-attenuated vaccines are in phase one of clinical trials. Popular alterations include deletion or substitution of the M2-2, NS2, or G proteins.

Two chimeric vaccines are in clinical trials and focus on delivering RSV protein genetic material as antigens. The Sendai Virus (SeV) is a modified mouse parainfluenza virus similar to human parainfluenza virus type 1 (Jones et al., 2014). The SIIPL, St. Jude Hospital SeV/RSV candidate currently in phase one utilizes the SeV virus to deliver RSV F protein genetic material to the vaccine recipient. In pre-clinical pediatric cotton rat model studies, this vaccine candidate was

able to elicit immune responses that were protective in the presence of simulated maternal antibody titers (Jones et al., 2014). The current phase one clinical trial (NCT03473002) will enroll up to 25 healthy participants to test the vaccine's safety, prolonged detectability, and immune response via ELISA (NIAID, 2018).

Another chimeric vaccine approach, Pontificia Universidad Catolica de Chile BCG/RSV, utilizes the tuberculosis BCG vaccine as a vector for the RSV N protein gene (Rey-Jurado, Soto, Gálvez, Kalgeris, 2017). Utilization of the BCG vaccine, already approved for newborns, to additionally induce RSV immunity, would provide newborns dual immunity to both respiratory diseases. The BCG vaccine has been shown to elicit a Th1 immune response, and pre-clinical data suggests the BCG/RSV vaccine promotes a protective T cell immune response via the recruitment of Th1 cells (Bueno et al., 2008; Cautivo et al., 2010). The phase one clinical trial (NCT03213405) is currently recruiting 24 males, aged 18-50 years old, to test three dosages of the BCG/RSV vaccine candidate against a conventional BCG dose (Pontificia Universidad Catolica de Chile, 2017).

3.3 Subunit Vaccine Candidates

Another broad vaccine platform being explored is the subunit vaccine type. This vaccine type is targeted towards elderly and maternal populations. Due to previous episodes of ERD elicited in animal models following subunit vaccination, this vaccine platform is unsuitable for infant populations (Murphy et al., 1990; Connors et al., 1992). Subunit vaccines utilize specific purified viral proteins often paired with an adjuvant to elicit immunity. There are currently five vaccine candidates in phase one of clinical trials and one candidate in phase two. Four of the candidates utilize the F protein as their antigenic material.

One recent late phase vaccine failure in 2016, MEDI-7510 (NCT02508194), a subunit vaccine candidate utilizing a Post-F conformation as its antigenic material, was thought to have failed because the antibodies generated in response lacked appropriate epitope specificity when induced by Post-F (Langley, 2017; Mazur et al., 2018). The majority of current vaccine candidates utilizing F are specified as the Pre-F conformation. The study was also thought to have failed due to the study population, 1900 adults aged 60 or older at varying RSV risk levels, not having a high enough incidence of laboratory-confirmed RSV infection to confirm or deny study endpoints (Mazur et al., 2018).

The phase two vaccine candidate, Pfizer RSV F Protein (NCT03572062), was recently suspended on October 10, 2018, during the enrollment period of nearly 474 healthy 60-85-year-old participants. An amendment is pending, but the original protocol was set to study the safety, tolerability, and immunogenicity of six vaccine candidate formulations administered alone or alongside seasonal inactivated influenza vaccine (Pfizer, 2018).

A novel subunit approach, Immunovaccine, VIB DPX-RSV-SH Protein (NCT02472548), has completed phase 1 trials. This vaccine candidate is a depot formulation utilizing the SH protein as its antigenic material. Forty healthy study participants aged 50-64 years old sustained measurable antigen-specific antibody levels and showed safe vaccine profiles despite pain complaints (Langley et al., 2018).

3.4 Recombinant Vaccine Candidates

The third major broad vaccine platform, recombinant vaccines, has the highest number of vaccine candidates in phase two of clinical trials. The majority of current RSV recombinant vaccine development has focused on using adenovirus as the attenuated viral vector for specific RSV genes. One of the recombinant adenovirus vaccine candidates, GlaxoSmithKline Adenovirus, includes genes for F, N, and M2-1 proteins as RSV antigenic material. When studied during phase one clinical trial (NCT02491463), 72 healthy adults' (mean age 30 years old) main complaints following vaccination during the first 0-6 days included general pain and fatigue. Blood work collected at time points 1, 3, 7, 30, 31, 33, 60, 180, 360 days post-vaccination did not show significant abnormalities to suggest adverse vaccine reactions (GlaxoSmithKline, 2015). This study has since progressed to phase two (NCT02927873) where safety, reactogenicity, and immunogenicity will be monitored in infants aged 12-23 months (GlaxoSmithKline, 2016).

Another recombinant adenovirus RSV vaccine candidate, Janssen Pharmaceutical Adenovirus, is currently being tested during phase two clinical trials (NCT03339713) for efficacy in pediatric and elderly populations. This vaccine uses the gene for the Pre-F conformation as antigenic material. The phase two clinical trial focused on elderly populations (>60-year-old) recently finished and aimed to discern if administration of the vaccine candidate paired with seasonal inactivated influenza vaccine was advantageous compared to seasonal influenza vaccination alone (Janssen Vaccines & Prevention B.V.a, 2017). Study results have yet to be released for the elderly study, but the phase two clinical trial (NCT03303625) for the pediatric targeted version of the Janssen Pharmaceutical Adenovirus vaccine candidate is currently vaccinating 60 participants in two age ranges: healthy adults 18-50 years old and RSV-seropositive toddlers 12-24 months old (Janssen Vaccines & Prevention B.V.b, 2017).

The other recombinant RSV vaccine candidate, Bavarian Nordic MVA, uses modified vaccine Ankara (MVA) a live-attenuated poxvirus derivative as its recombinant vector. This RSV recombinant vaccine candidate utilizes genes for F, G (both RSV subtypes), N, and M2 proteins as its antigenic material (Mazur et al., 2018). Phase two interim (NCT02873286) results show the vaccine candidate is well tolerated and induces humoral and T cell responses in older adults after a single vaccination. Planning has begun for the design of a phase three study (Bavarian Nordic, 2018).

3.5 Particle-Based Vaccine Candidates

The next relevant vaccine platform is particle-based vaccines. Novavax is a leader in this vaccine platform and utilizes a Sf9/insect baculovirus nanoparticle system to deliver antigenic particles of various diseases (Smith et al., 2012). The Novavax RSV F particle vaccine candidates, derived from infecting the ovary cells of fall armyworm insects with baculovirus engineered to generate recombinant Post-F protein, utilize their vector system to present Post-F protein conformation as an antigenic multimeric micelle similar in size to wild-type RSV within a host (Novavax, n.d.).

This approach has been used to target maternal, elderly, and pediatric populations. The maternal formulation is in a phase three clinical trial (NCT02624947), and the pediatric formulation is in phase one (NCT02296463). The elderly formulation had advanced to a phase three clinical trial (NCT02608502) and was found to be safe, but the vaccine failed to meet efficacious study

endpoints defined as percentages of subjects that developed moderate-severe RSV-LRI and RSV-acute respiratory disease during the 2015-2016 RSV season following administration of placebo and vaccine candidate treatments (Novavax, 2015; Novavax, 2016). Novavax claimed that the failure was due to a low attack rate of RSV during the season they monitored and failure was not due to data acquisition or the drug product itself (Novavax, 2016). According to Mazur et al., "Another proposed explanation for failure of this vaccine candidate is that the quantity of the immune response to vaccination might not represent effective immunity." Mazur et al. also noted that this failure suggests that late-phase clinical research should be conducted over multiple RSV seasons to prove efficacy. The trial has since been rolled over to phase two clinical trials (NCT03026348) where previously enrolled phase three participants were re-blinded and randomized between vaccine and placebo groups for a second round of vaccine administration (Novavax, 2016).

3.7 mAb Candidates

The other broad type highlighted on the PATH Snapshot is the immune-prophylaxis/combination platform focused on developing mAb for passive immunity. Palivizumab is approved in this category, but another mAb candidate, Medimmune, Sanofi Anti-F mAb, is currently in phase two of clinical trials and has shown immense promise. This mAb targets the Ø site on the Pre-F protein conformation of RSV, which has shown to be more neutralizing than the site II Post-F protein palivizumab targets (Robbie et al., 2013). The formulation of this mAb extends its half-life three-fold, and one administration would remain viable throughout a complete RSV season. Palivizumab has provided passive immunity for many years, but the mAb's financial burden has limited its use and relief. Pharmaceutical companies MedImmune and Sanofi have joined during the development of this prophylaxis tool and indicated that pricing would emulate vaccine markets (Mazur et al., 2018). If attained, this could provide reliable passive immunity for many who struggled before with RSV.

4. Conclusion

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

Although there is a significant amount of information that evades developers today, emerging knowledge and innovation will improve current points of development and allow researchers to maximize the effectiveness of their vaccine candidates. Moving forward, it appears based on the understanding of protein structure and pathogenicity that the RSV vaccine development field is moving towards targeting the F surface protein opposed to the G surface protein. While G protein may elicit a strong antibody response, this change is supported by superior F protein

conservation between RSV strains. Specificity for the F protein has also emerged with the Pre-F conformation being targeted opposed to the widely used Post-F conformation. This shift should elicit new information and has been supported through the success of recent Pre-F conformation targeted candidates in the vaccine PATH Snapshot. Although specificity for RSV proteins has recently developed, the population scope of vaccine candidates is still targeted towards the most vulnerable populations— pediatric and elderly. Due to the complications associated with the pediatric naïve or elderly weakened immune systems, these populations need vaccines targeted towards eliciting immune responses appropriate for their population's needs. Defining CoP in the future will help standardize the immune responses necessary for generating complete immunity in these populations and may range between antibody neutralizing effects and T cell recruitment. After a vaccine is developed and CoP established, other delivery modes should be compared across all vaccine platforms and populations. Administration time point regimens and dosages will also need to be established across all populations in response to the difficulty generated by waning RSV immunity after infection. Currently, these futuristic points of exploration remain second to developing a viable vaccine. The history of RSV vaccine development is vast; however, due to current advancements, a viable human RSV vaccine appears to be on the horizon.

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