Respiratory Syncytial Virus

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ABSTRACT

Respiratory syncytial virus (RSV) is one of most common cause of lower respiratory tract infection in infants less than 2 years of age and sometimes in elderly people. Bronchiolitis is the common presentation. Severe infection is seen in high-risk infants and preterm infants. More than 90% of mortality due to RSV infection occurs in developing countries. At present, no specific drug is available for treatment. Prevention of RSV infection can be done by giving palivizumab, which is a monoclonal antibody developed against RSV fusion protein. Phase 3 clinical trials are done with nanoparticle RSV protein F vaccine for pregnant mothers to protect the infant through transplacental transfer of RSV antibodies. Clinical trials are going on for live attenuated RSV vaccine, vector-based vaccines, chimeric vaccine, and subunit vaccine.

Keywords: Monoclonal antibody palivizumab, Respiratory infection, Respiratory syncytial virus, Respiratory syncytial virus protein F.

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BURDEN OF RESPIRATORY SYNCYTIAL VIRUS DISEASE

Respiratory syncytial virus (RSV) is the most important cause of viral ALRI in infants and in children less than 5 years of age all over the world. It can affect all age groups including those above 65 years of age. The incidence is high in infants aged less than 3 months of age and mortality is also highest among this age group. Most of the children will be affected with RSV before they reach 2 years of age. Natural infection will provide limited protection against reinfection. Disease transmission mainly occurs in winter in temperate areas but in tropical countries it may occur round the year. 33.1 million new episodes of RSV infections occur and RSV infection leads to about 3.2 million children admitted into hospitals and 118,200 deaths in under 5 years of age children, about 30 million RSV-ALRI episodes occurred LMIC. The burden of RSV is more than 10 times in low- and middle-income countries as compared to high-income countries (HICs).¹ In 2017, the WHO estimated that 23% of 2.6 million annual neonatal deaths were due to vaccine-preventable infectious diseases and RSV-ALRI prevention is a strategic priority in this age group. In India, various hospital based studies revealed that RSV detection varies from 5 to 54% per year. Studies using molecular techniques have shown 15–22% per year. In a study conducted by AIIMS, New Delhi, 17% of hospitalized children with ALRI are positive for RSV by centrifugation enhanced culture and RSV is a major pathogen, about 26%, in children with bronchiolitis.² In community-based studies from Ballabgarh near Delhi, the incidence rates of RSV infection were 234/1,000 child years (Broor et al.) and 420/1,000 child years (Krishnan et al.).^{3,4} Incidence of RSV is three times greater for preterm infants and 45% of hospitalization occur in less than 6 months of age.² About 99% of the global mortality due to RSV infection occur in LMIC and most of children are under 6 months of age.^{1,5}

VIROLOGY

Respiratory syncytial virus is a enveloped negative-sense RNA virus belonging to the order Mononegavirale, family Pneumoviridae, and

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genus *Orthopneumovirus*. The unsegmented RNA is about 15,200 nucleotide long and codes for 11 known proteins: 9 structural proteins and 2 nonstructural proteins. The envelope of the virus contains glycoproteins G, F, and SH. G protein is the cell attachment protein and F is fusion protein, the one that induces syncytia formation. Both G and F glycoproteins can induce neutralizing and nonneutralizing antibodies. Small hydrophobic (SH) proteins, whose function is not well understood. The matrix (M) protein serves as inner envelop protein. Four nucleocapsid-associated proteins, nucleoprotein (N), phosphoprotein (P), Large (L), and M2-1 proteins. M2-2 regulatory protein, encoded by downstream open reading frame of M2-1, is responsible for RNA synthesis. NS 1 and NS 2 nonstructural proteins are suspected to play a role in IFN-release inhibition from infected cell (Fig. 1).⁶

Fig. 1: Structure of respiratory syncytial virus⁶

MOLECULAR EPIDEMIOLOGY OF RESPIRATORY SYNCYTIAL VIRUS IN INDIA

Both group A and B RSV have been reported from India. Different genotypes of group A, GA2, GA5, NA1, ON1, and group B, GB2 SAB4 and BA9 and BA12, are reported.^{7,8}

RISK FACTORS OF RESPIRATORY SYNCYTIAL VIRUS INFECTION

Risk factors for RSV infection are preterm babies, children with chronic lung problems, congenital heart disease, cystic fibrosis, immunocompromised conditions such as HIV and malignancy, Down's syndrome, neuromuscular disease, and infants <6 months of age. These children may have severe RSV infection and at greater risk of death during hospitalization compared with healthier children.⁹ Low birth weight, male siblings, maternal smoking, history of atopy, non-breastfed, and overcrowding can increase the risk of RSV infection.¹⁰

CLINICAL FEATURES

Clinical features of RSV infection may present with mild upper respiratory tract illnesses or otitis media or may have severe respiratory illnesses. Incubation period is 3–5 days. Infections are more common in winter season but RSV transmission can occur all-round the year. Transmission of infection is by fomite and touching the face with contaminated hands.

The first symptom of RSV infection in infants is runny nose. Cough may appear along with rhinorrhea or over a period of 3 days.

Most common symptoms are runny nose, loss of appetite, cough, sneezing, fever, and difficulty in breathing. In very young infant with RSV, the only symptoms may be irritability, decreased activity, and breathing difficulty. Respiratory syncytial virus in infants aged less than 1 year can present with bronchiolitis, pneumonia, and sometimes croup. Severe disease may progress to tachypnea, cyanosis, and apnea. Chest X-ray may show hyperexpanded lungs, peribronchial thickening, and interstitial infiltrates. About 44% of children with influenza-like illness were found to be positive for RSV.^{11,12}

DIAGNOSIS

Respiratory syncytial virus infection can be diagnosed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). It is more sensitive than culture and antigen testing. Antigen testing is highly sensitive in children but not in adults.

Immunofluorescence assay can rapidly detect RSV antigen using a fluorescent-tagged primary or secondary antibody. Direct fluorescent antibody assay has a sensitivity of 94% and specificity of 96.8%. The modified ELISA method targeting RSV F protein can detect RSV within 25 minutes at low cost.

The sample of nasal washes or aspirates can be used for the rapid diagnostic test such as the lateral flow immunoassay (LFIA) method, which is based on immunochromatographic technique.

Multiplex real-time PCR can be done, which can detect multiple respiratory viruses simultaneously.¹³

TREATMENT

Treatment is mostly symptomatic. Hydration of the child should be maintained. Children who are hypoxic should be administered humidified oxygen in a nonthreatening manner if the child is

hospitalized. Nebulization with hypertonic saline, epinephrine, or β agonist is controversial. The Cochrane review on hypertonic saline nebulization concluded that it may modestly reduce the length of stay among the hospitalized infants with bronchiolitis and may reduce the hospitalization for those who are attending outpatient department or emergency department.^{14,15} The evidence is low to moderate. Corticosteroid therapy is not recommended except in older child with asthma. Antibiotics are not necessary. Ribavirin aerosol treatment did not show any use in routine therapy.¹⁶

Currently, two drugs are under clinical trials. ALS-008176 is a prodrug of a cytidine nucleoside analog, which can decrease the viral load and readily clear RSV when compared to placebo in a randomized, double-blind clinical trials in healthy adults but preexisting immune memory was not taken into consideration, which may also clear RSV.¹⁷ Another randomized double-blind phase 1 study assessing both single and multiple increasing dose of the drug in hospitalized infants was completed in February 2018.

Presotovir (GS-5806) is oral bioavailable agent supposed to inhibit conformational changes of protein F of RSV before fusion with host cell and block the entry of virus. It is in the phase 2 trial in adults.¹⁸

GENERAL APPROACHES TO VACCINE DEVELOPMENT

Due to huge burden of disease, the vaccine industry is relentlessly continuing the research to bring new vaccine. Till 2019, there are 13 vaccine candidates in phase 1, 4 candidates in phase 2, and 1 candidate has completed phase 3 trials. The candidates include mAbs and vaccines using different methods such as particle based, live attenuated, and chimeric, subunit, and vector based.

Out of these, RSV F vaccine (ResVax) for maternal immunization and MED18897 RSV mAb are in phase 3 trials.

Live-attenuated RSV vaccines for prevention of RSV infection in children are in process of development since many years. Live attenuated virus vaccine is not associated with vaccine-enhanced RSV disease. They are administered intranasally and stimulate innate, humoral, and cell-mediated immunity and give local and systemic immunity. The vaccine virus replicates even in the presence of maternal neutralizing antibodies derived from mother transplacentally.¹⁹

A novel approach of engineered virus with the M2-2 protein gene deletion mutant virus, which is attenuated and immunogenic, is tried. These M2-2 deletion mutant viruses are capable of producing more protein by transcription without much virus replication.²⁰ There are 12 live attenuated and live vectored vaccine candidates in clinical trials, 7 are in preclinical, and 4 in phase 1 trials.^{21,22}

Naturally attenuated chimeric viruses combining genes from RSV-related viruses such as Sendai, parainfluenza virus, and bovine RSV are also in development.²¹

Protein-based vaccine such as whole cell-inactivated virus, subunit antigens that associate to form aggregate particles, and nonparticle-based subunit antigens have been developed for the prevention of RSV infection in elderly populations from severe disease. These protein-based vaccines are sometimes clubbed with adjuvant. Particles can display viral proteins, peptides, or neutralizing epitopes with increased density to enhance B cell receptor binding. There are 30 vaccine candidates: whole inactivated 1, particle based 15, and subunit based 14 vaccine candidates. All vaccine candidates are in various stages of clinical

trials. Novavax RSV F nanoparticle for maternal and elderly population is under phase 3 trials.²³

Maternal vaccination during pregnancy with particle and protein subunit vaccines with safe adjuvant like alum or without adjuvant is in development. Vaccination of pregnant women can enhance the maternal antibodies against RSV, which are transferred transplacentally to fetus to protect the infant in early months. Clinical trial done by Muñoz et al. has demonstrated safety and immunogenicity of RSV fusion (F) protein nanoparticle vaccine in phase 2 trials.^{21,23} In February 2019, they announced their top-line data on completion of their phase 3 maternal RSV vaccine trial.

Novavax, a U.S.-based biotechnology company, has genetically engineered a novel F-protein antigen resulting in enhanced immunogenicity by exposing these antigenic sites. This RSV vaccine did not show any dose-related adverse events in phase 1 safety trial. Maternal RSV vaccine adjuvanted with aluminum phosphate was evaluated in 720 childbearing-age women with various formulations and followed by first-in-pregnancy study in 50 healthy pregnant women. It was found to be safe and immunogenic with a potential to protect infant early in life through transplacental transfer of maternal antibodies. On the strength of these studies in November 2014, the US FDA has granted Fast Track designation and allowed phase 3 study to assess the efficacy in phase 3 studies. For the licensure of vaccine in 2015, Novavax launched a global randomized, observer-blind, placebo-controlled trial enrolling 4,636 third-trimester pregnant women in northern and southern hemisphere in 5 continents, 11 countries, and 87 sites.²³ The trial was intended to determine the efficacy of maternal immunization with RSV F vaccine against medically significant symptomatic RSV lower respiratory infection through 90, 120, 150, and 180 days of life of infant. Though the clinical trial did not confirm the prespecified primary clinical endpoint, the efficacy of ResVax against the primary and two secondary endpoints in per protocol infants with RSV LRTI through 90 days of life was 39% against medically significant RSV LRTI (97.5% CI, 1–64%), 44% against RSV LRTI hospitalizations (95% CI, 20–62%), 48% against RSV LRTI severe hypoxemia (95% CI, 8–75%). There were no safety issues but the US FDA has recommended an additional phase 3 trial of ResVax to confirm the protection of infants born to vaccinated mother.

Respiratory syncytial virus surface antigen carried in the genome of vector viruses is being developed as vector vaccines. Alpha virus, adenovirus, and modified vaccinia virus Ankara are used as vector viruses for preparing the vaccine. These vector vaccines encoding RSV surface antigen are processed by MHC-1 and 2 pathways that stimulate humoral and cellular immunity response for the protection against RSV infection. There are 11 vaccine candidates. These are in preclinical and phase 1 trials.^{21,22}

DNA vaccines using plasmid genome encoding RSV antigen and messenger RNA encoding RSV antigen are in the process of development as they can induce humoral and cell-mediated immunity for protection against RSV infection in children and adults. There are four candidates in preclinical phase.^{21,22}

Research is going on to develop a modified D 25 monoclonal, which will have a longer half-life to sustain protection against RSV infection with one dose administration instead of monthly doses. It is specific for the epitope in the antigenic site of the prefusion conformation of the RSV fusion protein. Three clinical trials are undergoing; out the three, one is phase 2 trial.^{21,22}

RESPIRATORY SYNCYTIAL VIRUS VACCINE STRATEGIES²⁴

Depending on incidence of RSV infection, there two age groups to be targeted.

- 0–6 months
- 6–24 months

For the prevention of the RSV infection in infants younger than 6 months, two strategies can be adopted: one is maternal immunization, which will help for transplacental transfer of antibodies to the newborn, and other strategy is protecting the infant with vaccine like RSV neutralizing monoclonal antibodies.²⁴

Age-stratified seroepidemiology of RSV in India has shown that antibody positivity decline from 100% at birth to 71.3% at 3 months and 0.7% at 6 months of age. A significant rise was seen at 15 months of age to 16%, 16–24 months of age to 64.5%, and at 4 years to 95.2% with concomitant IgM anti-RSV positivity indicating of recent infection. In preterm infant's antibody, decline is higher compared to term infant. Positivity continued in higher age groups and elderly indicating repeated exposure to RSV. The results suggest early vaccination is essential for infants.²⁵

Respiratory syncytial virus disease burden in 6–24 months age group is associated with enhanced vaccine disease in children immunized with formalin-killed RSV vaccine in the United States in 1960.²⁶ Respiratory syncytial virus vaccine development research initiated after identification of virus in humans in 1957. The design and clinical trials have become a matter of concern for the enhanced respiratory infection following administration of formalin-inactivated vaccine in the year 1960. Most of the live attenuated RSV vaccines could not maintain balance between safety and immunogenicity. NIH, Serum Institute India, GSK, Novavax, Johnson and Johnson, and AstraZeneca are developing vaccines, but still they are in phase 1/2 and preclinical stage. Currently, there is no RSV vaccine licensed even after 60 years of first detection of virus.

LICENSED RESPIRATORY SYNCYTIAL VIRUS INTERVENTION PRODUCTS

Only two products are licensed for prevention of RSV infection for seasonal prophylaxis of preterm infants.

- RSV-IVIG (Respigam)
- Palivizumab-RSV mAB (Synagis).

RSV-IVIG was prepared from donors selected with high titers of RSV neutralizing antibody. It was discontinued in October 2003 because of cost and logistic difficulties of its use.

Palivizumab is a humanized monoclonal antibody (IgG 1k) against RSV protein F. It is developed by Medimmune USA. The USFDA has licensed this product in 1998. It is recommended for high-risk infants once a month during RSV season administered as intramuscular injection at a dose of 15 mg/kg Synagis. It has resulted in 55% reduction in hospital admissions for RSV illness compared to placebo. Average cost of treatment per season ranged from \$3,321 to \$12,568 per preterm infant.

The AAP recommendations for monthly prophylaxis during RSV season with palivizumab:²⁷

- In the first year of life, palivizumab prophylaxis is recommended for infants born before 29 weeks, 0 days' gestation.

Respiratory Syncytial Virus

Preclinical		Phase 1		Phase 2		Phase 3	Market approved
Codagenix, LID/NIAID/NIH (RSV)	LID/NIAID/NIH (RSV)	Intravac (RSV-ΔG)	Sanofi, LID/NIAID/NIH (RSV276, RSV ΔNS2, Δ1313/1314L, SIPL St. Jude Hospital (SeV/RSV) Pontificia Universidad Catolica de Chile (BCG/RSV)	Sanofi, LID/NIAID/NIH (RSV6120/ΔNS2/1030s)			
LID/NIAID/NIH (PIV-3/RSV)	Meissa Vaccines (RSV)						
Blue Willow Biologics (RSV)							
Fraunhofer (VLP)	Icosavax (VLP)	University of Massachusetts (VLP)	Novavax (RSV F Nanoparticle)	Novavax (RSV F Nanoparticle)	Novavax (RSV F Nanoparticle) For elderly	Novavax (RSV F Nanoparticle) Ph3 primary endpoint not met	
Georgia State University (VLP)	Sanofi (RSV F Nanoparticle)	Virometix (VLP)	For pediatric use			For maternal use	
Blue Willow Biologics (RSV F Protein)	Sciogen (RSV G Protein)	University of Saskatchewan (RSV F Protein)	Beijing Ad vaccine Biotechnology (RSV G Protein) for paediatric and elderly	Immunovaccine, VIB (DPX-RSV-SH Protein) for elderly	GlaxoSmithKline (RSV F Protein) for maternal use	Pfizer (RSV F Protein) For elderly and maternal	
Instituto de Salud Carlos III (RSV F Protein)	University of Georgia (RSV G Protein)		Glaxo Smith Kline (RSV F Protein) for elderly	NIH/NIAID/VRC (RSV F Protein) for elderly and maternal use			
Curevac (RNA)	Moderna (RNA)						
BravoVax (Adenovirus)	Vaxart (Adenovirus) for elderly			Bravarian Nordic (MVA) for elderly	Janssen Pharmaceutical (Adenovirus) for pediatric and maternal		
				GlaxoSmithKline (Adenovirus) for pediatric use			
Aridis (Anti-F-mAb)	Gates MRI (Anti-F-mAb)	Pontificia Universidad Catolica de Chile (Anti-F-mAb)		Merck (Anti-F-mAb) For pediatric use		AstraZeneca, Sanofi (Anti-F-mAb) for pediatric use	Astragenica, Sanofi (Anti-F-mAb, Synagis) for pediatric use
UCAB, mAb Xience (Anti-F-mAb)							

Fig. 2: RSV vaccine and mAb snapshot

- Palivizumab prophylaxis is not recommended for otherwise healthy infants born at or after 29 weeks, 0 days' gestation.
- In the first year of life, palivizumab prophylaxis is recommended for preterm infants with CLD of prematurity, defined as birth at <32 weeks, 0 days' gestation, and a requirement for >21% oxygen for at least 28 days after birth.
- May be administered palivizumab prophylaxis in the first year of life to certain infants with hemodynamically significant heart disease.
- May be administered up to a maximum of five monthly doses of palivizumab (15 mg/kg per dose) during the RSV season to infants who qualify for prophylaxis in the first year of life. Qualifying infants born during the RSV season may require fewer doses. For example, infants born in January would receive their last dose in March.
- Palivizumab prophylaxis is not recommended in the second year of life except for children who required at least 28 days of supplemental oxygen after birth and who continue to require medical intervention (supplemental oxygen, chronic corticosteroid, or diuretic therapy).
- Monthly prophylaxis should be discontinued in any child who experiences a breakthrough RSV hospitalization.
- Children with pulmonary abnormality or neuromuscular disease that impairs the ability to clear secretions from the upper airways may be considered for prophylaxis in the first year of life.
- Children younger than 24 months who will be profoundly immunocompromised during the RSV season may be considered for prophylaxis.
- Insufficient data are available to recommend palivizumab prophylaxis for children with cystic fibrosis or Down syndrome.
- The burden of RSV disease and costs associated with transport from remote locations may result in a broader use of palivizumab for RSV prevention.
- Palivizumab prophylaxis is not recommended for prevention of health care-associated RSV disease.

The other new-generation mAb motavizumab and suptavumab are not approved by the USFDA.

MED18897 RESPIRATORY SYNCYTIAL VIRUS mAb

It is an investigational recombinant human immunoglobulin G1 kappa (IgG1k) mAb with extended half-life, which can be administered as single shot before the season or at birth for prevention of severe RSV infection in infants. This monoclonal antibody has demonstrated potent antiviral activity against diverse panel of RSV A and B clinical isolates in preclinical *in vivo* and *in vitro* studies. Safety studies were in healthy adults initially.²⁸ Followed by phase 1b/2a study demonstrated extended half-life of MED18897 and protection from RSV infection for the duration of 5 months' season after a single dose of 50 mg intramuscular dose.²⁹ In phase 2b studies, the product has been found to be safe and has efficacy of 70% (52.3–81.2).²⁹ There are two multicentric phase 3 studies in the process in 3,000 healthy late preterm and term infants, and a double-blind study, palivizumab-controlled study evaluating the safety of MED18897 in 1,500 high-risk infants.^{30–32}

Palivizumab is not recommended as a treatment modality for RSV bronchiolitis. It has not shown any benefit in young children with acute RSV positive bronchiolitis.³³

SUMMARY

Respiratory syncytial virus can cause significant number of respiratory infections in infants contributing to infant mortality in LMIC. Some of Indian studies have shown that 40% of infants with flu-like illness may be due to RSV. Respiratory syncytial virus infection is seen mostly in less than 2 years' children. Palivizumab (mAb) therapy is not available in India probably due to very high cost. Indian studies demonstrated the decline of maternal antibodies against RSV in infants by the age of 6 months. Due to natural infections, antibodies level keeps increasing by the age of 2 years. In future maternal immunization with nanoparticle RSV F protein vaccine may be useful in protecting the infants in the initial few months after birth. In spite of initial setback with inactivated vaccine in 1960, many vaccine trials are going on with WHO coordination to develop vaccine (Fig. 2).²²

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