Development and Clinical Applications of Novel Antibodies for Prevention and Treatment of Respiratory Syncytial Virus Infection

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Abstract

Respiratory syncytial virus (RSV) remains a significant cause of morbidity and mortality in infants and young children, immunocompromised patients and the elderly. Despite the high disease burden, an effective and safe vaccine is lacking, although several candidates are currently in development. Current treatment for RSV infection remains largely supportive and RSV-specific options for prophylaxis are limited to palivizumab. In the past few years, novel therapeutic options including nanobodies, polyclonal and monoclonal antibodies have emerged and there are several products in preclinical and Phase-I, -II or -III clinical trials. The major target for antiviral drug development is the surface fusion (F) glycoprotein, which is crucial for the infectivity and pathogenesis of the virus. Solving the structures of the two conformations of the RSV F protein, the prefusion and postfusion forms, has revolutionized RSV research. It is now known that prefusion F is highly superior in inducing neutralizing antibodies. In this section we will review the stages of development and availability of different antibodies directed against RSV for the prevention and also for treatment of acute RSV infections. Some of these newer anti-RSV agents have shown enhanced potency, are being explored through alternative routes of administration, have improved pharmacokinetic profiles with an extended half-life, and may reduce design and manufacturing costs. Management strategies will require targeting not only high-risk populations (including adults or immunocompromised patients), but also previously healthy children who, in fact, represent the majority of children hospitalized with RSV infection. Following treated patients

longitudinally is essential for determining the impact of these strategies on the acute disease as well as their possible long-term benefits on lung morbidity.

INTRODUCTION

Respiratory syncytial virus (RSV) is the main cause of bronchiolitis and pneumonia, in infants and toddlers, accounting for ~ 60% of all lower respiratory tract infections (LRTI) in preschool-aged children worldwide. Globally, it is estimated that RSV causes about 34 million episodes of acute LRTIs in children under five years of age, resulting in ~ 3.4 million hospitalizations per year [1, 2]. In the developing world, RSV is associated with significant morbidity and represents the second most common cause of infant mortality [3]. In addition, RSV also causes significant disease in immunocompromised hosts and in the elderly [4, 5].

By their first birthday nearly 70% of infants have been infected with RSV at least once. Seropositivity is ~ 100% by 2 years of age. Despite the high disease burden, an effective vaccine or specific therapy is lacking, although there are several products at different stages of development [6]. Epidemiologic studies have identified specific groups of infants at high-risk for severe disease and mortality including premature birth, compromised cardiopulmonary function (chronic lung disease or congenital heart disease), Down syndrome, and immunodeficiencies. However, the majority of infants requiring hospitalization for RSV LRTI do not have any risk factors and are previously healthy [7, 8]. Of those, up to 20% will be treated in the pediatric intensive care unit (PICU). [9, 10]

RSV has also been associated with the development of persistent wheezing and asthma inception [11–13]. Thus, it is possible that interventions aimed at reducing the acute burden of RSV disease may also have an impact on the development of long-term pulmonary sequela [14].

VIRAL STRUCTURE AND TARGETS FOR MONOCLONAL ANTIBODIES

RSV is an enveloped, negative sense, single strand RNA virus that belongs to the family *Paramyxoviridae*, subfamily *Pneumovirinae*. Human RSV includes two antigenic subgroups, A and B, which can co-circulate during the same season and exhibit genome-wide sequence divergence. The RSV genome contains 15,222 nucleotides that encode eleven proteins (Figure 1). The RSV envelope contains three viral transmembrane surface glycoproteins: the attachment (G) protein, the fusion (F) protein, and the small hydrophobic (SH) protein. Of these proteins, SH is not required for initiating virus infection. On the other hand, the F and G 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 [15]. The F protein performs a dramatic structural rearrangement to cause membrane fusion, and is much more highly conserved among RSV strains than the G protein (Figure 2). For these reasons, the F protein represents the major target for antiviral drug development, both small molecules and neutralizing antibodies [6, 16].

The G protein initiates infection by targeting the apical surface of the ciliated cells of the airways and mediates adherence of the virus to these host cells [17]. Heparan sulfate,

surfactant protein A, annexin II and CX3CR1 have all been shown to bind the G protein and proposed as cellular receptors for RSV [18–21]. Recently, CX3CR1 was identified on the cilia of well differentiated human ciliated airway cells, acting as a cellular receptor for the G protein [22–24]. The F protein is essential for viral entry and initiates viral penetration by fusing the viral and cellular membranes [16]. In non-polarized laboratory cells, the F protein also promotes cell-to- cell fusion producing the characteristic syncytia. However, in polarized well differentiated human airway cells, the F protein does not cause cell-cell fusion probably because the F protein is limited to the apical domain [17, 25]. RSV lacking its G gene is infectious, though poorly, suggesting that the F protein may also have attachment activity [26]. Heparan sulfate, ICAM-1, TLR-4 and nucleolin have been proposed as cellular receptors for the F protein [26–30].

Our understanding of the RSV F protein was revolutionized in 2013 when McLellan et al. solved the crystal structure of its metastable, prefusion F (preF) conformation. Together with the crystal structure of the postfusion F (postF) protein [31], the beginning and end points of the F protein refolding became clear (Figure 2). The preF form is the active form of the F protein on the virion membrane surface. Antibodies that uniquely bind to the preF protein are much more efficient at neutralizing RSV compared to antibodies against the postF form [16, 32, 33]. In fact, some prefusion-specific monoclonal antibodies (mAbs) are able to stabilize the F protein in its prefusion state and in some cases this stabilization may be the mechanism by which the mAb prevents infection: they prevent the F protein from refolding [34]. The stages of development and the clinical applications for these antibodies will be discussed below.

POLYCLONAL ANTIBODIES

The field of antiviral therapy for RSV radically changed during the 1980s when prophylactic use of an intravenous (IV) polyclonal immunoglobulin preparation with high titers of neutralizing antibodies against RSV (RSV IVIG; Respigam) demonstrated efficacy in reducing hospitalizations due to severe RSV infections in high-risk children (PREVENT trial) [35, 36] (Table 1). Despite these positive results, RSV IVIG was associated with several limitations including fluid overload, adverse events in children with congenital heart diseases (CHD), need for intravenous access, development of hypoxemia or cyanosis, and the need to delay vaccination with live vaccines for ~ 9 months after the last dose. RSV IVIG was withdrawn from the market in 2004 but this preparation established the proof of principle that anti-RSV antibodies delivered IV can prevent severe RSV LRTI and prompted the development of mAbs protective against RSV. Subsequently, another IVIG preparation obtained from pooled plasma from donors with high titers of RSV (RI-001; ADMA Biologicals) was developed. RI-001 was tested in Phase-II clinical trials for the prevention of RSV LRTI in immunocompromised 2 to 65 year old patients who presented with upper respiratory tract symptoms with promising results [37]. Recently, the RI-001 formulation was replaced by RI-002, which in addition to high levels of RSV antibodies has naturally polyclonal antibodies against *Streptococcus pneumoniae* and *Haemophilus influenza type b*). A Phase-III open label clinical trial (www.clinicaltrials.gov; NCT01814800) has been completed using this second generation IVIG for the prevention of serious bacterial

infections in patients with primary immunodeficiencies. The role of RI-002 in preventing RSV infection in this population has not been reported.

MONOCLONAL ANTIBODIES

The main advantages of mAbs against RSV, compared to polyclonal preparations, are their greatly superior neutralizing activity with practically no risk of fluid overload or interference with vaccine schedules, and no possibility of blood-borne pathogen transmission. Most of the original anti-RSV mAbs that were developed and tested clinically are IgG antibodies directed against viral epitopes in the F protein (Table 1). During the early 1990s three mAbs were evaluated in clinical studies in humans. Two of them were IgG formulations that were administered systemically (SB209763 and MEDI-493/palivizumab) [38, 39]. Palivizumab was shown to be clearly superior to SB209763 and, since 1998, it has been the only mAb licensed for passive immunization in high-risk children [40–42]. A third neutralizing mAb, HNK20, was an IgA mAb administered intranasally. Although HNK20 appeared promising in non-human primates, it failed to provide clinical benefit [43].

Other novel mAbs have emerged and several products are currently in preclinical, or Phase I, II or III clinical trials. Of particular interest is MPE8, a human mAb directed against the preF protein, that potently neutralizes both human RSV and human metapneumovirus (hMPV), as well as two animal paramyxoviruses [44]. MPE8 has shown potent prophylactic efficacy in animal models of RSV and hMPV infection, and both a prophylactic and a therapeutic effect against pneumonia virus of mice. The conservation of this neutralizing epitope in the F protein of such diverse viruses suggests that this epitope is important to the function or structure of the F protein.

Targeting the F Protein

1. Palivizumab—In 1998 the US Food and Drug Administration (FDA) licensed the use of palivizumab, a humanized mAb directed against the F glycoprotein, for the prevention of serious LRTI caused by RSV in children at high risk for RSV disease. Palivizumab is a humanized IgG_1 mAb composed of the complement determining region from a murine mAb developed at the National Institutes of Health (NIH) and, transplanted into a human IgG_1 mAb using recombinant DNA technology [39]. It is > 95% human and recognizes the antigenic site II on the F protein of both RSV A and B subtypes. Palivizumab is significantly more potent than the polyclonal preparations. It is now known that palivizumab binds to both the preF and postF forms of the F protein, because it recognizes a site on the central region of preF whose structure does not change during the preF to postF transition [45].

In the original large, randomized placebo-controlled studies, the safety and efficacy of palivizumab (the primary endpoint was a reduction in RSV-related hospitalization rates) were established in infants with a history of premature birth (35 weeks' gestational age), children with chronic lung disease of prematurity (CLD), and children with hemodynamically significant congenital heart disease (CHD) [40, 41]. Since palivizumab approval in 1998, to provide guidance regarding the groups of infants at highest risk, the American Academy of Pediatrics (AAP) has updated the guidance on palivizumab prophylaxis, and increasingly limited its use [46]. Palivizumab has a half-life of 28 days and

thus it is administered once a month during the RSV season at 15 mg/kg intramuscularly. It should be initiated immediately before the start of the RSV season and continued monthly until the end. Although there is significant regional and year-to-year variability in the beginning and end of the season, administration of five monthly doses starting in November usually ensures adequate serum concentrations throughout the season.

2. Motavizumab—Subsequently, MEDI-524 (motavizumab), a second-generation recombinant IgG$_1$ mAb based on palivizumab and thus also directed against site II, was developed by directed mutagenesis of the complement determining regions and selection of the most effective version. It differed from palivizumab in 13 amino acid positions, selected for their 70 and 20- fold increased affinity and in vitro neutralizing activities respectively, as well as greater inhibition of RSV in the upper respiratory tract compared to palivizumab [47]. A large Phase III double blind active control study, in which 6,600 preterm infants with CLD were randomized to either motavizumab or palivizumab, at a dose of 15 mg/kg once a month for 5 months intramuscularly, showed that motavizumab was non-inferior to palivizumab at reducing RSV hospitalizations. However, it was associated with a 2.1% increase in cutaneous hypersensitivity reactions (7.2% vs. 5.1%) [48]. At the same dose, this second generation mAb was superior to palivizumab in further reducing RSV LRTI outpatients visits by 50%. The clinical efficacy of motavizumab was subsequently confirmed in two additional randomized clinical trials conducted in healthy US Native American Indians infants, and in children with hemodynamically significant CHD [49, 50]. Nevertheless, in 2010 the US FDA Antiviral Drug Advisory Committee did not grant motavizumab licensure and its development has been discontinued.

None of these antibody preparations, RSV IVIG, palivizumab or motavizumab, have been shown to be effective as treatments for acute RSV LRTI in previously healthy children [51, 52].

3. Motavizumab-YTE—Palivizumab and motavizumab have a half-life of 3–4 weeks and thus monthly injections are required to maintain protective levels during the RSV season. MEDI-557 (motavizumab-YTE) is a third generation, humanized mAb derived from motavizumab, in which three amino acid substitutions (M252Y/S254T/T256E [YTE]) were introduced in the IgG$_1$ Fc region, extending the serum IgG half-life [53]. A Phase-I randomized double blind, dose escalation study to evaluate the safety, tolerability and pharmacokinetics of this mAb showed that the clearance of motavizumab-YTE was significantly lower and the half-life up to 4-fold longer than motavizumab [54]. Further development of this mAb has also been discontinued.

4. MEDI-8897—MEDI-8897 is an investigational recombinant human IgG$_1$ mAb derived from D25, a human antibody with greater neutralizing potency than palivizumab, in which half-life has been extended using YTE technology. D25 targets antigenic site 0 that is unique to the preF conformation of the RSV F protein [32, 44]. In vitro, this drug has been shown to be 150-fold more potent than palivizumab, and 10-fold more potent in vivo. Phase-I clinical trials in adults confirmed its prolonged half-life [55]. Currently MEDI-8897 is undergoing Phase-II clinical trials in premature infants born between 32 and 35 weeks gestation. The long-term objective for MEDI-8897 would be to provide passive immunization for

prevention of LRTI caused by RSV not only to children with chronic lung disease (CLD) or CHD but also to all infants (preterm and full term) entering their first RSV season, using a fixed, single intramuscular (IM) dose.

Extended half-life mAbs represent a promising advance for the prevention of RSV by enhancing efficacy, avoiding compliance issues since repeated dosing would not be required, and lowering costs. In addition, this strategy represents a potential opportunity for application to other patient populations, including otherwise healthy children or adults.

5. REGN-2222—REGN-2222 is a fully human IgG_1 mAb that targets a well-conserved epitope of the F protein, different from site 0 or site II. In vitro, REGN2222 was 36-fold more potent than palivizumab at inhibiting RSV fusion to the cells, and 10 to 40-fold more potent than palivizumab in reducing lung and nasal viral loads in cotton rats. It is not clear if REGN-2222 is specific for preF. Phase-I ascending dose studies conducted in adults 18–60 years old demonstrated that the half-life of this mAb is longer that a typical IgG_1 mAb, with low immunogenicity [56]. A Phase-III randomized, double-blind, placebo-controlled clinical trials (www.clinicaltrials.gov; NCT02325791) is currently underway in premature infants born at 35 weeks of gestation who are 6 months of age at enrollment in whom palivizumab is not recommended. The primary endpoint is the prevention of serious lower respiratory tract infections associated with RSV. Similar to other mAbs, REGN-2222 is administered IM, however it is expected that one or two doses will be sufficient to provide coverage for one RSV season.

Targeting the Attachment (G) Protein

The G protein is the primary RSV attachment protein. The sequence identity of the G protein between RSV isolates can be as little as 60%, therefore most of the efforts in antiviral drug development has focused on the F protein which is well conserved among RSV A and B strains. Nevertheless, the G protein has also been implicated in the pathogenesis of RSV disease due to its ability to modulate the virus-induced immune response and lung pathology.

Studies in animal models using the RSV G mAb 131-2G, either as prophylaxis or in a treatment mode, showed that it decreased both RSV loads and lung inflammation when administered early in the disease course [57, 58]. This result was originally interpreted as being due to antibody dependent cellular cytotoxicity since 131-2G is a "non-neutralizing mAb" in HEp-2 cells. However, the G receptor in HEp-2 cells is heparan sulfate while the receptor on human ciliated airway cells, the natural target in the airway epithelium, is CX3CR1. Indeed, mAb 31-2G showed to prevent the G protein from binding to CX3CR1 and inhibited infection of well differentiated human airway epithelial cells, suggesting that it might also have neutralized RSV in the mouse experiments [22]. However, a prophylactic antibody against the G protein would need to recognize both RSV A and B strains. Despite the overall widely variable sequences of the G protein its central region contains a completely conserved 13 amino acid sequence followed by a cysteine noose also with conserved elements. MAbs, like 131-2G, that bind to this region may be able to neutralize both A and B strains of RSV.

NANOBODIES

Nanobodies are small (12–15 kDa) antibody fragments consisting of a single monomeric variable domain (heavy chain) that retain full antigen-binding capacity [59, 60]. The affinity of nanobodies is similar to that of conventional antibodies, however, their size and stability allowing intranasal delivery, their formatting flexibility into multivalent constructs, and their relatively straightforward production make these small proteins an attractive approach for both prophylaxis and treatment of respiratory viral infections.

ALX-0171 (Ablynx) is a nanobody, composed of three identical single variable chain antibody domains linked end to end by flexible peptides. Like palivizumab, this trivalent nanobody binds to antigenic site II in the preF and postF configurations of the F protein, and likely inhibits the conformational changes in the F protein that are required for membrane fusion (Figure 2). ALX-0171 design has allowed its delivery via the intranasal route (inhalation) for the treatment of RSV infection [60]. Its multivalent formatting has provided ALX-0171 with improved activity and coverage for RSV A and B strains, as well as neutralizing activity that is superior to palivizumab [60]. In the neonatal lamb model, treatment with nebulized ALX-0171 showed a significant reduction of RSV loads in bronchoalveolar lavage samples, decreased lung histological changes, and improvement of clinical symptoms in RSV-infected animals [61]. Preliminary results from a Phase-I/IIa randomized clinical trial (www.clinicaltrials.gov; NCT02309320) conducted in healthy infants and toddlers hospitalized with RSV infection showed that inhaled ALX-0171 administered once a day for 3 consecutive days was safe and well tolerated, had an antiviral effect with a reduction of viral loads compared to placebo, and showed a trend towards improvement of the clinical severity score [62, 63].

LONG TERM BENEFITS

Observational studies have shown that at least 30% of children who develop RSV LRTI as infants will develop recurrent wheezing during childhood [11, 13]. In the mouse model, RSV infection alone was associated with lung inflammatory changes and airway hyperresponsiveness that persisted for months after the acute infection [64]. In this model, administration of prophylactic mAbs against RSV was protective against the acute disease and significantly reduced the long-term pulmonary morbidity and the development of airway hyperresponsiveness [65, 66]. Prospective non-controlled studies followed by a randomized, double blind, placebo-controlled clinical trial in preterm infants who received prophylaxis with palivizumab, demonstrated a decrease in the development of persistent wheezing, that was independent of the atopic background [14, 67, 68]. Thus, data derived from animal and clinical studies suggest that the prevention of acute RSV LRTI may have implications in the development of recurrent wheezing.

SUMMARY

There have been recent advances in the pipeline for both treatment and preventive strategies for RSV infection. There are several newer RSV antibodies in different phases of development that have shown enhanced potency, improved pharmacokinetic profiles and

extended half-life, and these antibodies are being explored through alternative routes of administration. While the main barriers for mAb administration will likely be derived from their associated costs and the inconvenience of intramuscular dosing, inhaled nanobodies will need to be administered with caution in infants with an already inflamed airway. Nevertheless, the benefits associated with these interventions may well outweigh their potential side effects. Management strategies will require targeting not only high-risk populations, but also previously healthy children that represent the majority of hospitalized patients with RSV LRTI. In addition, ongoing studies should include the ability to follow these patients and determine the acute and possibly long term benefits associated with these compounds.

Acknowledgments

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HIGHLIGHTS

- Several promising candidates including monoclonal antibodies and nanobodies are in different stages of development for the prevention and treatment of acute RSV infection in the main target populations.

- Newer human monoclonal antibodies with enhanced potency and extended half-life have been developed, and represent a promising strategy for the prevention of RSV.

- Nanobodies delivered via the intranasal route represent an attractive approach for the treatment of acute RSV infections in young children.

- Evaluation of strategies aimed at preventing or treating RSV infections should include patient follow up to determine their impact on acute and long-term lung morbidity.

The RSV virion
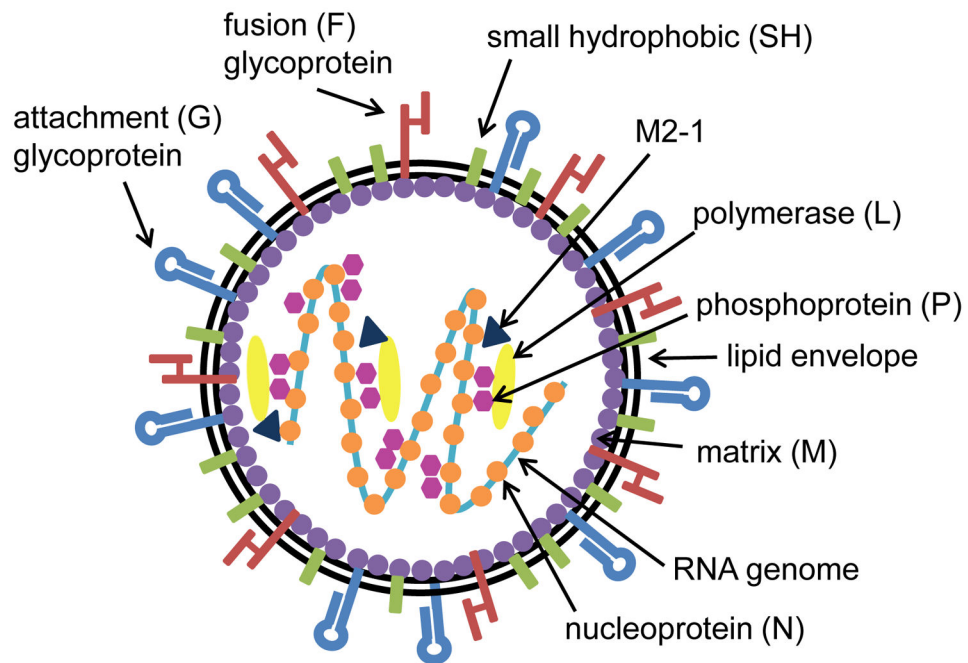
Figure 1. Anatomy of RSV

RSV is an enveloped, negative sense, single strand RNA virus whose genome contains 10 genes (15,222 nucleotides) encoding eleven proteins. Of the three transmembrane surface glycoproteins, the attachment (G) and fusion (F) proteins, are crucial for the infectivity and pathogenesis of the virus, and are the targets for neutralizing antibodies.
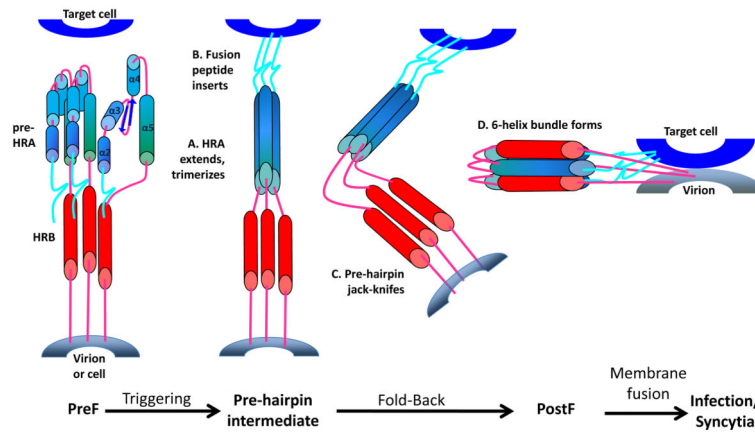
Figure 2. Refolding of the RSV F protein

The F glycoprotein is present in two forms, a metastable prefusion F (preF), the active form on the virion membrane, and the postfusion F (postF) after triggering, refolding and bringing the virus and target cell membrane together to initiate fusion and infection. It is not clear what causes the F protein to trigger.

Table 1

Anti-RSV antibodies at different stages of development

Antibody Class	Name/Company	Development phase	Route	Target	Class	Endpoints/Target Population	Comments
(1) Nanobodies	ALX0171 (Ablynx)	Phase-IIa RCT completed	Inhaled	F	Antibody nanobody	Treatment of infants and toddlers with RSV LRTI	• In healthy adult volunteers: no dose-limiting toxicity, no significant changes in lung function. • Potential for once-daily dosing.
	Palivizumab (Medimmune)	Phase-IV Marketed	IM	F	Humanized mAb	Prevention of RSV LRTI in high-risk infants	• Reduces RSV-related hospitalizations in high-risk infants. • Once per month dosing during RSV season (~ 5 doses).
	Motavizumab (Medimmune)	Phase-III RCT completed Not-licensed (no FDA approval)	IM	F	Humanized mAb	Prevention of RSV LRTI in high-risk infants	• 20 fold more potent than palivizumab. • Associated with increased type I skin hypersensitivity reactions • Decreased outpatient MALRI.
(2) Monoclonal antibodies (mAb)	Motavizumab-YTE (MEDI-557; Medimmune)	Phase-II RCT interrupted	IV	F	Humanized mAb derived from motavizumab with YTE technology	Prevention of RSV LRTI in high-risk infants	• Extended half-life. • Once per season dosing.
	MEDI-8897 (Medimmune)	Phase-II RCT ongoing	IM	Prefusion F	Human mAb derived from D25	Prevention of RSV LRTI in healthy and high-risk infants.	• 150 fold more potent than palivizumab. • Extended half-life. • Once per season dosing.
	REGN2222 (Regeneron pharmaceutics)	Phase-III RCT ongoing	IM	F	Human mAb anti-RSV	Prevention of RSV LRTI in premature healthy infants not candidates for palivizumab	• ~ 30 fold greater in vitro activity than palivizumab. • Extended half-life (1–2 doses per season).
(3) Polyclonal antibodies	RI-001 (ADMA)	Phase-II RCT completed	IV	Various viral epitopes	Polyclonal IG	Prevention of progression from RSV URI to LRTI in immunocompromised patients	Decreased RSV titers at day 18 from baseline. 4-fold rise in antibody titers.

Antibody Class	Name/ Company	Development phase	Route	Target	Class	Endpoints/ Target Population	Comments
	RI-002 (ADMA)	Phase-III RCT completed	IV	Various viral epitopes	Polyclonal IG	Prevention of RSV and serious bacterial infections in patients with PIDD	No adverse events and no serious bacterial infections. Unclear benefits for RSV specifically

DISCOVERY: (1) EV-046120 and EV-046135 (Evec, Inc): also targeting RSV and MPV; (2) Monoclonal antibodies (vanderBilt Univ): human mAb anti-F protein and human mAb anti G-protein.

PRECLINICAL: (1) AR-201 (Aridis Pharm) and Palivizumab Biosimilar (bioXPRESS Therapeutic, Celltrion, iBio, mAbxience & UCAB): mAb anti-F protein; (2) MPE-8 (Humabs BioMed): mAb anti prefusion-F protein. Possible prophylaxis and therapy of severe HRSV and HMPV infections; (3) Sym-003 (Symphogen): polyclonal antibody targeting various non- overlapping RSV epitopes (F and G proteins).

*
RCT: Randomized clinical trial; LRTI: Lower respiratory tract infection; FDA: Food and Drug Administration; MALRI: medically attended lower respiratory tract infections; URI: upper respiratory infection; IG: immunoglobulin; PIDD: Primary immunodeficiency diseases.