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DRUG PROFILE

RI-002, an intravenous immunoglobulin containing high titer neutralizing antibody to RSV and other respiratory viruses for use in primary immunodeficiency disease and other immune compromised populations

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ABSTRACT

Introduction: Novel immune globulin (IG) products (RI-002, RI-001) have been designed to provide protection against respiratory syncytial virus (RSV) mediated respiratory illness while at the same time meeting the manufacturing requirements established by FDA for antibody supplementation in immunocompromised subjects.

Areas covered: This review covers the manufacture and development of both RI-001 and RI-002, including the selection of plasma donors for IG preparation with high-titers of anti-RSV antibody, *in vitro*, and preclinical data in the cotton rat model *S. hispidus*, and clinical trials including Phase II and compassionate use studies of RI-001 and a multi-center, pivotal Phase III study of RI-002 in PIDD patients.

Expert commentary: The data demonstrate that RI-002 is efficacious in the prevention and treatment of RSV in preclinical normal and immune suppressed animal models and is safe and efficacious in the treatment of patients with various forms of primary immunodeficiency disease (PIDD). This product offers potential advantages over other available IG's for prophylaxis in immunocompromised patients requiring polyclonal immunoglobulin supplementation because of its unique antibody composition. In addition to its enhanced neutralizing anti-RSV activity and its polyclonal IG composition, there is preclinical data to support the use of RI-002 for humoral protection against other respiratory pathogens.

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1. Introduction

Supplementation with immunoglobulins (IG) has been considered the standard of care for the treatment of patients with a variety of antibody production disorders due to B cell or B and T cell abnormalities, collectively referred to as primary immunodeficiency diseases (PIDDs) [1]. While regular infusions with IG prevent the vast majority of serious bacterial infections within this population, there is no single prophylactic regimen that provides protection against the wide range of viral pathogens to which these patients are susceptible [2–8]. In the USA, there are several commercially available IG products that meet minimum standards of concentrations of antibodies to diphtheria toxoid (≥1.21 U/mL), measles (≥0.60 CBER reference), and polio (≥0.28 CBER reference) viruses in conformance with the US FDA guidance 21CFR 640 subpart J [9]. Although the FDA defines minimum concentrations of these antibodies, standards for antibodies against pathogens that are the most important to PIDD patients are not required. Titers of these antibodies in commercial polyclonal IG vary widely [10,11]. While the optimal dose of therapeutic polyclonal intravenous IG appears to be specific to the individual patient [4,12], increasing IG dosage is associated with better outcomes and a decrease in the incidence of infection [3,13]. Even with appropriate IG therapy, PIDD patients remain at an increased risk of infection, including those caused by upper and lower respiratory viral pathogens including RSV and influenza.

PIDD patients and patients with secondary immunodeficiencies (e.g. transplantation, cancer, etc.) are at high risk for respiratory syncytial virus (RSV) infections that often result in significant morbidity and mortality from bronchiolitis and pneumonia [3,14]. At present, the only prophylactic treatment available for RSV infection is palivizumab (Synagis®, MedImmune, Gaithersburg, MD), an anti-RSV monoclonal antibody that is used to confer seasonal passive immunity and has been shown to be effective in preventing RSV-related hospitalizations, particularly in low birthweight infants [15–17]. The American Academy of Pediatrics (AAP) recently revised its recommendation for use guidelines and available evidence suggest that use of this monoclonal antibody for RSV should be reserved only for patients born prematurely under 29 weeks gestation and under 1 year of age. There currently is no approved, recommended treatment for older age groups for the prevention of RSV disease. Although treatment of established disease is primarily supportive care, ribavirin (Virazole®) is the only FDA-approved treatment for RSV pulmonary infection in pediatric patients. Presently, the efficacy of ribavirin in RSV treatment is controversial and there is the

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potential for significant side effects in both patients and caregivers who administer it [18–23]. Because ribavirin is costly and must be administered in a hospital-based setting adding to the costs in the context of uncertain efficacy, an alternative therapy is needed. A polyclonal RSV-IVIG product (RespiGam®, MedImmune Inc., Gaithersburg, MD) enriched for antibodies against RSV derived from plasma donors with high-titers of anti-RSV antibodies was previously available and approved for the same indication as palivizumab, but was voluntarily withdrawn from the US market following approval of palivizumab. RI-002 was developed to meet an unmet medical need to provide both RSV protection and simultaneously supplement humoral immunity in patients with PIDDs or other immune compromised states.

2. Respigam® (RSV-IVIG, human IG, 5%)

RespiGam was marketed from 1996 to 2003 for prevention of RSV disease in infants and children younger than 24 months with chronic lung disease or a history of premature birth [24]. This hyperimmune globulin product was derived from a plasma pool of only a few hundred donors who had high neutralizing titers of anti-RSV antibody. The product was not developed for, nor was ever intended to be used in PIDD and as such, its manufacturing specifications did not adhere to FDA standards for collection of plasma from at least one thousand donors and was not tested for standard IG potency requirements. The product was never evaluated for efficacy in PIDD populations. Several randomized controlled trials demonstrated the efficacy of this RSV-IVIG product in preventing RSV infection. In a study of 249 infants and children with bronchopulmonary dysplasia due to congenital heart disease, or prematurity, 750 mg/kg of RespiGam reduced the incidence of lower respiratory tract infections (7 vs. 20 in the control group, $p = 0.01$), hospitalizations, hospital days, and days in intensive care [25]. In the PREVENT study, a randomized, double-blind, placebo-controlled, multicenter study conducted during the 1994–1995 RSV season, administration of 750 mg/kg RespiGam every 30 days reduced the incidence of hospitalizations due to RSV infection by 41% compared to placebo in 510 children with bronchopulmonary dysplasia and/or a history of prematurity [26]. In addition, administration of RespiGam in the PREVENT study also reduced hospitalization for respiratory illness of any cause by 38% suggesting a protective effect for other, non-RSV-related respiratory tract infections. Additionally, a *post hoc* analysis of 109 of the 249 children in the Groothuis study demonstrated a statistically significant decrease in episodes of acute otitis media compared to the placebo group (0 vs. 5, $p = 0.047$) following immunoprophylaxis with 750 mg/kg of RespiGam, suggesting that the protective effects of RSV enriched hyperimmune globulins may not be limited to RSV alone [27].

When RespiGam was commercially available, the AAP recommendations stated that, '*Palivizumab or RSV-IGIV immunoprophylaxis has not been evaluated in randomized trials in immunocompromised children. Although specific recommendations for immunocompromised patients cannot be made, children with severe immunodeficiencies (e.g. severe combined immunodeficiency or severe acquired immunodeficiency syndrome) may benefit from immunoprophylaxis. If these infants and children are receiving standard immune globulin intravenous (IGIV) monthly, physicians may consider substituting RSV-IGIV during the RSV season*' [28]. This recommendation and subsequent clinical practice failed to consider that RespiGam did not meet 21CFR640 subpart J requirements that are intended to ensure appropriate immunoprophylaxis in this high-risk population. While the product was available, there were numerous published reports regarding the successful use of RespiGam for treatment and prevention of RSV in various at-risk immunocompromised patient populations [25,27,29–31]. There have also been reports that the efficacy of combination therapy of ribavirin + RSV-IVIG may lead to better outcomes than either product used alone [32]. RespiGam was voluntarily withdrawn from the market in 2004, at least in part because the newly approved anti-RSV monoclonal antibody, palivizumab, did not require IV access. The discontinuation of RespiGam left a void in the options for RSV immunoprophylaxis, particularly for simultaneous anti-RSV activity and IG supplementation.

3. Ri-001

RI-001 was developed to address the therapeutic void following RespiGam discontinuation. RI-001 is an aqueous intravenous immune globulin preparation manufactured from the pooled plasma of healthy adult donors with high titers of neutralizing anti-RSV antibodies (>1.5 times the mean of commercial IVIG) as measured by a microneutralization assay. Product activity between RespiGam and RI-001 was shown to be comparable both in the cotton rat model and *in vitro* testing [Unpublished observations]. It is prepared using a process comparable to RespiGam employing Cohn Oncley cold-alcohol fractionation with a solvent/detergent treatment viral reduction step to eliminate enveloped viruses. An additional virus nano-filtration step to increase product safety and further reduce the probability of transmission of viral and prion contaminants (not part of the original RespiGam manufacturing process) was also included in RI-001 manufacture.

RI-001 efficacy was studied in a phase II randomized, double-blind, placebo controlled trial in immunosuppressed patients with RSV upper respiratory tract infection (URTI) confirmed by RT-PCR at the time of enrollment [33]. Patients between 2 and 65 years of age who had received a hematopoietic stem cell transplant or solid organ transplant within 2 years of enrollment were treated with study drug. Patients were treated concurrently with immunosuppressive treatment but were excluded if they had lower respiratory tract infection (LRTI) or required extracorporeal membrane oxygenation, CPAP, or other mechanical respiratory or cardiac support. Participants were randomly assigned 1:1:1 to each of the 3 treatment arms: RI-001 1500 mg/kg, followed by 750 mg/kg 2 days after first administration (high-dose group), RI-001 750 mg/kg followed by 750 mg/kg 2 days after first administration (low-dose group), or placebo. A total of 21 patients (7 patients per arm) with a mean age of 38.04 years (SD 25.03) were enrolled in the USA, Canada, Australia, and New Zealand.

The primary objective of this phase II study was to define the dose that produced a >fourfold rise in anti RSV

neutralizing titer at day 18 compared to baseline. In the low-dose group, the mean fold change was a 4.85 increase with 3/7 (43%) patients achieving a rise in titer ≥fourfold. A mean fold change of 1.42 was observed in the placebo group. The high-dose group met the primary end point, with a mean fold change in circulating titer of RI-001 of 9.24 and 6/7 (86%) patients achieving a rise ≥fourfold.

RI-001 was well tolerated with no study drug-related SAEs and a low incidence of AEs. The most frequently reported AEs in the active or placebo groups were cough, nausea, pyrexia, sinus congestion, diarrhea, platelet count decrease, and back pain. There was not a significant difference in the AE rate between the two groups. No significant risks associated with vital signs, laboratory assessments, or physical exams were identified.

RI-001 has also been evaluated in a nonrandomized, open-label compassionate use study in the USA, Australia, and New Zealand that collected data from December 2008 to February 2011 [33,34]. Compassionate use requests were accepted from patients with RSV LRTI considered at high risk of mortality who had either failed standard-of-care therapy (e.g. Ribivarin, standard IVIG, steroids, etc.) or had no alternative therapy available for their infection. Patients were diagnosed with RSV via rapid antigen test, RT-PCR, DFA, IFA, or viral culture. All patients received aerosolized ribavirin and some patients received infusions of IVIG, palivizumab, or steroids.

Data were collected from 15 patients who received compassionate use (5 in Australia, 9 in the USA, and 1 in New Zealand). They ranged in age from 2 months old to 71 years with a median age of 15 years. 53.3% of patients were less than <18 years of age. Nine patients were male and six were female. RI-001 was administered at a dose of 1500 mg/kg followed by an additional dose of 750 mg/kg after 2 days. All patients received at least 2 doses of RI-001. 11 patients (73.3%) survived and were discharged from the hospital, while four patients (26.7%) died. No deaths were related to administration of RI-001. Causes of death included respiratory failure attributed to RSV infection, RSV pneumonitis and acute respiratory distress, asystole following chest tube placement after development of left pneumothorax and hypoxic respiratory failure following withdrawal of care in a 71-year-old female with a dual infection of RSV complicated by acute influenza.

Infusion with RI-001 was generally well tolerated in the compassionate use experience with three infusion reactions noted that resolved upon completion of the infusion or after decreasing the infusion rate. Two patients experienced non-serious AEs of respiratory distress and there was a single nonserious AE of herpes simplex reactivation. All three AEs were considered to be not related or unlikely to be related to the RI-001. Four SAEs were documented including 3 of the deaths that were previously discussed and a single incident of dyspnea that resolved prior to discharge and was considered by the investigator to be not related to RI-001.

The data collected from the RI-001 trials is limited and represents a small sample size with heterogeneous demographics and disease characteristics. Although inconclusive due to limited data, several trends were observed in the compassionate use study. Possible seasonal variability was observed with all deaths occurring within the USA during the 2009–2010 and 2010–2011 RSV seasons.

No apparent gender bias was observed, nor did survival appear to be impacted by underlying condition including oncological status. Survival appeared to be influenced by age with higher mortality in the youngest and oldest patients, consistent with the potential for reduced immune response within these populations, as well as their generally more fragile health.

Importantly, there was a notable effect of early drug intervention on survival. First, patients that did not require ventilator support all survived (10 patients), while only one of five of those who required ventilator support survived. Further, the adult (≥18 years old) survivors were treated with RI-001, on average, 13.4 days after the onset of respiratory symptoms while the non-survivors were not treated until 29 days after symptom onset. A similar trend was observed in pediatric patients (<18 years old) with survivors receiving RI-001 treatment earlier (19.8 days) than non-survivors (39.5 days). Similarly, the time from positive RSV test to first treatment with RI-001 was also shorter in survivors (adults, 3.6 days; children, 4.7 days) versus non-survivors (adults, 12.5 days; children, 8.5 days). Of note, RI-001 was associated with significant increases in RSV serum neutralizing antibody titer in a subset of 7 immunocompromised adults with good clinical outcomes that were enrolled in the compassionate use study [35]. These subjects experienced a 3–151-fold rise in anti-RSV antibody titers compared to baseline for up to 30 days posttreatment. Taken together, these data suggest that early intervention with RI-001 is likely to be important in improving survival. Trends observed in this compassionate use study are consistent with a previous report as factors associated with a more severe clinical course and increased mortality in RSV disease [36].

4. Introduction to RI-002

Although RI-001 was considered a promising candidate to replace RespiGam in patients with RSV infection requiring immune supplementation, it was subject to the same limitations as RespiGam in PIDD and other patients who derive benefits from IG use. RI-002 (ADMA Biologics, Inc.) was developed to address the inconsistency in neutralizing anti-RSV titers found in commercial lots of IG while simultaneously meeting the FDA guidance requirements for treatment of PIDD patients defined in 21CFR640 subpart J. In addition to standardization with respect to minimum concentrations of antibodies required in the FDA guidance, RI-002 is manufactured according to a patented process to yield consistent and predictable levels of neutralizing anti-RSV antibodies obtained from a plasma pool of donors screened via a validated micro-neutralization assay for RSV A/2 and found to be hyperimmune to RSV [Unpublished observations]. The principal difference between RI-001 and RI-002 is that RI-001 was derived from a pool of plasma donors all of whom had elevated titers to RSV. RI-002 is derived from pooling plasma from high-titer donors with plasma from normal source donors to meet the FDA guidance stating that for treatment of patients with PIDD, the plasma pool needs to be derived from a minimum of 1000 unique donors.

A study was conducted to compare RSV neutralizing antibody titers in multiple lots of RI-002 to titers from a single available lot of RI-001. The overall average ratio of RI-002 to RI-

001 titer was 1.1 ± 0.2 with individual lots ranging from 0.7 to 1.5. Overall, anti-RSV titers observed in the study were considered to be equivalent between both IG products [Unpublished observations].

5. RI-002 preclinical studies

The cotton rat (*Sigmodon hispidus*) model of RSV disease was selected for preclinical evaluation of RI-002 as it is a widely used animal model that is accepted as an appropriate surrogate to predict efficacy of anti-RSV interventions as well as interventions for other respiratory pathogens including parainfluenza, metapneumovirus, and influenza [37–39]. Importantly, RSV infection in the *S. hispidus* model closely mirrors the course of natural infection in humans with respect to immunological response, time course, pulmonary inflammatory component, and clinical symptoms [36]. Further, *S. hispidus* exhibits susceptibility to infection across its lifespan and has been used to successfully model RSV infection in young, elderly, and immunosuppressed populations [37,40–43].

The *S. hispidus* model has previously demonstrated that anti-RSV antibody titers provided by standard IG prophylaxis are not sufficient to protect against RSV disease, while serum titers between 1:200 and 1:400 that can be provided by high-titer anti-RSV IG provide protection [44,45]. This finding is consistent with results in human infants that indicate that patients with high titers (1:400) of maternally acquired anti-RSV antibodies were less susceptible to RSV disease than infants with anti-RSV serum antibody titers from 1:100 to 1:200 [46]. This correlation suggests that the model can be used to predict the efficacy of immunoprophylaxis in addition to the dose required for immunoprophylaxis in human infants. Both RespiGam and palivizumab were advanced to clinical trials without the need for intermediate studies in nonhuman primates based on data from studies in *S. hispidus* [38,39]. To establish the relationship between protective titers in the cotton rat and *in vitro* microneutralization assays, human IVIG with elevated anti-RSV titers has been tested in animals with a strong correlation observed between neutralizing antibody titers and protection seen in the animal model. Human plasma donors were successfully identified as donors containing high-titer neutralizing anti RSV IG using this microneutralization assay [45]. The same assay is currently used for screening donors for the manufacture of RI-002.

5.1. RI-002 vs. standard IG, respiratory virus antibody titer evaluation

The antibody composition of RI-002 has been characterized and compared to commercial lots of IG in several *in vitro* studies. Following RSV infection, viral F protein (the target of palivizumab) mediates viral fusion and entry while G protein displays anti-inflammatory effects that dampen the host immune response to infection [47]. As neutralizing antibodies to both viral proteins may contribute to the therapeutic efficacy of RI-002, microneutralization assays were conducted to quantify anti-G protein and anti-F protein titers in 3 lots of RI-002 and 9 lots of commercial IG [Unpublished observations]. Enhanced levels of antibody to F protein and G protein (1.5-fold or greater compared to commercial IVIG lots) were observed in RI-002 lots compared to commercial IG, suggesting that selection of donors based on high anti-RSV titers in microneutralization assays enhances anti-RSV antibodies for multiple RSV surface proteins (Table 1).

As previously reported, RespiGam administration in the PREVENT study decreased the incidence of hospitalization for lower respiratory illness of any cause [26]. Although the donor pool for RI-002 is only screened for high anti-RSV antibody titers, enriching for RSV antibodies may be correlated with increases in antibody titers to other respiratory pathogens. Orange et al. performed ELISA to evaluate virus-specific IgG titers for 9 respiratory viruses in RI-002 produced from 3 plasma pools and 10 commercially available IVIG lots representing 7 brands (Table 2) [11]. Respiratory viruses tested include influenza A and B, RSV, parainfluenza virus serotypes 1, 2, and 3, hMPV, and coronavirus 229E and OC43. Aggregate mean titers across all viruses for RI-002 were 1.5-fold higher than those observed in commercial IG. The difference in titers for all viruses excluding hMPV achieved statistical significance with geometric mean ratios of RI-002/commercial IVIG ranging

Table 1. Comparison of anti-G protein antibodies (log_2) in RI-002 and 9 commercial lots of IVIG.

Analysis	Statistics	ADMA Lots	Commercial Lots
Antibody to F Protein	*n*	3	9
	Mean (SE)	18.010 (0.0000)	17.399 (0.1620)
	95% CI	NA	17.025, 17.772
	SD	0.0000	0.4859
	Median	18.010	17.510
	Min, Max	18.010, 18.010	16.510, 18.010
Antibody to G Protein A	*n*	3	9
	Mean (SE)	17.223 (0.1667)	16.390 (0.1443)
	95% CI	16.506, 17.940	16.057, 16.723
	SD	0.2887	0.4330
	Median	17.390	16.390
	Min, Max	16.890, 17.390	15.890, 17.390
Antibody to G Protein B	*n*	3	9
	Mean (SE)	16.840 (0.2887)	15.396 (0.1547)
	95% CI	15.598, 18.082	15.039, 15.752
	SD	0.5000	0.4640
	Median	16.840	15.340
	Min, Max	16.340, 17.340	14.340, 15.840

Table 2. Comparison of respiratory virus titers of RI-002 and commercial IVIG batches [11].

Virus	Ratio of geometric means (95% CI) (RI-002/commercial IVIG)[a]	*p*-Value[b]
RSV	1.861 (1.249, 2.771)	0.003
PIV 1	1.792 (1.282, 2.505)	0.001
OC43	1.610 (1.127, 2.301)	0.010
PIV 2	1.601 (1.160,2.210)	0.005
229E	1.494 (1.144, 1.950)	0.004
Flu A	1.402 (1.067, 1.843)	0.016
Flu B	1.316 (1.026, 1.688)	0.031
hMPV	1.264 (0.990, 1.613)	0.060
PIV 1 and 2	1.694 (1.250, 2.296)	0.001
OC43 and 229E	1.551 (1.237, 1.945)	<0.001
All viruses[c]	1.529 (1.227, 1.907)	<0.001

[a]Three randomly selected RI-002 batches and seven unselected commercial lots of IVIG from four different manufacturers/brands

[b]Two-group t-test for null hypothesis of no difference between in the groups in geometric means (i.e. ratio of geometric means = 1).

[c]Pooled RSV, respiratory syncytial virus; Flu A, influenza A; Flu B, influenza B; hMPV, human metapneumovirus; PIV 1, parainfluenza virus serotypes 1; PIV 2, parainfluenza virus serotypes 2; OC43, coronavirus CoV OC43; 229E, coronaviruses CoV229E.

from 1.3 to 1.9. Titers to respiratory viruses were consistent across RI-002 lots for all viruses tested but varied widely among IVIG products. A direct correlation was observed between RSV titers and titers to the other tested respiratory viruses with Pearson linear correlation coefficients ranging 0.29–0.67 $\log_2$ scale ($p < 0.05$). This positive correlation suggests that elevated titers to respiratory viruses may be a consistent feature of each lot of RI-002. The protective efficacy of these elevated titers against respiratory pathogens other than RSV remains to be determined.

While there is no data to support a hypothesis that underlies the enhanced antibody titers to other non-RSV viruses, there are a number of possibilities to consider. It is possible that the donors who have elevated titers to RSV may have been living in an area or workplace where they were exposed over long periods of time to many other respiratory viruses and thus have elevated titers to multiple respiratory viruses. It may also be possible that those individuals with high-titer neutralizing antibody to RSV have an immune response gene that encodes for elevated antibody responses to respiratory viruses where immune response is regulated by a common histocompatibility locus.

5.2. Cotton rat – prophylaxis model

RI-002 has also undergone extensive *in vitro* and preclinical testing in the cotton rat model and a summary of these studies is provided in Tables 3 and 4. First, a prevention study was performed in this model with either saline, RI-002 500 mg/kg, RI-002 750 mg/kg, RI-002 1000 mg/kg, or RespiGam administered intraperitoneally to five animals in each of the groups (Day 1) as prophylaxis [50]. One day following dosing, animals were challenged with 10^5 pfu of RSV-A Long strain via the intranasal route. Serum was collected on Day 4 and animals were euthanized for harvesting of lung and nasal tissue. RSV was undetectable in the lungs of animals that had received RI-002 at all doses and in 4/5 animals that received RespiGam. Virus was undetectable in nasal tissue of all animals receiving RI-002 1000 mg/kg, below quantifiable levels in all animals at the other doses, and detectable in 2/5 animals receiving RespiGam. Anti-RSV microneutralization assays established that sera from all groups reached neutralizing titers that would be considered protective in humans ($10\log_2$) through Day 4 [50], Unpublished observations. This experiment established the efficacy of RI-002 in the cotton rat model and showed that the higher dose of 1000 mg/kg is required to sterilize the nasal tissue of animals challenged with RSV as compared to lower doses required for sterilization of pulmonary tissue. Further, a dose–response relationship was apparent for both nasal infection and neutralizing titers in *S. hispidus* sera.

5.3. Cotton rat – immunocompromised treatment model

Because RI-002 is intended to be utilized in an immunocompromised population, cotton rats were treated with cyclophosphamide 50 mg/kg for 18 days resulting in approximately 80% suppression of white cell count and circulating Ig compared to untreated animals [50,52]. Following intranasal infection with RSV A-Long Strain (10^5 pfu/100g body weight), 10 animals in each group were treated with 3 doses (Days 1, 4, and 7) of either saline, RI-002 1500 mg/kg for all doses, or RI-002 1500 mg/kg followed by 750 mg/kg for subsequent doses. Control animals were also infected and received a single dose of either saline, RI-002 1500 mg/kg, or RI-002 750 mg/kg. Cyclophosphamide treated animals were euthanized on Day 10 and untreated animals were euthanized on Day 4. RI-002 was effective in reducing RSV viral titer by about 2–3 logs compared to saline in both the lungs and nasal tissue of treated normal and immunosuppressed animals, with a greater reduction in viral titer observed in the cyclophosphamide treated animals that received the higher dose. RSV gene expression measured by qPCR confirmed systemic dissemination of RSV in lung, liver, and kidney as well as undetectable RSV RNA in lungs and diminished RNA levels in liver and kidney following treatment with RI-002. In the same study, pulmonary histopathology of immunosuppressed cotton rats treated with high-dose RI-002 was approximately equivalent to that observed in normal uninfected rats unlike the extensive inflammatory infiltration observed in lungs of saline-treated animals (Figure 1). RSV neutralizing titers were achieved with all RI-002 dose regimens that would be considered protective in humans. An equivalent prophylaxis study, in which immunosuppressed cotton rats were treated with RI-002 1500 mg/kg or 750 mg/kg 24 h prior to RSV challenge, showed a 3–4 $\log_{10}$ reduction in RSV viral titers from lung homogenates at both doses demonstrating that a single dose of RI-002 prior to exposure is sufficient to protect cotton rats from infection.

Table 3. Summary of *in vitro* Studies for RI-002.

Study	Design	Key results	Ref.
In vitro clarification of the unique antibody composition of RI-002	Comparison of RI-002 to 9 commercial IG lots for anti-RSV antibodies	Enhanced antibodies to multiple RSV surface proteins (viral F protein, viral G protein A and B) compared to commercial IG products	Unpublished observations [48]
In vitro quantification of antibodies to respiratory viruses – RI-002 compared to commercial IVIG batches	Comparison of 3 RI-002 lots to 10 commercial IG lots for anti-RSV antibodies	Consistently higher titers against RSV, influenza A and B, parainfluenza virus serotypes 1,2, and 3, and coronaviruses 229E and OC43. Aggregate ratio of geometric mean titers (RI-002/IVIG) was 1.529 (1.227, 1.907). Although human metapneumovirus titers were elevated, significance was not reached.	[11]
In vitro neutralization titers to influenza virus	Hemagglutination inhibition titers in 2 manufactured RI-002 lots Separate HAI conducted for 6 lots of RI-002	HAI titers elevated (>160) for commonly circulating strains H1N1 pdm09, H3N2 (Switzerland), H3N2 (Texas), B-VIC, B-YAM. In the second assay, reported inhibition titers against same strains ranged from 160 to 640. Protective titers were not observed for 5 non-circulating influenza strains.	Unpublished observations [49]
In vitro bridging of RI-001 to RI-002 (TEC-16–002)	Comparison of anti-RSV neutralizing titers between RI-001 and RI-002 lots	Overall average ratio of 1.1 ± 0.2 for anti-RSV neutralizing antibody titers (RI-002/RI-001).	Unpublished observations

Table 4. Summary of preclinical studies for RI-002.

Study	Design	Key results	Ref.
S. hispidus prevention study (TEC-13–003-RPT)	Animals dosed then challenged with RSV-A Long strain. Euthanized on Day 4. 5 animals per group: RI-002: 500 mg/kg, 750 mg/kg, 1000 mg/kg RESPIGAM: 750 mg/kg Saline	RSV viral titers undetectable in lungs for all RI-002 groups. Virus detectable in 5/5 animals treated with saline and 1/5 with reference IVIG. Nasal titers were below detectable levels in all animals receiving RI-002 mg/kg, only 2/5 animals with RESPIGAM, and 5/5 with saline. Serum neutralizing titers reached protective levels.	[50]
S. hispidus immune suppressed therapeutic study (TEC-14–002-RPT)	Cyclophosphamide treated and untreated animals infected with RSV-A Long strain, then treated on Days 1 only (normal) or Days 1, 4, and 7 (immunosuppressed). Euthanized on Day 4 or 10. 10 animals per group: Immunosuppressed Saline Immunosuppressed RI-002 1500/1500/1500 mg/kg Immunosuppressed RI-002 1500/750/750 mg/kg Normal Saline Normal RI-002 1500 mg/kg Normal RI-002 750 mg/kg	2–3 $\log_2$ reduction in lung RSV viral geometric mean titer on Day 4 and 10 with both RI-002 regimens compared to saline in immunosuppressed animals. Similar reduction in viral titers with RI-002 in normal animals with no detectable viral titers in either RI-002 group on Day 10. Reduction in RSV gene expression by qPCR in lung, liver, and kidney of immunosuppressed animals comparable to normal animals receiving saline. Restoration of pulmonary histopathology to normal uninfected histopathology in immunosuppressed animals treated with higher dose RI-002. Elevated RSV neutralizing titers in normal and immunosuppressed animals at all RI-002 doses.	[50]
S. hispidus immune suppressed prophylactic study	Immunosuppressed animals received single dose of RI-002 or saline then challenged after 24 h with RSV-A Long strain. 10 animals per group: RI-002 1500 mg/kg RI-002 750 mg/kg Saline	3–4 $\log_2$ reduction in lung RSV viral geometric mean titer on Day 4 and 10 with both RI-002 doses compared to saline. Reduction in RSV gene expression by qPCR in lung, liver, and kidney of immunosuppressed animals dosed with RI-002.	[50]
S. hispidus wild-type and palivizumab-resistant RSV prophylaxis study	Animals treated with palivizumab 15 mg/kg, RI-002 1500 mg/kg, or saline then infected with wild type RSV/A/Tracy strain or palivizumab-resistant RSV/A/Tracy strain.	Palivizumab reduced RSV lung titers by greater than 2 logs with wild-type strain but did not reduce titers for palivizumab resistant strain compared to saline. RI-002 reduced lung titers of both palivizumab-resistant and wild-type virus by greater than 2 logs compared to saline. Serum neutralization titers were fourfold higher for RI-002 compared to palivizumab.	[48,51]
S. hispidus nasal tissue titers treatment study	Animals infected with RSV/A/Long strain and treated with either saline, RI-002 1000 mg/kg, RI-002 1500 mg/kg, or palivizumab 15 mg/kg	Treatment with RI-002 1000 mg/kg reduced RSV lung titers by 2 logs while RI-002 1500 mg/kg and palivizumab 15 mg/kg eliminated detectable virus. A reduction in RSV titers was observed in nasal tissue with all 3 dosing arms compared to saline with the greatest reduction observed for RI-002 1500 mg/kg. Post-infusion serum neutralizing antibody titers were confirmed to be higher with RI-002 at both doses compared to palivizumab	Unpublished observations
S. hispidus influenza study	Animals challenged with influenza/A California 07/2009 (H1N1) one day following prophylaxis with RI-002 1500 mg/kg or saline	RI-002 1500 mg/kg group had improved lung histology, reduced viral mRNA in pulmonary tissue, and reduced chemotactic chemokine (CXCL10 and RANTES) mRNA compared to saline treated animals.	Unpublished observations [49]

5.4. Cotton rat – RI-002 and palivizumab-resistant RSV

RI-002 was also tested against a wild-type RSV/A Tracy strain and a palivizumab-resistant RSV/A Tracy strain with a single-amino acid mutation at position 262 [51,52]. Since a polyclonal antibody contains thousands of antibodies of different antigenic specificities, the concentration of each specificity can never be as high as that of a monoclonal which is of a single specificity. For this reason, higher doses of a polyclonal antibody would have to be administered compared to a monoclonal antibody to achieve comparable final concentrations of a given specificity. Palivizumab 15 mg/kg reduced wild-type RSV/A Tracy lung titers by greater than 2 logs but this decrease was not observed with the palivizumab-resistant strain. RI-002 1500 mg/kg reduced lung titers by greater than 2 logs in both wild-type and palivizumab-resistant strains compared to palivizumab ($p \leq 0.00001$). An independently validated microneutralization assay established that mean RSV serum neutralizing titers from *S. hispidus* were increased by 1.5–1.9 $\log_2$ following prophylaxis with RI-002 versus palivizumab. These results were confirmed by a third laboratory that found an approximate 2 log reduction in viral load following prophylaxis with RI-002 1000 mg/kg and elimination of detectable virus with RI-002 1500 mg/kg. In the same study, virus was also eliminated from lung tissue by palivizumab 15 mg/kg (Figure 2). Reduction of RSV/A Long strain viral titer in the nasal tissue of normal cotton rats was also observed with both RI-002 doses and palivizumab at 15 mg/kg, with the greatest decrease achieved with RI-002 at 1500 mg/kg [38].

5.5. RI-002 and influenza prophylaxis

RI-002 activity against influenza is supported by *in vitro* studies demonstrating neutralizing activity against influenza virus and *in vivo* data in the *S. hispidus* model showing significant reduction in influenza virus load in the lung of influenza-infected cotton rats. The protective activity of RI-002 against multiple influenza strains has also been investigated in hemagglutination inhibition assays (HAI) and in the *S hispidus* model [49]. It is well established that protective

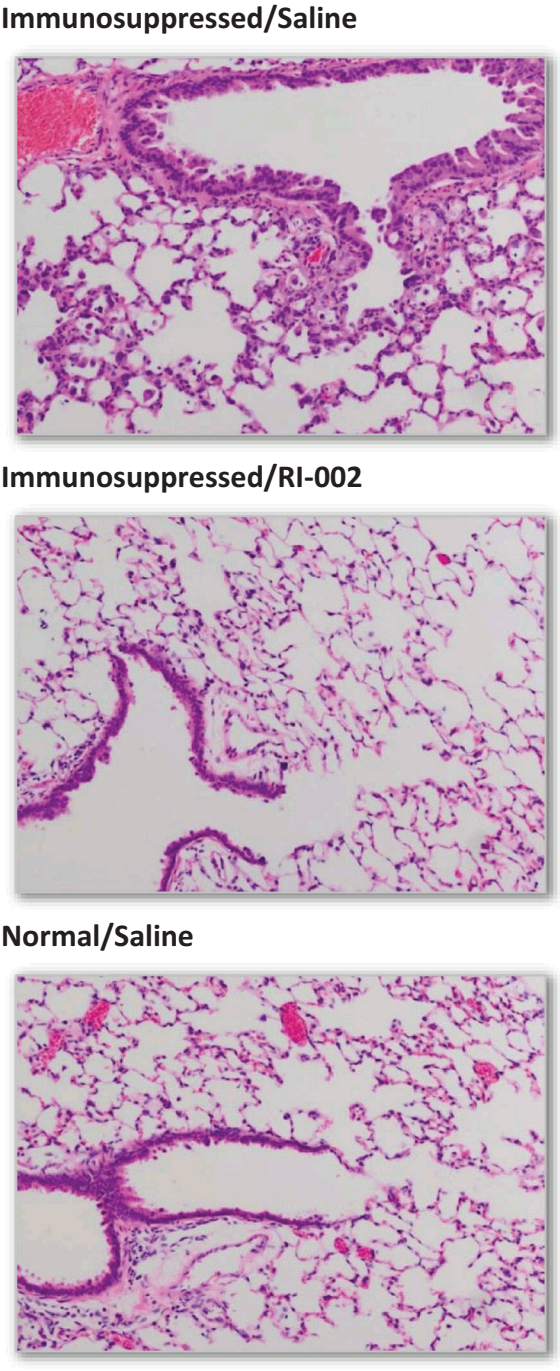

Figure 1. Pulmonary histopathology of immunosuppressed *S. hispidus* challenged with RSV/A/Long on Day 1 then treated with 3 doses of saline or RI-002 (1500 mg/kg) and normal *S. hispidus* treated with saline without challenge.

activity against influenza correlates with HAI inhibition titers greater than 1:40 [53,54]. HAI inhibition titers from six lots of RI-002 were measured against 10 influenza strains. Protective titers ranging from 1:160 to 1:640 were observed for A (H1N1)pdm 09 control antigen (A/California), Influenza A (H3) (A/Texas/50/2012), Influenza B, Yamagata lineage (B/Phuket/3073/2013), and Influenza B Victoria lineage (B/Brisbane/60/2008). Protective titers were not observed for influenza strains to which the donor population would be unlikely to be exposed including A/Anhui/1/13 (H7N9):PR8, E3, HA:256, H5 SVP from Medign, A/Viet Nam/1203/2004, H9 SVP from Medigen, A/Hong Kong/33,982/2009,0, H10 VLP from Medigen, and H10N1 (A/teal/Egypt/12,908-NAMRU3/2005 H10N1). The HAI titers present in RI-002 suggest that donors selected for exposure to RSV may also have elevated neutralizing titers and provide protective immunity against prevalent strains of influenza.

S. hispidus were treated intraperitoneally with 1500 mg/kg of RI-002 one day prior to infection with 106 TCID50 Influenza/A California 07/2009 (H1N1) and sacrificed 1 or 4 days later [49]. Lung histology showed a significant reduction in perivasculitis and alveolitis four days after infection in animals treated with RI-002 compared to saline. Influenza mRNA in the pulmonary tissue of RI-002 pretreated animals was significantly reduced on both Day 1 and Day 4. Further, chemotactic chemokine mRNA including IFN-gamma-inducible protein 10 (CXCL10) and RANTES (CCL5) were reduced in the pulmonary tissue of RI-002 treated animals suggesting that inflammation inducing infection was prevented or markedly attenuated.

5.6. RI-002 human clinical efficacy and safety

An open-label phase clinical trial was conducted to evaluate efficacy, safety, and pharmacokinetics of RI-002 in subjects with PIDD [55]. Subjects were given intravenous infusions of RI-002 at doses of 300–800 mg/kg every 3–4 weeks based on their pre-study infusion schedule for approximately 1 year. Infusion rates started at 0.5 mg/kg/min and were incrementally increased every 15 min up to a maximum of 8 mg/kg/min. Dose adjustment to maintain trough IgG concentrations >500 mg/dL was permitted throughout the study. The mean dose administered was 505.2 (4.84) mg/kg with a range of 284–1008 mg/kg with 95.8% of infusions administered at the maximum rate.

Subjects were 3–74 years of age, inclusive, with a confirmed diagnosis of PIDD who had been receiving a stable dose of IVIG (no change >50% of mean mg/kg dose) every

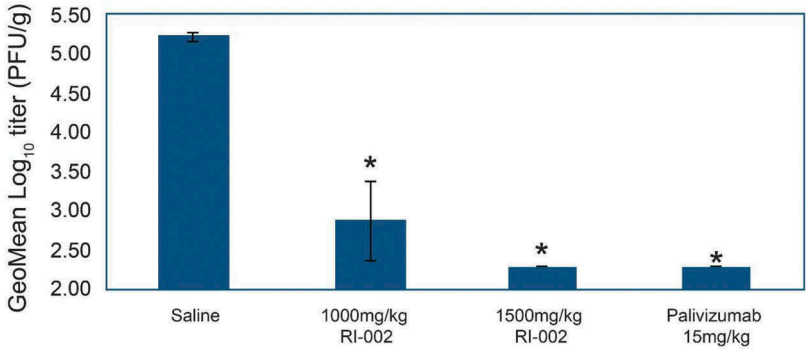

Figure 2. Lung titers in S. hispidus receiving RI-002 and palivizumab then challenged with RSV/A/Long.

3–4 weeks for ≥3 months. Subjects were excluded for a history of adverse reactions to blood-derived products, selective IgA deficiency with IgE anti-IgA, abnormal liver function, a history of deep vein thrombosis, hemolysis, positive Coombs test, or current pregnancy or lactation. 59 subjects (28 males, 31 females) with a mean of 8.66 years since diagnosis and an average age of 41.8 years were enrolled. All subjects who received at least one dose of RI-002 were included in the safety population. Demographic data was comparable for the three and four week dosing schedule subjects.

The primary efficacy end point of the study was the acute serious bacterial infection (aSBI) rate over the 12-month treatment period. Consistent with FDA recommendations, an aSBI rate of less than 1.0 infection per person-year was considered efficacious. Over 55.99 subject years, zero aSBIs were observed. Secondary end points included the incidence of all infections of any kind (3.436 infections per subject per year), number of days lost from work/school/usual activity due to infection (1.66 days per subject per year), unscheduled visits to a physician or ER due to infections (0.97 visits per subject per year), hospitalizations due to infection (0.018 hospitalizations per subject per year), number of days of antibiotic therapy (32.9 days per subject per year). A summary of clinical efficacy data is provided in Table 5. Taken together, these data attest to the efficacy of RI-002 in preventing serious infections and minimizing unscheduled medical visits and hospitalizations in this PIDD population.

Overall, RI-002 was well tolerated and met the target (<0.40) for proportion of study infusions with a Temporally Associated Adverse Event (TAAE, 0.142). Overall, 43 (72.9%) subjects experienced a TAAE in 113/793 (14.2%) infusions. 618 treatment emergent AEs were documented in 58 subjects during the study of which 55 TEAEs in 26 (44.1%) subjects were recorded as related to study drug. The most frequently reported AEs unrelated to the study drug, were headache, sinusitis, diarrhea, viral gastroenteritis, nasopharyngitis, and URTI with headache being the most frequent. No study drug-related, serious adverse events (SAEs) were reported although two SAEs (postoperative wound infection and migraine) were documented. 31 adverse infusion reactions in 18 (30.5%) subjects in 29 (3.7%) infusions were reported with headache and myalgia being most frequent. Four infusion site reactions in two subjects were also documented (infusion site extravasation and pain). A summary of clinical safety is presented in Table 6.

The relationship between dose, trough level, and risk of serious and nonserious infections as well as total trough IgG and specific antibody levels was also investigated. Pharmacokinetic sampling was performed in a subset of patients beginning at infusion 7 or 9 to ensure washout of

Table 6. Summary of temporally associated AEs (TAAEs) in phase III trial of RI-002.

	Total	3-week cycle	4-week cycle
Total number of subjects	59	19	40
Total number of infusions	793	294	499
Number of infusions with ≥1 TAAEs	113	36	77
Total number of TAAEs	158	47	111
Mean number of TAAEs per infusion	0.199	0.160	0.222
Proportion of infusions with ≥1 TAAEs (one-sided 95% upper limit)	0.142 (0.164)	0.122 (0.156)	0.154 (0.182)
Subjects with ≥1 TAAE, n (%)			
Within 1 h	28 (47.5)	7 (36.8)	21 (52.5)
Within 24 h	40 (67.8)	13 (68.4)	27 (67.5)
Within 72 h	43 (72.9)	15 (78.9)	28 (70.0)
Subjects with ≥1 study drug-related TAAE, *n* (%)			
Within 1 h	14 (23.7)	5 (26.3)	9 (22.5)
Within 24 h	21 (35.6)	7 (36.8)	14 (35.0)
Within 72 h	21 (35.6)	7 (36.8)	14 (35.0)
TAAEs reported by ≥5% total subjects, *n* (%)			
Headache	14 (23.7)	3 (15.8)	11 (27.5)
Sinusitis	6 (10.2)	2 (10.5)	4 (10.0)
Nausea	5 (8.5)	1 (5.3)	4 (10.0)
Acute sinusitis	4 (6.8)	1 (5.3)	3 (7.5)
Fatigue	4 (6.8)	2 (10.5)	2 (5.0)
Muscle spasms	4 (6.8)	1 (5.3)	3 (7.5)
Adverse drug reaction	3 (5.1)	0	3 (7.5)
Bronchitis	3 (5.1)	0	3 (7.5)
Diarrhea	3 (5.1)	1 (5.3)	2 (5.0)
Epistaxis	3 (5.1)	0	3 (7.5)
Myalgia	3 (5.1)	2 (10.5)	1 (2.5)
Oropharyngeal pain	3 (5.1)	0	3 (7.5)
Pain in extremity	3 (5.1)	0	3 (7.5)
Pruritus	3 (5.1)	0	3 (7.5)

Table 5. Summary of primary and secondary efficacy endpoints for phase III trial of RI-002.

	Total	3-week cycle	4-week cycle
Number of mITT Subjects	59	19	40
Total number subject-years	55.9	17.3	38.6
Infections			
Number of Serious acute bacterial infections (SBIs)	0	0	0
Rate of SBIs per person per years (one sided 99% upper bound)	0.000 (<1.0)	0.000 (<1.0)	0.000 (<1.0)
Total infections of any kind/seriousness	192	62	130
Infections per subject per year (one-sided 95% upper bound)	3.436 (3.869)	3.584 (4.417)	3.370 (3.893)
Antibiotic use for therapy			
Number of subjects, n (%)	37 (62.7)	12 (63.2)	25 (62.5)
Days per subject per year	32.9	41.2	29.2
Days off school/work/day care due to infection			
Number of subjects, n (%)	23 (39.0)	7 (36.8)	16 (40.0)
Total days	93	27	66
Days per subject per year (one-sided 95% upper bound)	1.66 (1.97)	1.56 (2.14)	1.71 (2.09)
Unscheduled visits to the ER and physician due to infection			
Number of subjects, *n* (%)	24 (40.7)	9 (47.4)	15 (37.5)
Total days	54	18	36
Days per subject per year (one-sided 95% upper bound)	0.97 (1.21)	1.04 (1.53)	0.93 (1.23)
Hospitalization due to infection			
Number of subjects, *n* (%)	1 (1.7)	0	1 (2.5)
Number of days	5	0	5
Hospitalizations per subject per year	0.018	0	0.026

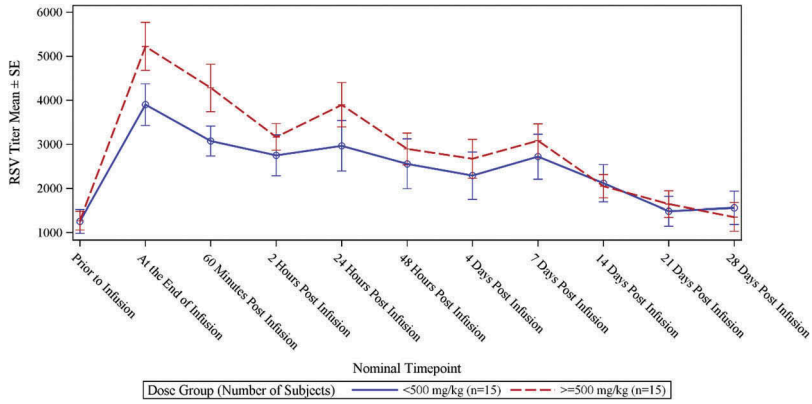

Figure 3. Neutralizing antibodies to RSV in Phase 3 RI-002 study before and post-infusion by dose and nominal time point – PK Evaluable Set (N = 30).

previous IG products. Administration of RI-002 for both treatment intervals resulted in increases in specific antibodies to CMV, tetanus, Hib, measles, RSV, and *S. pneumonia*. Figure 3 shows the profile of anti-RSV antibodies following infusion that are maintained throughout the dosing interval. As expected, the greatest increase was observed in RSV neutralizing antibodies with a post-infusion mean (95% CI) fold change from baseline (C_{max}/baseline) of 5.47 (4.37, 6.56) overall and fold changes of 4.23 (2.95, 5.51) and 6.79 (5.11, 8.47) for doses <500 mg/kg and >500 mg/kg, respectively. No apparent differences were observed between 3 and 4-week dosing intervals for mean IgG concentrations or C_{max}. There was no sex or age effect on IgG pharmacokinetics.

6. RI-002 – current status

A Biologics License Application (BLA) for RI-002 was filed with FDA's Center for Biologics Evaluation and Research (CBER) in 2015. ADMA Biologics has also filed United States Patent 9,107,906 entitled 'Compositions and methods for the treatment of immunodeficiency' [55]. The patent covers ADMA's method for producing pooled plasma containing elevated RSV neutralizing antibodies and antibodies directed against one or more respiratory pathogens. The process includes assays to screen donors for high RSV neutralizing titers, selection of the highest assayed donors where donors have an IG fraction neutralizing titer of 1800 or above, the pooling of plasma from 1000 or more donors selected in the previous steps, and the cold alcohol fractionation process for purification of the finished product. As previously discussed, the process outlined in this patent ensures consistency from product lot-to-lot, anti-RSV neutralizing titers at least twofold higher than control IG products, and enhanced binding activity against RSV and other respiratory viruses.

7. Conclusion

RI-002, and its predecessor RI-001, are novel immune globulins manufactured using a unique approach that meets specific criteria for plasma donor selection and plasma pool formulation. RespiGam, the only previous hyperimmune globulin product enriched for anti-RSV activity, is no longer marketed for several reasons including the approval of the monoclonal anti-RSV antibody palivizumab. Although it demonstrated efficacy in preventing RSV infection, it had a number of limitations on administration and was not indicated as a sole therapy for immune supplementation in the PIDD population. Although palivizumab is a current commercially available therapeutic option for patients that require prophylaxis against RSV, a monoclonal antibody, cannot provide immune reconstitution in the PIDD population-like polyclonal products, nor does it have the potential to provide protection against other respiratory viruses or palivizumab-resistant strains of RSV which are becoming more prevalent. In addition, polyclonal antibodies have the advantage of providing anti-inflammatory activity that is triggered by RSV while the monoclonal does not [56].

RI-001 and RI-002 were developed by ADMA Biologics to fill the void left by the market withdrawal of RespiGam. RI-001 was well tolerated in a phase II trial of immunocompromised patients with RSV URTI and achieved the primary efficacy end point, with a mean fold change in circulating RSV neutralized titer ≥fourfold in 6/7 (86%) patients in the high-dose arm [35]. An open-label, compassionate use treatment study was conducted in 15 patients with RSV LRTI at high risk of mortality with 73.3% survival [35]. Although no conclusions can be drawn due to limits in sample size, several encouraging trends toward improved clinical outcomes were observed. Factors associated with survival included age, early intervention, absence of need for ventilator support (10/10 patients), fewer average days from onset of respiratory symptoms to first treatment with RI-001, and shorter time from positive RSV test to first treatment with RI-001. RI-001 was also associated with significant increases in RSV serum neutralizing antibody titer in a subset of 7 immunocompromised adults with favorable clinical outcomes that were enrolled in the compassionate use study [35]. Although promising, from an efficacy and safety perspective, RI-001 was subject to the same limitations as RespiGam and did not meet FDA requirements for supplementation in the PIDD population [9].

RI-002 is currently under regulatory review and is manufactured using the novel approach of pooling plasma from more than 1,000 donors who exhibit anti-RSV neutralizing antibody titers [57]. In comparison to RI-001 titers [Unpublished observations], RI-002 meets FDA IG supplementation requirements for minimum titers of antibodies to diphtheria toxoid, measles, and polio viruses in immunocompromised populations including PIDD patients [9]. The company also has anti-RSV potency

testing manufacturing standards that are performed for each batch of RI-002. Although it has not yet been approved, comprehensive evaluation of the preclinical and clinical data collected suggests that RI-002 may provide an important option for patients benefitting from IG supplementation who are at risk of viral-induced respiratory illness.

Microneutralization assays conducted to quantify IgG titers against RSV surface proteins (F protein and G protein) demonstrated that the manufacturing process for RI-002 selects for substantially higher anti-RSV antibody titers against multiple RSV antigens compared to commercially available IG products [11]. Further, this enrichment is not limited to RSV but also yields consistently higher (1.5-fold higher aggregate mean) neutralizing titers against a host of other respiratory pathogens including influenza A and B, parainfluenza virus serotypes 1, 2, and 3, hMPV, and coronavirus 229E and OC43 [11]. Although prophylactic efficacy in human populations against these additional pathogens has yet to be established, it is probable that RI-002 may impact the incidence of respiratory infections of any cause within immunocompromised populations with a similar trend to that observed in the RespiGam PREVENT study [26]. Serum from *S. hispidus* RI-002-treated animals was tested against 10 strains of influenza by HAI inhibition assays and was considered protective against 4 prevalent circulating strains, with titers ranging from 1:160 to 1:640 [51]. Similar protection was not observed for noncirculating influenza strains, suggesting that the donor selection process used to manufacture RI-002 only enriches humoral antibodies against common respiratory pathogens when donors have been exposed. *S. hispidus* challenged with influenza/A California 07/2009 (H1N1) one day following prophylactic administration of RI-002 1500 mg/kg had significantly improved lung histology, reduced viral mRNA in pulmonary tissue, and reduced chemotactic chemokine (CXCL10 and RANTES) mRNA compared to saline-treated animals suggesting that the observed titers may be adequate to provide improved protection against circulating influenza strains [49].

In the *S. hispidus* model, RI-002 administered intraperitoneally at a dose of 500 mg/kg, 750 mg/kg, or 1000 mg/kg prior to challenge prevented RSV disease in healthy young animals. RSV was undetectable in the lungs of animals at all doses and in nasal tissue of all animals receiving 1000 mg/kg with neutralizing titers that would be considered protective in humans. In a treatment setting, *S. hispidus* that were immunosuppressed with cyclophosphamide had approximately 2–3 log reductions in RSV viral titer in lungs and nasal tissue compared to saline-treated animals [50]. Importantly, pulmonary histopathology of immunosuppressed animals treated with RI-002 1500 mg/kg was similar to the histopathology of normal uninfected animals. In a prophylaxis study of immunosuppressed animals, a single dose of RI-002 was sufficient to protect animals from infection [38].

The open-label phase III clinical trial conducted to evaluate efficacy, safety, and pharmacokinetics of RI-002 in subjects with PIDD provides strong evidence regarding the efficacy of this product against viral respiratory pathogens and serious bacterial infection in PIDD patients [49,55,58]. Overall, RI-002 was well tolerated in these patients with few adverse events and no SAEs related to the study drug. RI-002 met the primary efficacy end point of the study, achieving an aSBI rate of less than 1.0 infection per person-year with no aSBIs observed during approximately 56 subject-years. The secondary end points also supported the efficacy of RI-002 against infection, with a low incidence of infection of any kind and few days lost from usual activity, unscheduled medical visits, hospitalizations due to infection, and days of antibiotic therapy. These clinical outcomes correlated with elevations in IgG and antibodies to CMV, tetanus, measles, and *S. pneumonia*. As anticipated from the donor screening methods using to manufacture RI-002, the greatest increase was observed in post-infusion mean titers for RSV neutralizing antibodies, with the magnitude of the change dependent on dose. While it is clear that RI-002 confers passive immunity against bacterial infections and some respiratory viruses including RSV, the prophylactic efficacy of RI-002 for other respiratory viruses in humans is yet to be established.

7.1. Expert commentary

There is a gap in therapeutic options for immune globulin supplementation in PIDD patients who are at high risk of viral respiratory infections. The preclinical and clinical data collected for RI-002 supports the use of this product in a population that has few available options. Commercial IG products are standardized against pathogens that are not common within the population and are approved based on reduction in the incidence of aSBI. RI-002 extends the coverage of these products to include RSV and possibly other respiratory viruses that are commonly acquired in patients at high risk of RSV disease. The broad range of protection provided by RI-002 suggests that it will be an important agent to supplement the physician's arsenal for immunocompromised patients during RSV season and for patients that might benefit from the broad coverage of this product.

The previous AAP recommendation for use of RespiGam as a replacement for commercial IG products in immunocompromised children during RSV season did not take standardization of RespiGam into account [28]. While RespiGam was not appropriately indicated for use in this population without additional supplementation, RI-002 may be uniquely poised to fill this therapeutic role. Data collected in the *S. hispidus* model for multiple agents has proven its reliability in predicting the efficacy of new therapeutic agents against RSV and, importantly, this model can be extended to young, elderly, and immunosuppressed populations. RI-002 achieved protective titers in *S. hispidus*, protected animals from infection, and was successful in maintaining normal lung histology and in eliminating infection in treated animals [11,33,34,50]. These findings extended to both immunocompromised animals and animals infected with palivizumab-resistant RSV [48,50–52]. Data collected in *S. hispidus* are similar to the trends observed in the phase II and compassionate use study of RI-001, which provided equivalent anti-RSV neutralizing titers compared to RI-002. The neutralizing titers that were reached in the animal

model were on average twofold higher than that achieved with the clinical dosing regimen of palivizumab. Although limited in size, these studies demonstrate that RI-002 achieves high RSV neutralizing antibody titers that can be considered protective in humans and could result in improved outcomes in RSV-infected patients when treatment begins soon after the onset of symptoms.

Importantly, RI-002 meets IG antibody standardization requirements and its phase III primary efficacy end point, with zero serious bacterial infections observed over 56 person-years, and is therefore suitable for use in supplementation of PIDD patients [9,11,55]. In addition to reduction in the incidence of serious bacterial infection and RSV, neutralizing antibody titers against other respiratory pathogens are also elevated [11]. In *S. hispidus*, RI-002 achieved protective titers against four strains of commonly circulating influenza, improved lung histology post-infection, reduced or eliminated influenza viral mRNA, and suppressed chemotactic signaling [49,50]. Taken together, these data suggest that a comparable effect is possible in humans although this remains to be determined. The process of enriching for anti-RSV antibodies within the donor pool is strongly correlated with increased antibody titers against other respiratory viruses and this finding is reproducible in all lots tested [10]. The impact of increased humoral protection against a range of commonly circulating respiratory viruses that pose a risk to PIDD patient is not yet known, although consistent supplementation with IG against respiratory illness is likely to be of clinical benefit based on previous data generated by the PREVENT study group.

7.2. Five-year view

RI-002 has potential to redefine treatment within immunocompromised populations where vaccines are often ineffective. While commonly used prophylactic agents provide protection against a limited spectrum of specific pathogens, the potential for broad-spectrum protection against respiratory viruses that have previously required additional prophylactic agents or for which no protective agents are available will be an invaluable to physicians seeking to optimize protection of these patients with enhanced passive immunity to the range of commonly encountered pathogenic viruses. In addition to these patients, it could be used in patients who have been hospitalized with severe RSV respiratory infections who have not responded to conventional therapy. The observed correlation between selection for donors with high-titer RSV neutralizing antibodies and elevated antibody titers against commonly circulating viruses may pave the way for additional IG products with expanded coverage.

Key issues

While a single prophylactic option for RSV is currently marketed, no product has been previously developed that provides both RSV prophylactic activity and meets minimum FDA requirements for antibody standardization for use as an IG supplement in PIDD patients.

- RI-001, an immune globulin product manufactured from pooled plasma of donors with elevated RSV neutralizing titers, was well tolerated in a Phase II study and achieved a fold-change in circulating RSV neutralizing titer ≥ 4-fold in 6/7 (86%) patients at a dose of 1500 mg/kg. An open-label compassionate use study was also conducted that suggested that RI-001 may provide a survival benefit to subjects, particularly when treatment is initiated early.
- RI-002 is currently under development and is manufactured using the pooled plasma of more than 1000 donors that had the highest titers of anti-RSV neutralizing antibodies. In comparison to RI-001, RI-002 is standardized with equivalent anti-RSV titers using a proprietary standard. RI-002 meets FDA immunoglobulin supplementation requirements for minimum titers of antibodies to diphtheria toxoid and measles and polio viruses in immunocompromised populations including PIDD patients.
- The manufacturing process for RI-002 selects for higher titers of anti-RSV antibodies against multiple RSV antigens compared to commercially available IG products. Further, this selection also yields consistently higher (1.5-fold higher aggregate mean) neutralizing antibody titers against other respiratory pathogens.
- Evidence from *S. hispidus* preclinical testing demonstrates that RI-002 is effective for RSV treatment and prophylaxis in both normal and immunocompromised hosts and also shows some efficacy against commonly circulating strains of influenza. Further, RI-002 is protective against palivizumab-resistant RSV, probably because RI-002 contains antibodies targeting multiple RSV surface proteins.
- RI-002 was well tolerated in a Phase III clinical trial and met the primary efficacy endpoint of <1 serious bacterial infection per person year, with no serious bacterial infections documented over 56 person-years.

Information resources

Additional information regarding the status of RI-002 can be found on the ADMA Biologics, Inc. website, http://www.admabiologics.com/

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Declaration of interest

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Geolocation information

North America

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