High Titer Anti-RSV Polyclonal Antibody (RI-002) Prevents Infection with Palivizumab Resistant RSV in Cotton Rats and Achieves Greater Neutralizing Anti-RSV Activity as Compared to Palivizumab

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Introduction

Palivizumab (PZ) is a monoclonal antibody and the only product that is FDA approved for the prevention of RSV pulmonary disease in premature-born children under the age of 2. Many Investigators have demonstrated that when RSV is grown in vitro in the presence of PZ, mutations arise which are primarily located in the F gene and which render the virus resistant to PZ. Furthermore, under in vivo conditions of prolonged pulmonary RSV replication in the immune suppressed cotton rat animal model, the presence of PZ also has been documented to induce the emergence of RSV escape mutants that are resistant to neutralization by PZ. PZ-resistant RSV mutants have been described in various clinical settings and their frequency reported in the literature can vary from 1%-9%. **(Table 1)**

Table 1: Incidence of Palivizumab Resistance Among RSV Clinical Isolates

Clinical Study	Rate
Papenburg et al. Emerg Inf Dis 2012;18:120-4.	8.7%
Zhu et al. J Inf Dis 2011;203:674-82.	5.4%
Boivin et al. J Clin Virol 2008;42:52-7.	9.0%

In a recently completed Phase III trial in Primary Immune Deficiency Disease (PIDD) patients, RI-002 successfully achieved the primary endpoint and compared very favorably in the secondary endpoints to other commercial immune globulin products.

Objective

To study whether PZ-resistant strains of RSV can be neutralized in vitro with RI-002, which is an IVIG obtained from plasma donors tested to be hyperimmune for RSV that was manufactured to meet FDA criteria for IVIG, as well as contain standardized and elevated levels of neutralizing anti-RSV antibodies. We also wished to determine whether RI-002 when used in vivo could prevent RSV induced pulmonary disease in cotton rats infected with a PZ-resistant strain of RSV.

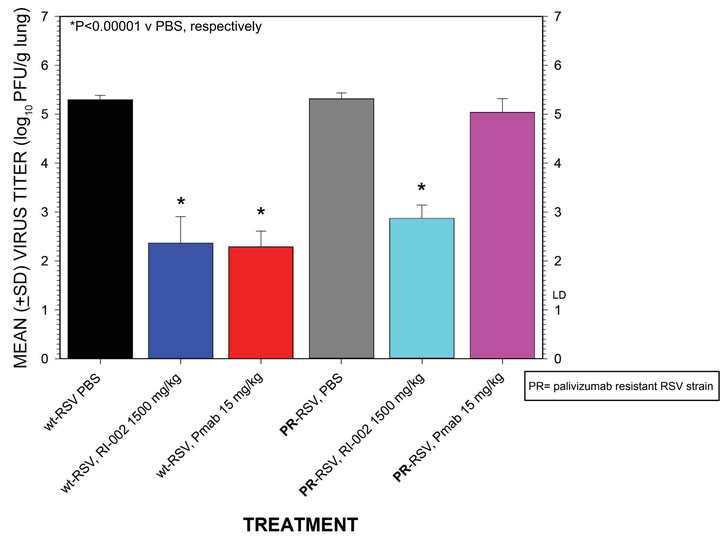
Method

PZ-resistant mutants of RSV were generated at Baylor College of Medicine in the laboratory of Dr. Pedro Piedra and Dr. Brian Gilbert after serial passage of the virus in increasing concentrations of PZ. The PZ-resistant mutant virus had a single amino acid mutation at position 262. Cotton rats were injected on day 0 with PZ at 15mg/kg or with RI-002 at 1500 mg/kg and one day later challenged intranasally with 2 x 10^5 RSV or 2 x 10^5 PZ resistant RSV. Four days later animals were bled, sacrificed, lung lavage cultured for the presence of RSV and serum tittered for neutralizing activity against the wild type RSV and the PZ-resistant strain of RSV.

Results

While PZ at the clinically approved dose of 15mg/kg reduced RSV lung titers by greater than 2 logs, it was completely ineffective in reducing lung titers in the cotton rats infected with the PZ-resistant strain. In contrast, when RI-002 was administered at 1,500 mg/kg, there was a significant decrease in RSV lung titers in the cotton rats infected with the PZ-resistant strain. **(Figure 1)**

Figure 1: Effect of RI-002 IVIG on RSV Lung Lavage Virus Titers



When serum anti-RSV neutralizing titers were measured in the serum of cotton rats treated with RI-002 or with PZ using a microneutralization assay developed and validated at Baylor College of Medicine, the neutralizing titers achieved by RI-002 at 1500 mg/kg were approximately 2 $\log_2$ greater than that achieved by PZ at 15mg/kg ($p \leq 0.00001$). **(Table 2)**

Table 2

Group	Treatment/ Challenge Virus	RSV neutralization titer ($\log_2$) in cotton rat							T test/2 v. Group 1*
		#1	#2	#3	#4	#5	Mean	SD	
1	PBS/wt-RSV	2	2	2	2	2	2	0	---
2	RI-002/wt-RSV	9.0	9.0	9.5	9.0	8.0	8.9	0.5	<0.00001
3	Pmab/wt-RSV	7.0	7.0	7.5	7.5	7.5	7.3	0.3	<0.00001
4	PBS/**PR**-RSV	2	2	2	2	2	2	0	---
5	RI-002/**PR**-RSV	9.0	9.0	8.5	9.5	9.0	9.0	0.4	<0.00001
6	Pmab/**PR**-RSV	7.0	7.0	7.0	8.0	7.0	7.2	0.4	<0.00001

Pmab= palivizumab 15mg/kg; PR= palivizumab resistant RSV

This experiment demonstrated the comparable efficacy of palivizumab and RI-002 in diminishing viral load in RSV infected cotton rats and that RI-002 was also effective in preventing RSV infection with a palivizumab-resistant strain of RSV. Serum neutralization titers achieved by palivizumab at 15mg/kg were 4-fold lower than those achieved by 1500mg/kg of RI-002. Since this dose of palivizumab at 15mg/kg has been shown to be effective in humans, as well as the dosing regimen has been documented to be effective in the cotton rat animal model and correlated with effective dosing in humans, the fact that higher neutralizing titers are achieved with RI-002 would predict and suggests that a dose of 1500mg/kg of RI-002 would be effective in preventing RSV disease in humans.

Pre-Clinical Invitro

While antibodies to the F protein of RSV (the target of palivizumab) have been shown to inhibit viral fusion and entry of RSV into cells, G protein has demonstrated the ability to mediate multiple anti-inflammatory effects (Borchers, 2013). G protein plays a substantial role in dampening the host immune response to RSV infection as well as inducing other steps that contribute to RSV inflammatory disease.

To further evaluate components in RI-002 which could account for the anti-RSV neutralizing activity to PZ-resistant RSV that displayed altered F protein expression, we compared the anti-G antibody titers present in 3 manufactured lots of RI-002 to 9 commercial lots of IG **(Table 3)**. There were enhanced levels of antibodies to protein G in RI-002 as compared to commercial lots of IG, suggesting that donors who have high titers to RSV as measured by microneutralization assay also have high titers to multiple determinants on the RSV surface. This may explain why RI-002 was effective in preventing RSV disease even when animals were challenged with a PZ-resistant strain of the virus which had a single amino acid substitution on the F protein.

Table 3: Comparison of RSV Anti G Protein Antibodies in 3 RI-002 Lots and 9 Commercial Lots of IG

Analysis	Statistics	ADMA RI-002 Lots	Commercial IG Lots
Antibody to F Protein	n	3	9
	Mean (SE)	263967.4 (0.0000)	181315.7 (19143.44)
	SD	0.0000	57430.32
	Median	263967.4	186653.1
Antibody to G Protein (A)	n	3	9
	Mean (SE)	154987.2 (16768.71)	89830.37 (10865.20)
	SD	29044.26	32595.59
	Median	171755.9	85877.94
Antibody to G Protein (B)	n	3	9
	Mean (SE)	122056.9 (24063.56)	44898.76 (4099.654)
	SD	41679.31	12298.96
	Median	117312.7	41476.31

Summary and Conclusion

There have been many reports in the literature of patients who have failed palivizumab treatment and who have been shown to harbor palivizumab resistant strains of RSV. The extent to which the emergence of PZ-resistant RSV strains may account for the many cases of hospitalized infants and children who have failed prophylaxis with palivizumab is unknown. This is primarily due to the fact that at the present time, investigators do not routinely pursue genotyping of the infecting RSV in patients who have failed palivizumab treatment. With the adoption of routine testing and genotyping, a more defined rate of infection could be determined.

RI-002 is an IVIG manufactured from the plasma of donors who have naturally elevated neutralizing antibody titers to RSV, and are thus defined to be hyperimmune. In a recently completed Phase III trial in 59 PIDD patients with RI-002, there were zero serious bacterial infections observed and the secondary endpoints were generally more favorable when compared to other data from published IG Phase III trials.

The data shown in this poster demonstrates that RI-002 has neutralizing activity on an RSV strain that is resistant to the monoclonal anti-RSV antibody palivizumab, possibly reflecting the elevated anti G protein antibodies that are present in the RI-002 IG preparation. Moreover, the neutralizing activity in the serum of cotton rats treated with RI-002 was fourfold higher than that observed in the serum of cotton rats that received palivizumab. Since palivizumab at 15mg/kg has been shown to be effective not only in preventing infection in cotton rats but also in preventing RSV infection in humans, the encouraging results obtained with RI-002 in this animal model warrant additional studies in humans for confirmatory purposes.