# PROTECTION AGAINST PALIVIZUMAB RESISTANT RSV WITH AN IVIG CONTAINING HIGH TITER ANTI-RSV NEUTRALIZING ANTIBODIES

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## Introduction

Although palivizumab has been successfully utilized as prophylaxis for RSV infection for the past 17 years, an increasing number of reports have appeared in the literature in the last few years raising the specter of the emergence of palivizumab-resistant variants of RSV (Bates 2015, DeVincenzo 2004, Zhu 2012)[1,2,3]. Hospitalizations due to RSV infection in subjects who received palivizumab, while generally rare, are nonetheless increasingly well described. Breakthrough infection is more likely during the first and second injection interval or when palivizumab administration is performed in a clinic or office instead of the hospital or specialty clinic (Parnes 2003, Frogel 2008)[4,5]. When RSV infects patients when trough levels of palivizumab are low the virus is subject to selection pressure which may lead to resistant variants of the virus (Adams 2010)[6]. ADMA has produced an immune globulin product (RI-002) that contains high titer anti-RSV neutralizing antibodies, which recently completed a Phase III pivotal trial in 59 patients with primary immune deficiency disease (PIDD) and which met the primary endpoint[7].

[1]Bates, J. T., et al. 2014. *Virology* 454-455: 139-144.
[2]DeVincenzo, J. P., et al. 2004. *Antiviral Res* **62**(1): 47-51.
[3]Zhu, Q., et al. 2012. *J Infect Dis.* **205**(4): 635-638.
[4]Parnes, C., et al. 2003. *Pediatr Pulmonol* **35**(6): 484-489.
[5]Frogel, M., et al. 2008. *J Perinatol* **28**(7): 511-517.
[6]Adams, O., et al. 2010. *Clin Infect Dis.* **51**(2): 185-188.
[7] Mond, J. J., et al. 2015. J Allergy Clin Immunol. **135**(2): AB89.

## Experimental Aim

The goal of these experiments was to compare the effectiveness of the anti-RSV monoclonal antibody, palivizumab, to the high titer anti-RSV neutralizing polyclonal IVIG, RI-002 when administered prophylactically to respiratory syncytial virus (RSV) infected cotton rats (CR). Cotton rats were infected with either wild type (wt)-RSV/A/Tracy **(wt-RSV/A/Tracy)** or a palivizumab-resistant RSV/A/Tracy **(PR-RSV/A/Tracy)**. Viral titers in lung lavage fluids of the RSV infected cotton were compared to untreated cotton rats.

## Methods

Palivizumab-resistant mutants of RSV/A/Tracy was generated after serial passage of the virus in increasing concentrations (40, 80, 160 and 320 µg/mL) of palivizumab (Pmab). Palivizumab-resistant mutant virus had a single amino acid mutation at position 262. Parental strains had asparagine at amino acid 262 and the resistant mutants had a tyrosine substitution. The palivizumab resistant mutant infected cotton rats comparable to that seen with the parent virus, however, the mutant virus was resistant to the effects of palivizumab immunoprophylaxis. Cotton rats were injected i.p. with RI-002 (1500mg/kg) or palivizumab (15mg/kg) one day prior to RSV infection. Virus quantification was performed on lung lavage fluid by semi-quantitative plaque assay. RSV-specific neutralizing antibody levels in sera of cotton rats given RI-002 or palivizumab was determined by a microneutralization assay on days 0 and +4 using the wt-RSV/A/Tracy.

**Serum neutralization assay** Tests for serum neutralizing antibodies to RSV/A/Tracy (RSV/A) were performed in 96-well microtiter plates with HEp-2 cells. Serial two-fold dilutions in duplicates starting at 3 $\log_2$ were performed to determine the neutralizing antibody (NtAb) titer for each sample. The neutralizing antibody titer is defined as the serum dilution at which >50% reduction in viral cytopathic effect (CPE) is observed. CPE is defined as tissue destruction and is determined visually after the wells are fixed with 10% neutral buffered formalin and stained with crystal violet.

**Plaque Assay on lung fluid** Plaque assays on lung lavage fluid was performed using 24-well tissue cultures plates containing nearly confluent monolayers (20 to 40 x$10^4$ cells/well) of HEp-2 cells. Sample was added to wells in duplicate and allowed to adsorb for 90 min with occasional gentle agitation. After the inoculum was removed, monolayers were overlayed with 0.75% methylcellulose in 2% FBS-MEM containing antibiotics, vitamins and other nutrients. The plates were placed in a 36°, 5% $CO_2$ incubator for six days and then plates were stained with 0.01% crystal violet/10% formalin solution (1.5 mL/well) and allowed to sit for 24-48 hr at room temperature and plaques were counted and virus titers calculated as total $\log_{10}$ PFU/g for lungs.

### Table 1. Neutralizing activity of RI-002 on a wild type RSV (RSV wt) and a Palivizumab resistant strain of RSV (RSV PR)

| Anti RSV source | RSV strain | EC 50 (ug/ml) |
|---|---|---|
| RI-002 | RSV wt | 3.1 |
| Palivizumab | RSV wt | 0.39 |
| | | |
| RI-002 | RSV PR | 3.9 |
| Palivizumab | RSV PR | >1000 |

### Table 2. RSV titers in lung lavage fluids when given palivizumab or RI-002 24 hours prior to RSV infection

| Group | Treatment/ Challenge Virus | RSV titer ($\log_{10}$ PFU/g lung) in cotton rat | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | | Mean | SD |
| | | Palivizumab sensitive RSV | | | | | | | |
| 1 | PBS/wt-RSV | 5.42 | 5.18 | 5.35 | 5.23 | 5.25 | | **5.29** | 0.10 |
| 2 | RI-002/wt-RSV | 2.11 | 2.23 | 2.79 | 2.08 | 3.12 | | **2.47** | 0.46 |
| 3 | Pmab/wt-RSV | 2.27 | 1.73 | 2.53 | 2.35 | 2.67 | | **2.31** | 0.36 |
| | | Palivizumab resistant RSV | | | | | | | |
| 4 | PBS/PR-RSV | 5.30 | 5.51 | 5.29 | 5.27 | 5.19 | | **5.32*** | 0.12 |
| 5 | RI-002/PR-RSV | 2.57 | 3.20 | 3.06 | 2.89 | 2.59 | | **2.86*** | 0.28 |
| 6 | Pmab/PR-RSV | 4.60 | 5.01 | 5.07 | 5.39 | 5.11 | | **5.03** | 0.28 |

wt wild type RSV
PR palivizumab resistant RSV
Pmab Palivizumab
*P values (Student t test, two-tailed): Group 4 v 5, Group 5 v 6, P<0.00001.

### Table 3. RSV/A serum neutralization titers with cotton rat serum taken immediately after infusion

| Group | Treatment/Challenge Virus | RSV/A neutralization titer ($\log_2$) in cotton rat | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | 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 |
| 3 | Pmab/wt-RSV | 7.0 | 7.0 | 7.5 | 7.5 | 7.5 | **7.3** | 0.3 |
| | | | | | | | | |
| 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 |
| 6 | Pmab/PR-RSV | 7.0 | 7.0 | 7.0 | 8.0 | 7.0 | **7.2** | 0.4 |

*P values (Student t test, two-tailed):
Group 2 v 3 P<0.00066; Group 5 v 6, P=0.00011.

### Table 4. RSV/A serum neutralization titers with cotton rat serum taken 4 days after infusion

| Group | Treatment/Challenge Virus | RSV/A neutralization titer ($\log_2$) in cotton rat | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | Mean* | SD |
| 1 | PBS/wt-RSV | 2 | 2 | 2 | 2 | 2 | **2** | 0 |
| 2 | RI-002/wt-RSV | 9.0 | 8.5 | 8.5 | 8.5 | 8.0 | **8.5** | 0.4 |
| 3 | Pmab/wt RSV | 7.0 | 6.0 | 7.0 | 7.0 | 6.5 | **6.7** | 0.4 |
| | | | | | | | | |
| 4 | PBS/PR-RSV | 2 | 2 | 2 | 2 | 2 | **2** | 0 |
| 5 | RI-002/PR-RSV | 8.8 | 7.5 | 8.5 | 7.5 | 7.5 | **8.0** | 0.6 |
| 6 | Pmab/PR-RSV | 5.5 | 6.0 | 6.5 | 7.0 | 5.5 | **6.1** | 0.7 |

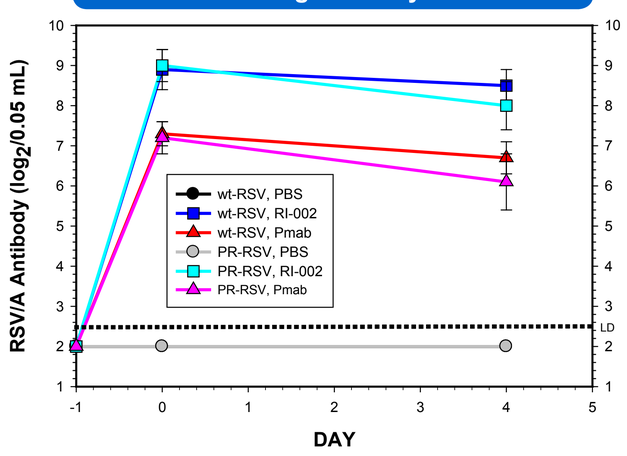
* Student t test, two-tailed. Minimal detection = 2.5; for statistical analysis a value of <2.5 was counted as 2. Additional significant P values (Student t test, two-tailed): Group 2 v 3, 4, 6, P<0.00011; Group 3 v 4, 5, P < 0.007; Group 4 v 5, 6, P<0.00001; Group 5 v 6, P=0.0019.

### Table 5. RSV/B/18537 serum neutralization titers with cotton rat serum taken immediately after infusion

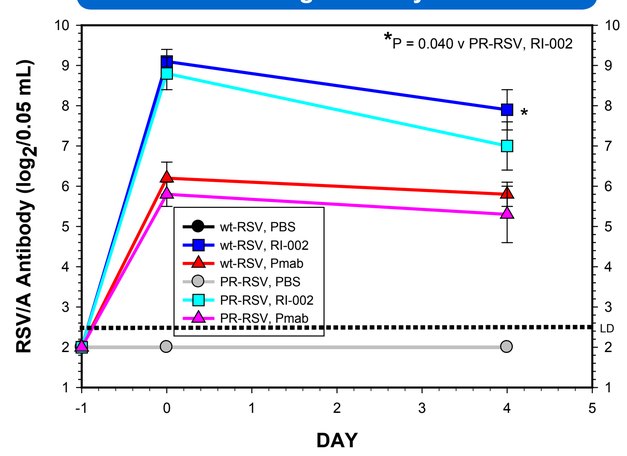
| Group | Treatment/Challenge Virus | RSV/B neutralization titer ($\log_2$) in cotton rat | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | Mean* | SD |
| 1 | PBS/wt-RSV | 2 | 2 | 2 | 2 | 2 | **2** | 0 |
| 2 | RI-002/wt-RSV | 9.0 | 9.0 | 9.5 | 9.0 | ns | **9.1** | 0.3 |
| 3 | Pmab/wt-RSV | 6.0 | 6.0 | 6.0 | 6.0 | 7.0 | **6.2** | 0.4 |
| | | | | | | | | |
| 4 | PBS/PR-RSV | 2 | 2 | 2 | 2 | 2 | **2** | 0 |
| 5 | RI-002/PR-RS | 9.0 | 9.0 | 8.0 | 9.0 | 9.0 | **8.8** | 0.4 |
| 6 | Pmab/PR-RSV | 5.5 | 5.5 | 6.0 | 6.0 | 6.0 | **5.8** | 0.3 |

*P values (Student t test, two-tailed): Group 2 v 3, P<0.00001; Group 5 v 6, P<0.00001.

### Fig. 1 Effect of IP RI-002 IVIG on RSV/A/Tracy Serum Neutralizing Antibody Titers




### Fig. 2 Effect of IP RI-002 IVIG on RSV/B/18537 Serum Neutralizing Antibody Titers




## Conclusion

IVIG containing high titer neutralizing antibodies to RSV can prevent infection in cotton rats infected with a palivizumab resistant strain of RSV. Moreover, the anti-RSV neutralizing antibody titers that were attained in the serum of cotton rats using the IVIG product were between 4-8 fold greater than that achieved in the sera obtained from cotton rats injected with Palivizumab. This data reflects another aspect of the unique antibody profile of RI-002.

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