



**Department of Health and Human Services**  
**Public Health Service**  
**Food and Drug Administration**  
**Center for Biologics Evaluation and Research**

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**To:** BLA, BL 125590/0  
**From:** Evi Struble, Ph.D.  
**Through:** Dorothy E. Scott, M.D.  
**Applicant:** ADMA Biologics  
**Product:** ASCENIV®, Immune Globulin Intravenous (Human) 10%  
**Subject:** Final Memo, Nonclinical Pharmacology/Toxicology

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**Brief Description and Main Findings**

ASCENIV® (also referred to as RI-002 preparation) is a liquid formulation of 10% plasma derived human immune globulin (IgG). Primary Immune Deficiency Disorders (PIDD) 300-800 mg/kg every 3-4 weeks infused intravenously at a rate up to 0.5 mg/kg/min (0.005 mL/kg/min).

ADMA biologics claims that high level of (b) (4) are present in the preparation and has submitted several non-GLP animal studies to justify adding a label claim that acknowledges this. Absent a clinical trial demonstrating safety and efficacy of this preparation in (b) (4) disease, these studies are not sufficient to support a label claim.

**Excipients and Impurities**

The specifications for the excipients are shown in Table 1 (modified from Quality Control Release Specification). From these Polysorbate 80 (PS80, highlighted in table) stands out as an excipient of some concern based on the higher than usual concentration.

Table 1. Specifications for excipients and impurities

TEST	SPECIFICATION
(b) (4)	(b) (4)
Chloride	100 - 140 mM
Glycine	200 – 290 mM
(b) (4)	(b) (4)
pH	4.0 – 4.6
Polysorbate 80	0.15 – 0.25 %
Residual Triton X-100	(b) (4)
Residual Tri-n-Butyl Phosphate (TnBP)	(b) (4)

Several IGIV preparations contain PS80 as an impurity following solvent detergent treatment, but in 10x or lower levels (Table 2) compared to ASCENIV®. Another product, Bivigam® contains the same amount of PS80 and, based on results from animal studies (1, 2) it was noted during the review of this BLA that the possibility exists for cardiovascular adverse events in the clinic. A post-marketing study is underway for this product to assess these effects. This study has not been completed.

Table 2 Concentration of PS80 in IGIV Products and ASCENIV®

Product Name/Concentration (Sponsor)	PS80 Concentration
Gammaplex/10% (BPL)	0.006%
(b) (4)	(b) (4)

(b) (4)	(b) (4)
IgPro20/20% (CSL)	0.003%
Bivigam®/10% (Biotest)	0.25%
ASCENIV®/10% (ADMA)	0.25%

Glycine is another excipient used in ASCENIV®. It is also used in other approved products as shown in Table 3, including Bivigam® and Gammagard® at similar amounts.

Table 3 Concentration of Glycine in Approved Products and ASCENIV®

Product Name/Concentration (Sponsor)	Glycine Concentration
Gamunex (Talecris)	0.24 M
Gammagard Liquid/10% (Baxter)	0.25 M
Gammagard S/D/5% (Baxter)	0.30 M
Bivigam®/10% (Biotest)	0.29 M (~2.2%)
ASCENIV®/10% (Biotest)	0.29 M (~2.2%)
Alphanate/150 IU/mL (Grifols)	1.8 M

### *Conclusions*

Based on the excipient profile of ASCENIV®, the possibility exists for cardiovascular adverse events in the clinic. From the nonclinical toxicology data, it is recommended that the BLA be approved for the proposed indication with a post marketing commitment for assessing PS80 related toxicity.

### **Labeling**

ASCENIV® formulation contains a 10 times higher concentration of PS80 when compared to other IGIV products, thus carrying a risk for cardiovascular, hepatic and renal adverse events in the clinic. These effects were not seen in the clinical study performed in support of this BLA. Until the post-marketing study is complete, the risk should be noted in the label.

### *Letter Ready Comments for Labeling*

1. Please update section 13 in the PI for ASCENIV. An example of information that should be included in this section is shown below. These or other pertinent publications can be cited in this section: a) National Toxicology Program Technical Report 415 and b) Pestel et al, Effect of commonly used vehicles on gastrointestinal, renal, and liver function in rats, J Pharmacol Toxicol Methods. 2006 Sep-Oct;54(2):200-14.

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, mutagenesis, impairment of fertility

*No animal studies were conducted to evaluate the carcinogenic or mutagenic effects of ASCENIV® or its effects on fertility.*

### 13.2 Animal Toxicology and/or Pharmacology

*No animal studies were conducted to evaluate possible toxicity of ASCENIV® in animals.*

*ASCENIV contains Polysorbate 80 at a concentration of up to 2.5 mg/mL. Intravenous administrations of Polysorbate 80 in multiple species have been linked with a decrease in blood pressure. In rats, single doses of Polysorbate 80 that were up to 25 times higher than the amount from 800 mg/kg ASCENIV resulted in an increase of liver enzymes and total bilirubin.*

## **Proof-of-Concept Pharmacology Studies**

### *Summary*

RI-002 preparation was tested in RSV infection cotton rat models using three strains of RSV ((b) (4) [REDACTED]). A justification on why cotton rats are appropriate models for RSV infection efficacy studies in animals was submitted and showed convincing evidence for the appropriateness of this model. There is regulatory precedence in using this model as proof of concept study in the approval of RSV monoclonal antibody palivizumab.

The studies submitted were performed by a CRO for ADMA and, even though a signature of quality assurance director is included, there is no claim for GLP adherence in these studies.

Two different clinical scenarios were assessed pre-clinically:

1. Infected normal or immunosuppressed cotton rat IgG treatment
  - a. Single and multiple (3x every 3 days) doses of IgG were used
  - b. Dose in the most complete study (TEC-14-002) was 1500 mg/kg
  - c. Analysis for viral titers in lung and nose were performed 4 and 10 days after challenge and for replication 10 days after challenge; blood RSV antibody titers were measured on day 0, 4 and 10 using MNA. There is no differentiation between administered human RSV IgG and CR anti-RSV antibodies in non-immunosuppressed CR.
  - d. The treatment with IgG preparation lowers virus titers and improves pathology scores. Palivizumab was reported to only reduce viral titers, but did not improve pathology. There is a slight indication of dose-response in the IgG effect, especially in pathology score and viral levels on day 10.
2. Infected immunosuppressed cotton rat prophylactic IgG administration
  - a. In study TEC-13-003 both ADMA IgG (500, 750 and 1500 mg/kg) and RespiGam® (750 mg/kg) were administered as a single dose and analyses were performed four days after infection (five days after IgG administration). All these doses showed reduction in viral load in lungs and nose with the lowest dose of ADMA IgG showing a lower effect.

Similar antibody levels were measured in IgG high dose and RespiGam® dose.

*Conclusions*

Based on these pharmacology studies there is enough evidence to perform a clinical study in intended population, i.e. subjects with RSV infection. If such studies were to be initiated, a justification of the efficacious starting dose should be submitted. However, there is no PK analysis included in animal studies, and the neutralizing titers for only day 4 and day 10 after infection have been performed/submitted.

*Complete Review*

**Technical report TEC-14-002-RPT II-89: Normal and Immunosuppressed Cotton Rat: ADMA Anti-RSV Treatment Study and Determination of Serum RSV Neutralization Titers**

Aim: To evaluate the efficacy of ADMA RI-002, as therapeutic treatment in normal and immunosuppressed cotton rats challenged with RSV-(b) (4)

Performing laboratory: In-life phase was performed by CRo for ADMA (b) (4)

Date: 01/09/2015

Design: 50 experimental CR were divided into 7 groups; 4 groups with 10 animals each and assigned Group A, B, C or D; 2 groups with 5 animals each and assigned Group E or F; see Table 2

Groups A, B, C were immunosuppressed for 18 days and on D21 (infection d0) infected IN with  $10^5$  pfu/100 g body weight of RSV-(b) (4) strain followed by treatment on d1 with either saline or RI-002 (Table 2). In one arm, n=5 animals were sacrificed and tissues and blood collected. In the second arm, CR received IgG or saline on d3 and d7 and terminated for analysis on d10.

Groups D, E and F were challenged IN with  $10^5$  pfu/100 g body weight of RSV-(b) (4) strain followed by treatment on d1 with either saline or RI-002 at two different doses (table 2). Groups E and F and n=5 from group D were sacrificed on d4 for analysis.

**Table 2 Cotton Rat Therapeutic Treatment Groups and Dosing Chart**

Group	# of Animals	Treatment	Days	Volume per 100 g body weight, i.p.
A Immunosuppressed	10	Saline	d1 d4 d7	1.5/1.5/1.5 mL
B Immunosuppressed	10	RI-002 1500/1500/1500 mg/kg	d1 d4 d7	1.5/1.5/1.5 mL
C Immunosuppressed	10	RI-002 1500/750/750 mg/kg	d1 d4 d7	1.5/0.75/0.75 mL
D Normal	10	Saline	d1	1.5 mL
E Normal	5	RI-002 1500 mg/kg	d1	1.5 mL
F Normal	5	RI-002 750 mg/kg	d1	0.75 mL
G Immunosuppressed	3	TBD*		

\*TBD: Group G is extra 3 animals to undergo cyclophosphamide treatment. These animals will be used as possible replacements for potential mortalities in Groups A, B or C. Group assignments determined on day of RSV infection (d0).

Outcome measures: Viral titration in lung and nasal tissue homogenates, qPCR for RSV (b) (4) protein in lung, liver and kidney tissue, neutralizing assay (MNA) in blood.

Results: Viral titers using plaque assay plaque forming units (pfu) per gram of lung or nasal tissue homogenate show decreased viral titers in IS CR receiving 3 doses of IgG (1,500 mg/kg and 750 mg/kg) 4 and 10 days after infection. Decreased titers are also seen in normal CR 4 days after infection. Levels of RSV RNA detected by PCR were lower in the lungs of IS CR treated with IgG compared to saline controls 10 days after infection, but not as low as the normal CR 10 days after infection. Better pathology scores, especially less epithelial damage, are seen in day 10 in treated animals versus untreated.

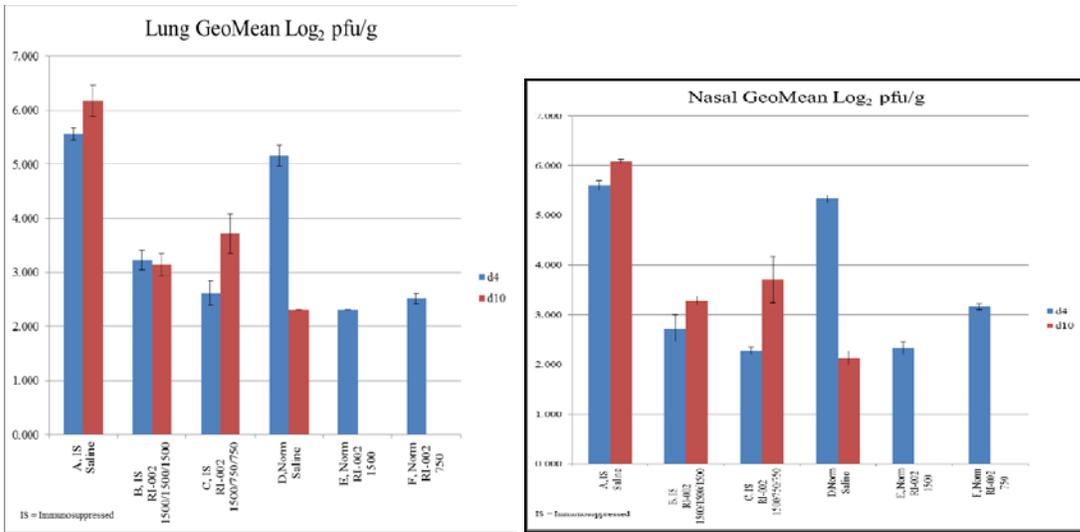
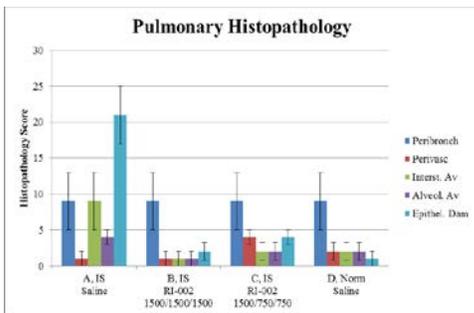
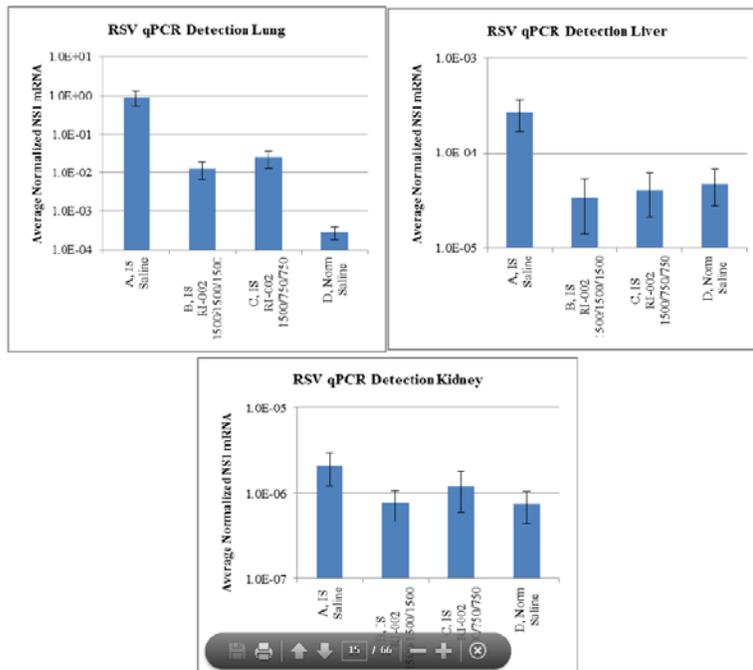


Figure 5 RSV Gene Expression by qPCR in Lung, Liver and Kidney



**Technical report TEC-13-003-RPT II-04: Evaluation of ADMA Biologics RSV-IGIV preparations for efficacy in the Cotton Rat Model of RSV and determination of serum RSV Neutralization Titers.**

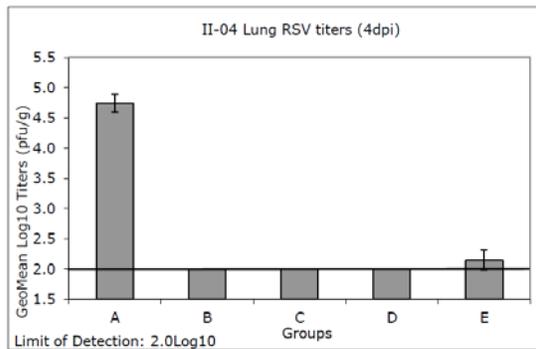
Purpose: To study the efficacy of ADMA preparation in preventing RSV infection in the cotton rat model, to compare its efficacy to RespiGam and to report on the serum neutralizing titers in the cotton rat recipients of this product.

Performing laboratory: CRO for ADMA: (b) (4) ., RSV MNA in CR performed in the laboratory of (b) (4)

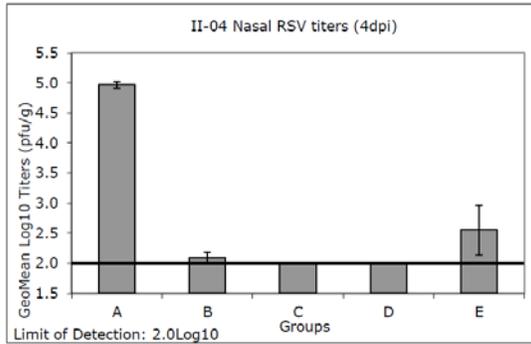
Study design: Twenty five young male cotton rats were divided into 5 groups of 5 animals each and assigned to receive IP administration of saline (Group A), ADMA IVIG at doses 500 (group B), 750 (group C), and 100 (group D) mg/kg, or RespiGam at dose 750 mg/kg (group E). The animals were inoculated with  $10^5$  pfu of RSV-(b) (4) strain. On day 4 (d4) blood was collected and all cotton rats were euthanized and the nose and lungs were harvested for RSV viral titer determination.

Results: RSV pfu/g tissue (both lung and nasal) were lower in animals being treated with test article or control versus saline negative controls. Neutralizing titers were also demonstrated on day2 and day4.

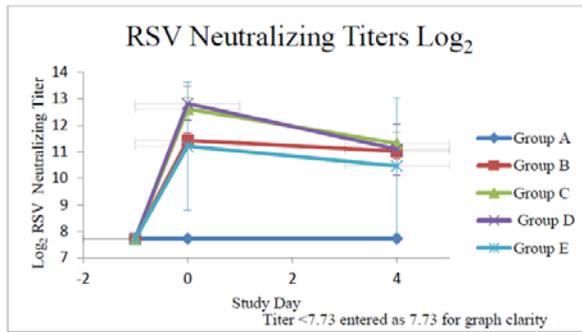
Figure 1 RSV Lung Titers



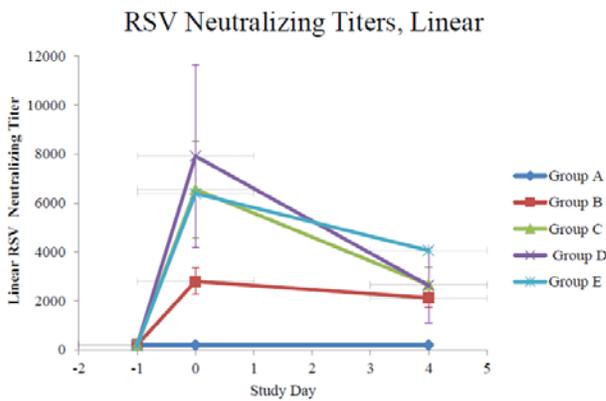
2 RSV Nasal Titers



3 RSV Neutralizing Serum Titers, Log<sub>2</sub>



4 RSV Neutralizing Serum Titers, Linear



**Title Technical Report TEC-14-004-RPT; II-82: Immunosuppressed Cotton Rat: ADMA Anti-RSV Prophylaxis Study and Determination of Serum RSV Neutralization Titers**

Aim: To evaluate the efficacy of ADMA RI-002, when used prophylactically in immunosuppressed cotton rats challenged with RSV-(b) (4) strain).

Performing laboratory: CRO for ADMA: (b) (4), RSV MNA in CR performed in the laboratory of (b) (4)

Design: There were two arms in the study. In total, 10 cotton rats/group were treated on D0, D2, D4, D7, D9, D11, D14, D16 and D18 with 50 mg/kg of cyclophosphamide to induce leukopenia as an immunosuppression model. On D20 animals were treated with either RI-002 1500 mg/kg, 750 mg/kg or saline as negative control. On D21 animals were challenged with RSV (b) (4) at  $10^5$  pfu/100 g BW.

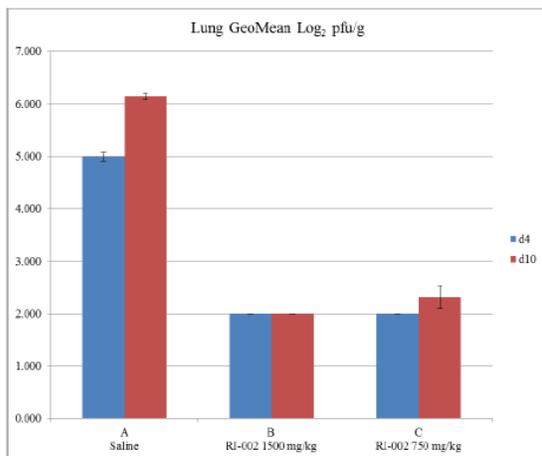
First arm: n= 5 animals were terminated four days after challenge (D25)

Second arm: n=5/group were treated with two more doses of IgG or negative control, respectively 4 and 8 days after inoculation and then terminated 10 days after challenge.

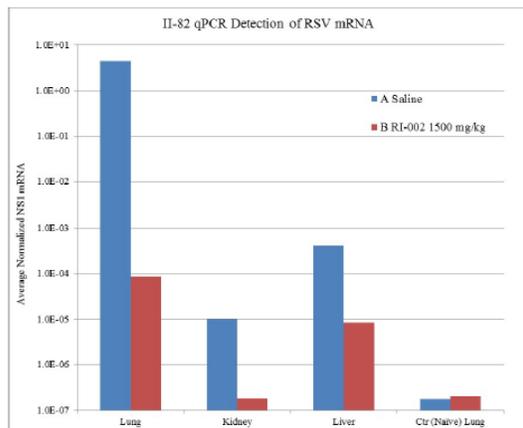
Outcome measures: blood for WBC count and MNA, lung, liver and kidney homogenates for plaque assay, RNA analysis and histopathology.

Results: Lower plaque forming units were measured in the lungs of immunosuppressed CR pretreated with ADMA RI-002 with single dose or with three doses of IgG. Lower levels of RSV viral RNA was measured in the lungs of the animals 10 days after challenge with RSV and following three doses of IgG; undetectable levels were seen on D4.

**RSV Viral Titers in Lung Homogenates in RI-002 Treated and Untreated Immunosuppressed Cotton Rats**



**RSV Gene Expression by qPCR in Lung, Liver and Kidney**



Lung pathology demonstrated evidence of RSV disease in control animals but the high dose animals did not show such changes.

**Title: Technical Report: TEC-15-008-RPT Prophylactic Administration of RI-002 (IGIV) Administered by Intraperitoneal Injection for Antiviral Activity in the Palivizumab-resistant (PR) RSV (b) (4) infected Cotton Rat Model**

Revised report signed 05/21/2015

Performing Laboratory: (b) (4) (contractor for ADMA)

Aim: To evaluate the effectiveness of RI-002 when administered by IP injection at 1.5 g/kg to RSV-infected cotton rats (CR).

Design: RI-002 (1,500 mg/kg) and palivizumab (pmab, 15 mg/kg) were administered IP or IM, respectively onto 5 cotton rats (CR) per group, 24 hrs before challenge IN with

wild-type (wt)-RSV ( $1.35 \times 10^5$  PFU) or palivizumab-resistant (PR)-RSV ( $2.04 \times 10^5$  PFU ).

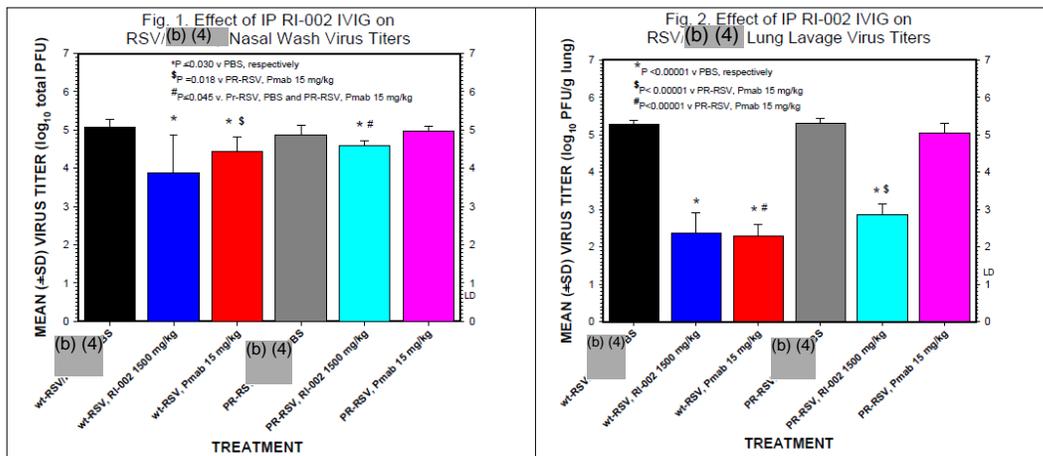
**Table 1: Dosage, Lung and Sample Weights on Day (+4)**

Group	Treatment/Challenge Virus	Mean±SD Dose (mg/kg) <sup>1</sup>
1	PBS/wt-RSV	0
2	RI-002/wt-RSV	1,518±197
3	Pmab/wt-RSV	16.1±1.4
4	PBS/PR-RSV	0
5	RI-002/ PR-RSV	1,503±136
6	Pmab/ PR-RSV	15.1±2.4

<sup>1</sup>There was no statistically significant difference between matched groups (Student t test, two-tailed).

Outcome measures: virus titers in lung lavage (3 mL) and nasal wash (2 mL) by semi-quantitative plaque assay. Also RI-002 RSV-specific neutralizing antibody levels in lung lavage fluids and in sera determined by a microneutralization (MNA) assay against RSV (b) (4) on the day of inoculation and four days after inoculation (+4).

Results: virus titers in both nasal and lung lavages were significantly lower in RI-002 treated CR four days after challenge with both WT-RSV and PR-RSV (Fig 1 and 2).

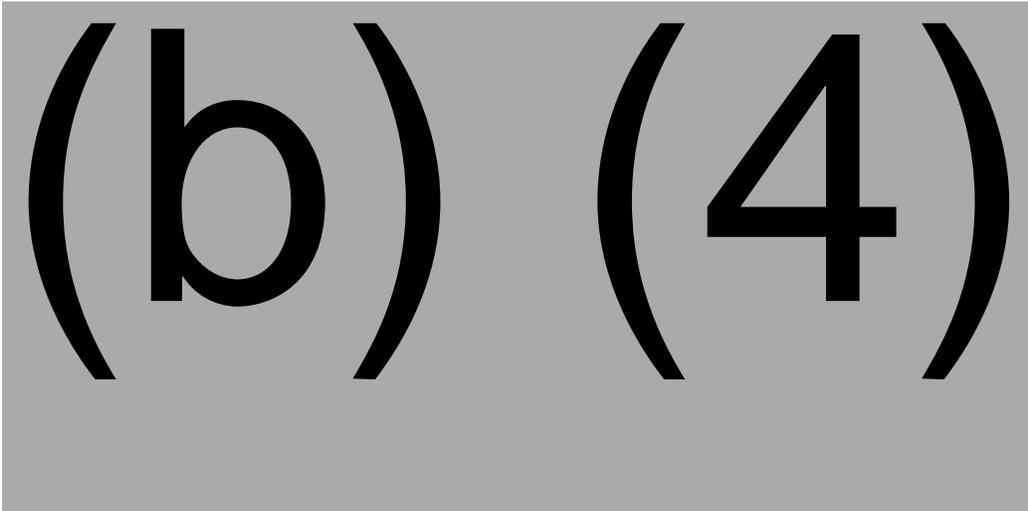


**An analysis of immune determinants of ASCENIV®**

The different titers in ASCENIV® in comparison with 9 commercial IGIV products were submitted. Both preparations contain antibodies against F protein and G protein A and B, with ADMA lots having higher titers. (The table in RSV antibody titer justification pg 29, 30 out of 68 is unclear and is shown below as an example.)

**Table 4 Comparison of Anti G protein antibodies in RI-002 and 9 commercial lots of IG**

Data Scale	Analysis	Statistics	ADMA Lots (A,G,K)	Commercial Lots
Linear	Antibody to F Protein	n	3	9
		Mean (SE)	263967.4 (0.0000)	181315.7 (19143.44)
		(b) (4)		
		SD	0.0000	57430.32
		Median	263967.4	186653.1
		(b) (4)		
	Antibody to G Protein A	n	3	9
		Mean (SE)	154987.2 (16768.71)	89830.37 (10865.20)
		(b) (4)		
		SD	29044.26	32595.59
		Median	171755.9	85877.94
		(b) (4)		
Antibody to G Protein B	n	3	9	
	Mean (SE)	122056.9 (24063.56)	44898.76 (4099.654)	
	(b) (4)			
	SD	41679.31	12298.96	
	Median	117312.7	41476.31	
	(b) (4)			



## **References**

1. National Toxicology Program Technical Report 415
2. Pestel et al, Effect of commonly used vehicles on gastrointestinal, renal, and liver function in rats, J Pharmacol Toxicol Methods. 2006 Sep-Oct;54(2):200-14