PHARMACOLOGIST'S REVIEW

BLA: 97-1359
SPONSOR: MedImmune, Inc.
PRODUCT: humanized monoclonal antibody to the F protein of respiratory syncytial virus; MEDI-493; Palivizumab; Synagis®
FORMULATION/CHEMISTRY: A humanized MAb directed to the antigenic site A of the conserved fusion (F) protein of RSV; constructed using molecular techniques to insert the antigen-binding regions (complementarity determining regions or CDRs) from an F protein-specific murine MAb (MAb 1129) into a human variable framework domain. The functional protein is made up of 2 heavy chain (IgG_1) and 2 light chains (Kappa), with a MW of 147 kD. Expressed in a NSO (murine myeloma) cell line. The final sterile lyophilized product contains 47 mM histidine, 3 mM glycine, and 5.6% mannitol when reconstituted. The clear/slightly opaque solution is supplied in single dose vials [1 mL] containing 100 mg/mL.
PROPOSED INDICATION: Prophylaxis against RSV infections in pediatric patients who are at high risk of developing RSV infections.
ABBREVIATIONS: respiratory syncytial virus = RSV; intravenous = IV; intramuscular = IM; monoclonal antibody = MAb; human RSV-specific hyperimmune globulin preparation = RSV-IGIV = Respigam®; bronchopulmonary dysplasia = BPD

Table of Contents

INTRODUCTION:

RSV is an enveloped single negative-strand RNA virus which belongs to the Paramyxoviridae family and to the genus, Pneumovirus. The RSV genome encodes for at least 10 unique viral polypeptides (9.5 to 160 kD MW). Two glycosylated surface proteins, the F and G proteins, play a role in the infectivity

and pathogenesis of the virus. The F protein appears to initiate viral penetration and cell-cell fusion, while the G protein appears to mediate attachment of the virus to host cells. Two major antigenetically distinct subgroups of RSV are group A or B. Evaluation of strains from these groups have shown that the major antigenic differences are on the G protein.

RSV is the major cause of serious lower respiratory tract disease in children, manifested as bronchiolitis and pneumonia. The peak incidence of hospitalization of RSV-associated illness is in infants 2-5 months old. Primary RSV infection occurs before one year of age, with 95% of the children displaying serologic evidence of infection by 2 years, and 100% by adulthood. RSV infection occurs in seasonal outbreaks, peaking during the winter in temperate climates and during the rainy season in warmer climates. Repeated infections are common, but development of neutralizing antibodies results in significant protection. There is presently no vaccine for RSV, however, monthly infusions of 750 mg/kg of RSV-IGIV (also a MedImmune product) is associated with reduced risk of developing severe RSV disease and reduced rates of hospitalization for RSV.

MEDI-493 is specific for the antigenic site A on the highly conserved F protein on the surface of RSV, has potent neutralizing and fusion-inhibiting activity, and is cross reactive to strains from both A and B subtypes of the virus. The MAb is pharmacologically effective in vitro at blocking RSV infection and syncytia formation. MEDI-493 appears to be 50-100 times more potent both in vitro and in vivo compared to RSV-IGIV, thus prophylaxis with MEDI-493 may require smaller doses.

The proposed clinical indication [per the package insert] for MEDI-493 is for the prevention of serious lower respiratory tract disease caused by RSV in infants and children with a history of premature birth (≤35 weeks gestation) with or without BPD who are under 24 months old at the time of first administration. The sponsor claims that MEDI-493 has proven to be safe and effective in reducing the incidence and days of RSV hospitalization, as well as the severity of the RSV illness in this patient population. The proposed treatment regimen for MEDI-493 [per the package insert] is IM injection (anterolateral aspect of the thigh) of 15 mg/kg/dose, once a month during anticipated periods of RSV prevalence in the community. The monthly dose = patient weight x 15 mg/kg ÷ 100 mg/mL = amount to be injected. **Note** that injection volumes over 1 mL are to be administered as a divided dose.

MEDI-493 is manufactured by MedImmune, Inc. Labeling of this vialed MEDI-493 will be performed ~~~~~ MedImmune has also contracted with Thomae to manufacture, fill, lyophilize, label. and package MEDI-493 ~~~~~

Preclinical Pharmacology Studies

In vitro

Kinetic Analysis of Binding
MEDI-493 [humanized MAb1129IgG$_1$, Kappa], with a K_d of ~1 nM [$K_d = K_{diss}/K_{assoc}$], bound with a slightly higher affinity compared to chimeric 1129 [an isotype matched chimeric version of the parent MAb]:

Chimeric 1129 - K_d = 1.7 nM
MEDI-493 - K_d = 0.96 nM

Microneutralization Assay of RSV by MEDI-493 [Humanized 1129] and Chimeric 1129 MAbs
Using an ELISA-based microneutralization assay to detect virus replication, MEDI-493 showed comparable in vitro inhibition activity relative to the parental MAb 1129, against Long strain virus. Exposure to approximately 20 ng/mL MEDI-493 resulted in 50% inhibition [EC_{50}] compared to a concentration of 50 ng/mL for chimeric MAb 1129.

Plaque Reduction Neutralization Assay
Dilutions of MEDI-493 (1-1000 μg/mL) were tested against RSV-Long (A subtype) and against RSV-18537 (B subtype). The polyclonal RSV-IGIV was used as the positive control. The number of plaque forming units (pfu) was lower (i.e., neutralized) for both A & B subtypes of RSV compared to RSV-IGIV. Lower concentrations of MEDI-493 were needed to reduce pfus (i.e., higher potency).

Neutralization of Clinical Isolates
MEDI-493 (400 ng/mL) was tested against a panel of 57 clinical isolates for different geographic areas of the USA (isolated between 1987 and 1993). The polyclonal RSV-IGIV was used as the positive control and a non-neutralizing human antibody developed by the sponsor served as the negative control. Using the ELISA-based microneutralization assay, MEDI-493 inhibited all 57 isolates. Another virus generated by plaquing & passaging in the presence of MEDI-493 was also tested and found to be susceptible to neutralization by RSV-IGIV, but not by MEDI-493.

In vivo

IV Treatment in Cotton Rats

Methods: Cotton rats (*Sigmodon hispidus*, 4/grp) were infected with 10^5 pfu of RSV-Long strain, followed three days later by IV injection of MEDI-493 at 0, 0.63, 1.25, 2.5, 5, or 10 mg/kg or 500 mg/kg of RSV-IGIV (positive control) or BSA (negative control). The rats were killed one day later and circulating human IgG levels and pulmonary RSV titers determined.

Findings: Data showing effective reduction in viral burden levels at ≥5 mg/kg MEDI-493 are depicted:

Table 7.2.1-1 Therapy of RSV Infection by MEDI-493 in Cotton Rats

Sample	Dose mg/kg	Serum [Ab] µg/ml	Lung RSV Titer pfu/g (mean $\log_{10}$±SE)
BSA	10.0	0.0	5.99±0.1
MEDI-493	0.63	4.1	5.13±0.16
MEDI-493	1.25	18.3	4.74±0.08
MEDI-493	2.5	20.2	4.94±0.19
MEDI-493	5.0	60.2	3.37±0.13
MEDI-493	10.0	106.0	2.81±0.27
RSV-IGIV	500.0	3100.0	<2±0

IM Prophylaxis in Cotton Rats

Cotton rats (4/grp) were IM dosed with MEDI-493 at 0.56, 1.67, or 5 mg/kg, followed by challenge 24 hours later with 10^5 pfu of RSV-Long strain and kill 4 days later. RSV-IGIV doses were 16.7, 27.8, 50, 83.3, or 250 mg/kg.

Findings: A 50-fold increase in potency for MEDI-493 compared to RSV-IGIV was noted and a >2-log reduction in RSV titer was present at 1.67 mg/kg, with MEDI-493 serum levels of 11-13 µg/mL.

Table 7.2.2-1 Intramuscular Prophylaxis of RSV in Cotton Rats: Experiment III-41

Compound	Number of Animals	Dose mg/kg	Serum [Human IgG] at Challenge ug/ml	Lung Viral Titer pfu/g (mean $\log_{10}$)
MEDI-493	4	0.56	2	4.66 ± 0.07
MEDI-493	4	1.67	11	2.66 ± 0.38
MEDI-493	4	5.0	30	2.17 ± 0.17
RSV-IGIV	4	16.7	125	4.48 ± 0.04
RSV-IGIV	4	50	298	3.87 ± 0.11
RSV-IGIV	4	250	1450	2.29 ± 0.29
BSA	4	5.0	0	5.20 ± 0.07

Table 7.2.2-2 Intramuscular Prophylaxis of RSV in Cotton Rats: Experiment III-42

Compound	Number of Animals	Dose mg/kg	Serum [Human IgG] at Challenge ug/ml	Lung Viral Titer pfu/g (mean $\log_{10}$)
MEDI-493	4	0.56	4	3.75 ± 0.24
MEDI-493	4	1.67	13	2.15 ± 0.09
MEDI-493	4	5.0	47	2.08 ± 0.08
RSV-IGIV	4	27.8	64	4.32 ± 0.08
RSV-IGIV	4	83.3	295	3.49 ± 0.19
RSV-IGIV	4	250	1400	2.0 ± 0
BSA	4	5.0	0	4.80 ± 0.08

Figure 7.2.2-1 Intramuscular Prophylaxis of RSV in Cotton Rats: Experiment III-41

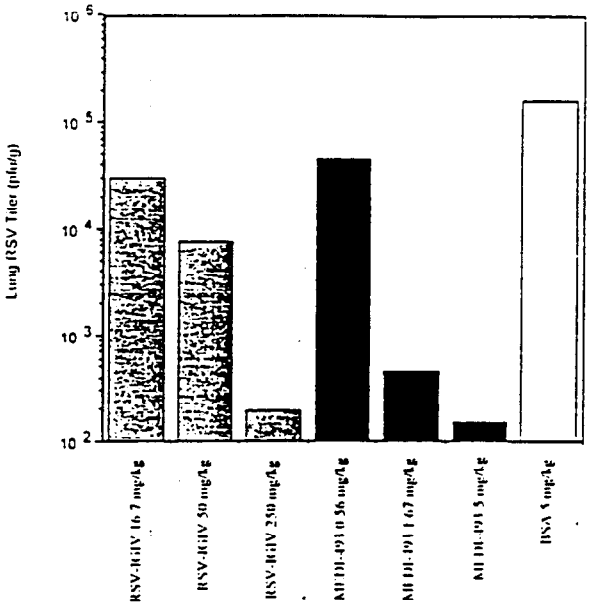


Figure 7.2.2-1 This figure depicts prophylaxis of RSV infection by RSV-IGIV or MEDI-493 administered intramuscularly prior to challenge with RSV.

Figure 7.2.2-2 Intramuscular Prophylaxis of RSV in Cotton Rats: Experiment III-42

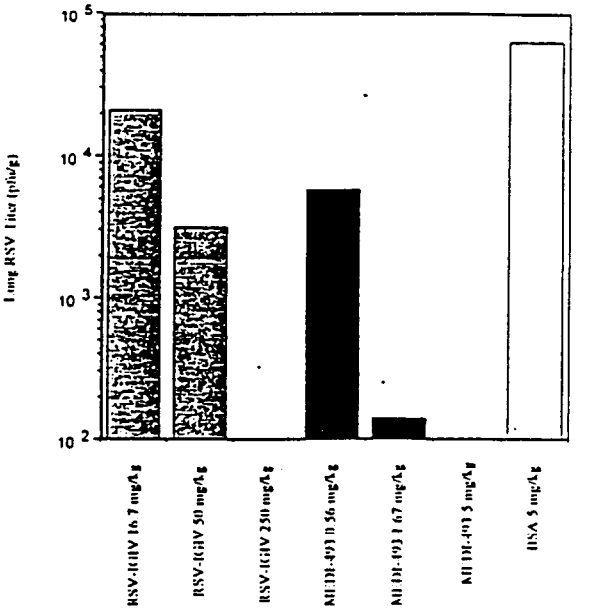


Figure 7.2.2-2 This figure depicts prophylaxis of RSV infection by RSV-IGIV or MEDI-493 administered intramuscularly prior to challenge with RSV.

Cotton rats IM dosed with MEDI-493 at 0.625, 1.25, 2.5, or 5 mg/kg, followed by challenge 24 hours later with 10^5 pfu of RSV-Long or RSV-18537, showed a 50-fold increase in potency for MEDI-493 compared to RSV-IGIV and a >2-log reduction in RSV titer at 1.67-2.5 mg/kg:

Figure 7.2.2-3 Intramuscular Prophylaxis of RSV A & B Subtype Viruses in Cotton Rats

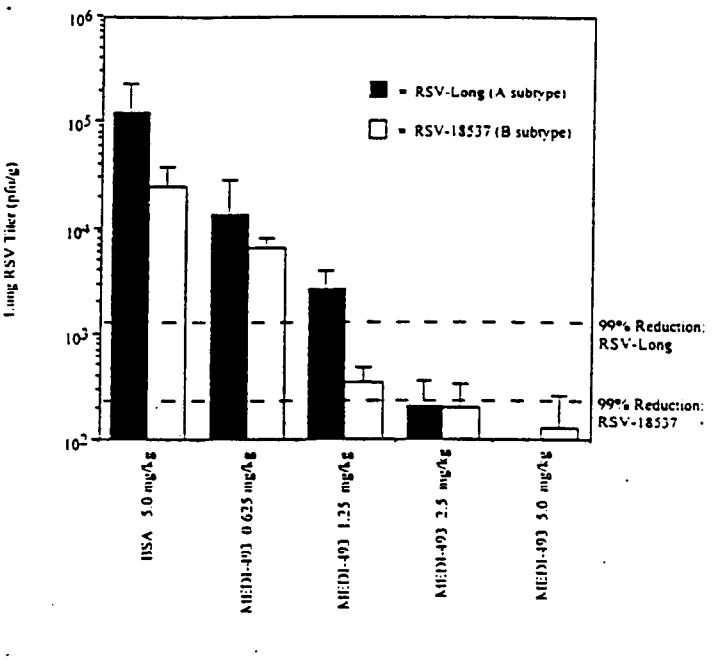


Figure 7.2.2-3 This figure shows the ability of MEDI-493 administered intramuscularly to protect cotton rats against both A and B subtypes of RSV.

IV Prophylaxis of RSV Infection in Cotton Rats

Methods: Cotton rats were IV injected with 0.31, 0.63, 1.25, 2.5, 5.5, or 10 mg/kg MEDI-493 or 31.3, 62.5, 125, 250, or 500 mg/kg RSV-IGIV at 24 hours prior to infection with RSV [BSA (10 mg/kg) serves as the negative control]. The rats were challenged intranasally with 10^5 pfu of Long strain RSV, followed (4 days later) by their kill. Lung tissue was collected and pulmonary viral titers determined by plaque titration using HEp-2 cells (limit of detection = 100 pfu/g tissue). Sera MEDI-493 concentrations at the time of challenge were measured via a sandwich ELISA.

Findings:
Dose-responsive reduction in lung viral titers was evident. A dose of 5 mg/kg [corresponding to MEDI-493 sera levels of ~30 µg/mL] produced comparable reductions in viral titer, at 2 logs, to 250 mg/kg RSV-IGIV.

Table 7.2.3-1 Intravenous Prophylaxis in Cotton Rats: ALL EXPERIMENTS COMBINED

Compound	Number of Animals	Dose mg/kg	Mean±Std Error Concentration of Human IgG ug/ml	Geometric Mean±Std Error Lung Viral Titer pfu/g
BSA	15	10	0	1.3x10^{7}±1.2
MEDI-493	7	0.312	2.67±0.60	4.6x10^{6}±1.5
MEDI-493	17	0.625	5.27±0.27	2.7x10^{6}±1.5
MEDI-493	18	1.25	10.1±0.29	3.3x10^{5}±1.4
MEDI-493	17	2.50	28.6±2.15	9.6x10^{3}±1.5
MEDI-493	15	5.0	55.6±3.43	1.3x10^{3}±1.2
MEDI-493	18	10.0	117.6±5.09	1.0x10^{2}±1.0

Table 7.2.3-2 Intravenous Prophylaxis in Cotton Rats: EXPERIMENT III-47

Compound	Number of Animals	Dose mg/kg	Mean±Std Error Concentration of Human IgG ug/ml	Geometric Mean±Std Error Lung Viral Titer pfu/g
BSA	4	10	0	1.4x10^{7}±1.7
MEDI-493	3	0.312	3.83±1.1	2.1x10^{6}±2.1
MEDI-493	3	0.625	5.27±0.37	7.7x10^{5}±1.6
MEDI-493	4	1.25	9.15±0.16	3.4x10^{5}±1.3
MEDI-493	3	2.50	23.4±2.3	1.4x10^{3}±1.7
MEDI-493	2	5.0	42.4±13.4	4.6x10^{2}±4.6
MEDI-493	4	10.0	141.1±14.4	1.0x10^{2}±1.0

Table 7.2.3-3 Intravenous Prophylaxis in Cotton Rats: EXPERIMENT III-47A

Compound	Number of Animals	Dose mg/kg	Mean±Std Error Concentration of Human IgG ug/ml	Geometric Mean±Std Error Lung Viral Titer pfu/g
BSA	4	10	0	1.9x10^{7}±1.2
MEDI-493	4	0.312	1.8±0.12	8.5x10^{6}±1.2
MEDI-493	4	0.625	4.0±0.19	5.0x10^{6}±1.6
MEDI-493	4	1.25	11.3±0.68	1.9x10^{5}±1.4
MEDI-493	4	2.50	18.9±2.0	5.5x10^{3}±1.6
MEDI-493	3	5.0	55.6±2.5	1.6x10^{3}±1.3
MEDI-493	4	10.0	109.7±5.22	1.0x10^{2}±1.0

Table 7.2.3-4 Intravenous Prophylaxis in Cotton Rats: EXPERIMENT III-58

Compound	Number of Animals	Dose mg/kg	Mean±Std Error Concentration of Human IgG ug/ml	Geometric Mean±Std Error Lung Viral Titer pfu/g
BSA	10	10	0	1.1x10^{7}±1.2
MEDI-493	10	0.625	5.78±0.32	1.6x10^{6}±1.2
MEDI-493	10	1.25	9.82±0.23	1.6x10^{5}±1.3
MEDI-493	10	2.50	34.1±2.11	4.3x10^{3}±1.6
MEDI-493	10	5.0	58.3±4.48	1.0x10^{3}±1.0
MEDI-493	10	10.0	111.5±5.04	1.0x10^{2}±1.0

Findings: Data are shown:

Table III. Serum Levels of MEDI-493

Week 0 (ug/ml)	Week 2	Week 4	Week 6	Week 8
145.71±35.01	2.73±1.91	0.44±0.29	0.05±0.04	0.01±0.01

Table IV. Re-challenge of previously infected cotton rats.

Group	Dose	1° RSV challenge (mean log pfu/gm ±SE)	2° RSV challenge (mean log pfu/gm ±SE)	Animals with RSV Associated Pathology	Range of Severity (N-4)*
1	10 mg/kg BSA	5.51±0.05	< 2±0	0/7	0
2	10 mg/kg MEDI-493	< 2 ± 0	< 2±0	0/5	0-1
3	none	Mock Challenged	2.46±0.14	1/8	0-1

* See Table V below for scores of individual animals

Table V. Re-challenge of previously infected cotton rats. Analysis for potential enhancement of RSV associated histopathology

	Group1 (10 mg/kg-BSA) 1	2	3	4	5	6	7
Lung Diagnosis	N	N	N	N	N	N	N

	Group 2 (10 mg/kg MEDI-493) 1	2	3	4	5
Lung Diagnosis	N		N	N	N
Inflammation, focal,chronic-etiology foreign material		1			

	Group 3 (untreated/no primary RSV) 1	2	3	4	5	6	7	8
Lung Diagnosis	N	N	N		N	N	N	
Bronchiolitis, focal/multifocal, subacute				1				
Inflammation, focal,chronic-etiology foreign material		1						
Histiocytosis, focal								1
Pigmentation, focal								1

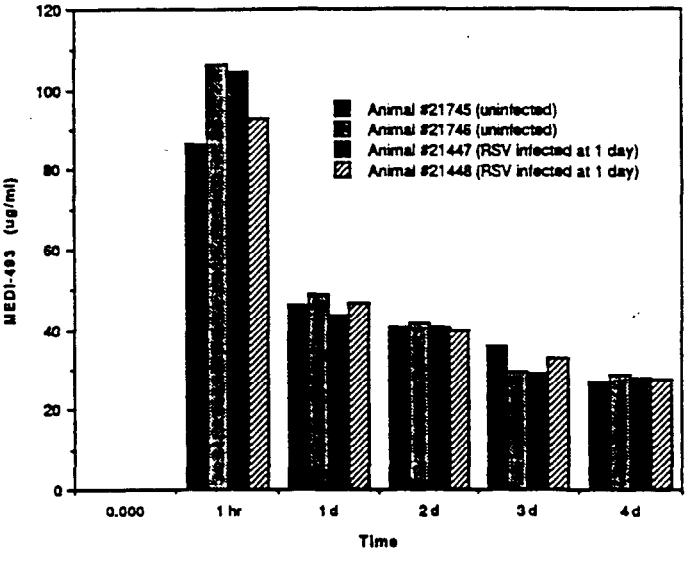
* N (Normal), 1 (Minimal), 2 (Mild), 3 (Moderate), 4 (Marked)

Data for the mock-challenged rats show minimal pulmonary lesions upon a low primary challenge - 10^3 pfu.
No enhancement of viral replication or pathology was noted upon secondary infection.

Effect of RSV Infection on Serum Levels of MEDI-493 in Cotton Rats

Four cotton rats were IV dosed with 5 mg/kg MEDI-493, followed by challenge 24 hours later with 10^5 pfu of RSV-Long strain. Sera levels of MEDI-493 were measured using an ELISA assay specific for human IgG.

Findings: Peak titers were achieved at one hour postdose - 87-107 μg/mL. MEDI-493 levels were similar for (RSV) uninfected and infected animals:



BLA #97-1359

Biological Half-Life ($t_{½}$) of MEDI-493 in Cotton Rats
The PK profile of samples from lots of protein from different sized bioreactors and from pooling different sizes of bioreactors was evaluated. Rats (5/grp) were prebled, then dosed with 1.5 mg of MEDI-493, and samples collected out to 96 hrs. Evaluation of samples via a sandwich ELISA resulted in the following:

Table 7.2.7-1

Lot	Bioreactor	$t_{½}$ (hr)	SD	MDN (hr)	Range (hr)
L96H002	7 x 20L	83.2	36.0	78.7	17.6 - 98.1
L96K004	9 x 20L	85.5	10.5	92.4	71.7 - 94.3
L96J071	5 x 45L	83.5	2.6	85.7	80.3 - 86.3
L96E009	200 L	90.5	27.7	81.3	67.2 - 138.5
L96J091	200 L	101.8	34.4	80.9	73.6 - 151.1
L96T012	5 X 20 L 1 X 100 L 2 X 45 L 1 X 200 L	93.6	11.2	87.7	67.0 - 91.8
L97A044	3 X 20 L 1 X 100 L 1 X 45 L	91.3	22.2	87.5	60.0 - 145.7
	Overall =	85.0			

MDN = median
SD = standard deviation

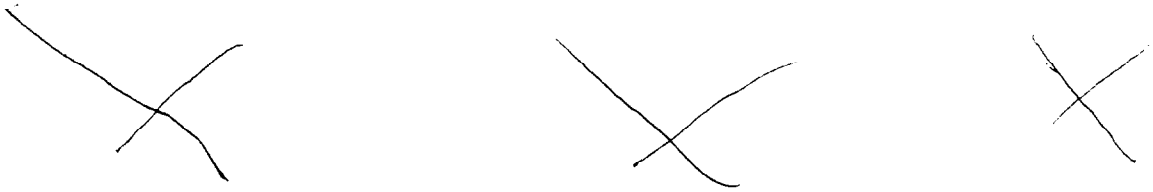
Comparability studies indicated no significant biochemical differences in the glycosylation between the lots. The sponsor suggests that microheterogeneity involving the component sugars (fructose, mannose, galactose, glucose) of the glycoproteins may be occurring & that such differences may influence the half-life. The overall $t_{½}$ = 85 hrs (3.5 days).

BLA #97-1359

PK/ADME Studies

A Single Dose PK Study of MEDI-493 in the Cynomolgus Monkey
Species: 2 ♀ cynomolgus monkeys - prescreened for RSV-specific antibodies
Dose Levels: 10 mg/kg/dose
Route/Duration: IV/single dose

Methods:
Single dose (15-minute infusion), followed by blood sampling up to 20 days postdose
Clinical pathology - baseline and day 21



Findings:
No abnormal clinical pathology findings.

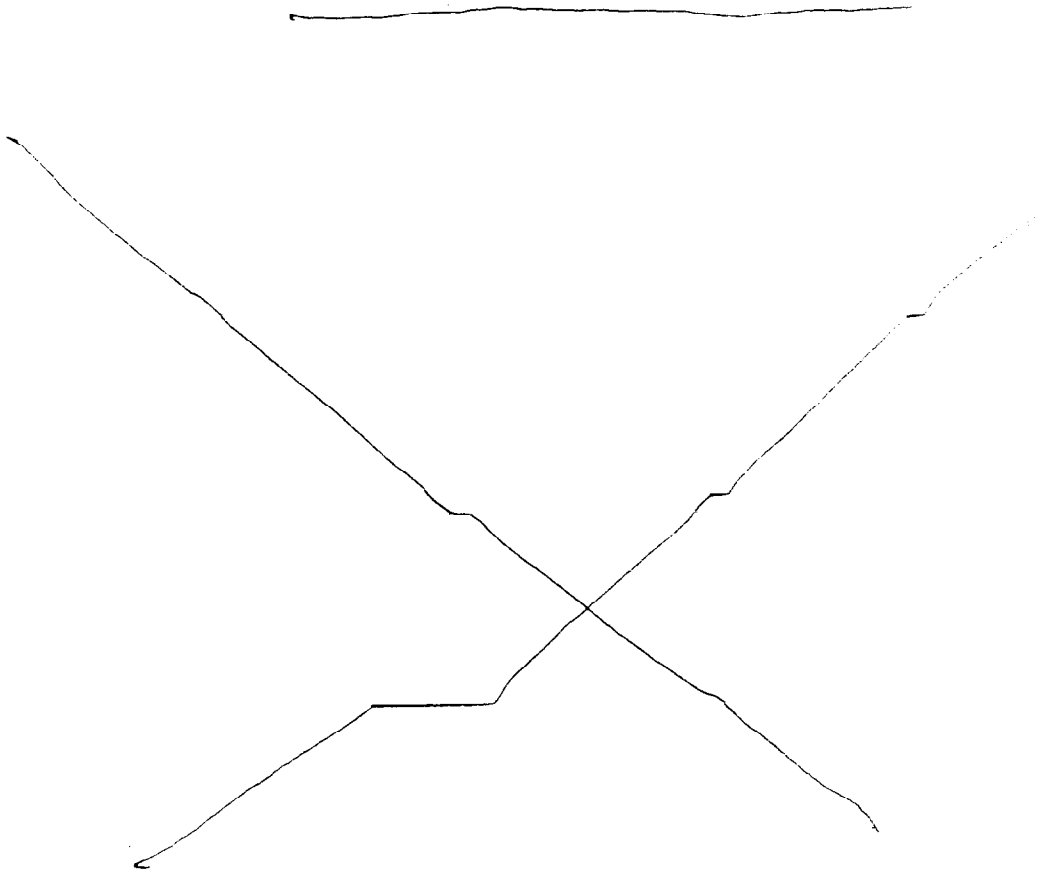
No immunogenicity was detected in the monkeys (via a sandwich ELISA assay).

PK Profile:

Pharmacokinetic Analysis

PARAMETER	FEMALE 1724	FEMALE 1854
A (µg/ml)	156.31 ± 7.29	137.50 ± 8.31
B (µg/ml)	93.78 ± 4.48	97.00 ± 6.25
Cmax	250.1 ± 6.5	234.5 ± 6.6
α (hr^{-1})	0.0791 ± 0.0099	0.0548 ± 0.0091
ß (hr^{-1})	0.0033 ± 0.0002	0.0033 ± 0.0002
$t_{1/2}$ α (hr)	8.77 ± 1.10	12.64 ± 2.10
$t_{1/2}$ ß (hr)	210.51 ± 10.58	212.61 ± 13.87
$t_{1/2}$ EFF (hr)	197.37 ± 9.18	197.02 ± 11.40
AUC_{∞} (µg-hr/ml)	30456.24 ± 739.17	32259.65 ± 917.19
$AUMC_{\infty}$ (µg-hr^2/ml)	8674149 ± 556194	9171551 ± 723309
Cl_{ss} (ml/hr/kg)	0.33 ± 0.01	0.31 ± 0.01
MRT (hr)	284.81 ± 13.24	284.30 ± 16.45
V_c (ml/kg)	39.99 ± 1.03	42.64 ± 1.20
V_{ss} (ml/kg)	93.51 ± 3.47	88.12 ± 4.02

Peak MEDI-493 levels achieved were about 200 µg/mL. MEDI-493 has a very low plasma clearance and a long elimination half-life of about 8.6 days. [Note that the half-life of human IgG is about 23 days in man.]



Toxicology Studies

1. Acute Dose Toxicity Study of a MAb Administered IV to Cynomolgus Monkeys
Species: cynomolgus monkeys (2/sex/group)
Dose Levels: 0, 10, 30 mg/kg/dose
Route/Duration: IV (15-minute infusion)/single dose + 14 or 29 days of nontreatment observation

Methods:
Clinical signs, body weights, food consumption, blood pressure, rectal temperatures, clinical pathology (baseline and days 2, 3, 4, 8, 15, 29), and gross and microscopic pathology were determined.

Findings:
All animals appeared normal for clinical signs, body weight, appetite, indirect blood pressure, and body temperature.

Clinical pathology - ↓ red cell mass - relative to baseline - days 2-4 - control & Rx groups [likely due to repeated phlebotomy] - recovery noted by day 8 (↑ retics)

↑ ALT, AST, CK, LDH - variable for baseline and day 2 - control & Rx groups - relative to historical values - considered by the lab to be due to the restraint procedure (slings) during dosing

Immunogenicity - (via ELISA) - slight positive for one 30 mg/kg ♀ (killed on day 30) [likely a non-specific response, as reaction occurred with all Abs tested]

PK Profile -

Text Table 3
Sex-Averaged Pharmacokinetics of MEDI-493
Group 2 - 10 mg/kg

PARAMETER	*Male 2001 Female 2101	*Male 2002 Female 2102
A (µg/mL)	323 ± 93	333 ± 54
B (µg/mL)	198 ± 10	117 ± 37
C_{max} (µg/mL)	521 ± 95	450 ± 46
α (hr^{-1})	1.7624 ± 0.6606	0.0837 ± 0.0331
β (hr^{-1})	0.0073 ± 0.0005	0.0038 ± 0.0014
$t_{1/2}$ α (hr)	0.39 ± 0.15	8.28 ± 3.27
$t_{1/2}$ β (hr)	95.12 ± 6.46	184.31 ± 68.64
AUC (µg-hr/mL)	27349 ± 1467	35068 ± 6844
AUMC (µg-hr^2/mL)	3727989 ± 415705	8316133 ± 4324120
Cl_{ss} (mL/hr/kg)	0.37 ± 0.02	0.29 ± 0.06
MRT (hr)	136.31 ± 9.22	237.14 ± 85.26
V_c (mL/kg)	19.19 ± 3.48	22.24 ± 2.30
V_{ss} (mL/kg)	49.84 ± 2.47	67.62 ± 17.10

Peak MEDI-493 levels achieved were from 286 µg/mL (10 mg/kg) to 595 µg/mL (30 mg/kg). The PK curves were biphasic, with an elimination half-life ranging from 4.2-10.2 days.

Day 15 Kill
Organ weight data were very variable between groups - no conclusive compound effect could be documented. There were no apparent gross or microscopic correlates to the organ weight changes.

Day 15 Kill
Histomorphologic changes noted:
Injection site - SC hemorrhage (mild to moderate), acute inflammation (minimal to mild) - control & Rx groups

Liver - subcapsular fibrosis, chronic inflammation - 30 mg/kg ♂ - of traumatic etiology (per pathologist)

Kidneys, liver, spleen, cecum, adrenal cortex, mesenteric lymph node, bone marrow - golden brown pigment - consistent with hemosiderin - 30 mg/kg ♀

Note that hemosiderin is usually seen when there is enhanced breakdown of RBC - but there was no hemolysis noted

Kidneys - birefringent crystals in renal tubules - 30 mg/kg ♀ [No evidence of chronic renal tubulo-interstitial disease]

No evidence of hyperuricemia (thus no urate crystal formation)

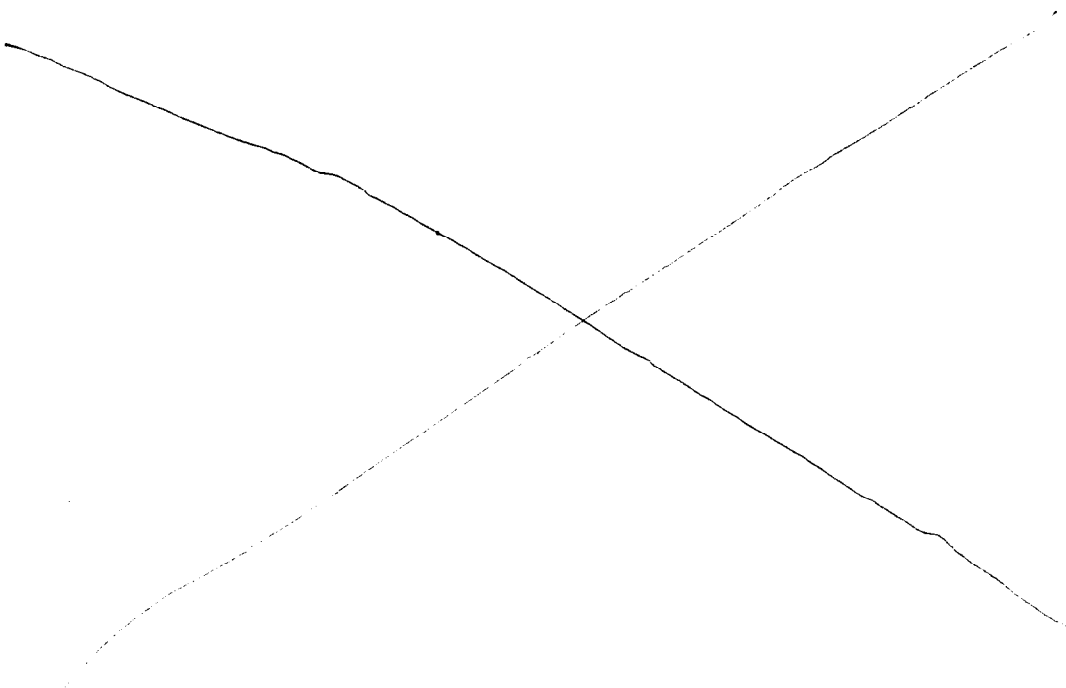
Yellow perinuclear pigment in tubular epithelial cells - consistent w/ lipofuscin - control ♀

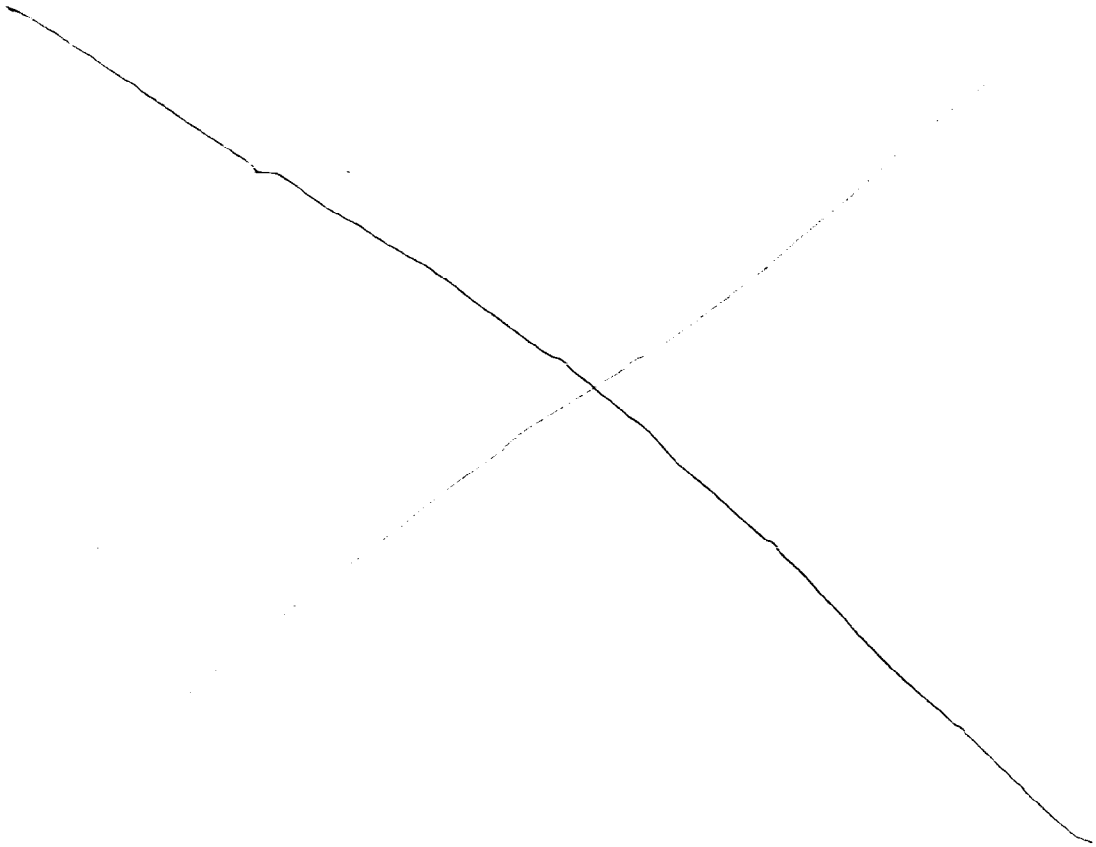
Day 30 Kill
Histomorphologic changes noted:
Injection site - SC hemorrhage (mild to moderate), acute inflammation (minimal to mild) - control & Rx groups

Kidneys - yellow perinuclear pigment in tubular epithelial cells - consistent w/ lipofuscin - control ♀, 10 mg/kg ♀, 30 mg/kg ♂

Spleen - focal aggregate of macrophages adjacent to an arteriole within the white pulp - 30 mg/kg ♀

The NOAEL = 30 mg/kg/dose





2. Single Dose IM and SC Toxicity Study of MEDI-493 Formulation in Rabbits
Species: NZW rabbits (2-4/sex/group)
Dose Levels: 0, 15, 50 mg/kg/dose
Route/Duration: IM/SC/single dose + kills on day 4 & 15

Methods: Clinical signs, BWs, gross & histopathology (injection sites only) were performed

Findings:
Erythema - very slight - 1/8 rabbits (15 mg/kg, SC - days 2 & 3)
Erythema - slight - 1/8 (50 mg/kg, IM - day 2), decreasing to very slight on days 3 & 4

No MEDI-493 related abnormalities in BWs or microscopic evaluation of the injection site

3. 14-Day Toxicity Study of MEDI-493 Administered to SD Rats via IV Injection
Species: SD rats (6/sex/group)
Dose Levels: 0, 210, 420, 840 mg/kg/dose
Route/Duration: IV/single injection + kill on day 14

Methods:
Clinical signs, body weights, food consumption, ophthalmology, clinical pathology (baseline and days 3, 14), and gross and microscopic pathology (0, 210, 840 mg/kg grps only) were determined.

Findings: No apparent MEDI-493 related adverse effects were noted. Sporadic ophthalmic findings were noted, but determined to be a result of the blood collection procedure.

4. Cross-Reactivity of Biotinylated Humanized MAb MEDI-493 with Human Tissues
Methods:
Biotinylated MEDI-493 was incubated with frozen normal human tissue specimens (all major organs, including adrenal, brain, eye, kidney, bone marrow, liver, lung, etc...) for measurement of cross-reactivity. Tissues were obtained from two adult and one neonate donor.
A dilution of 1:800 - 2.5 μg protein/mL - was found to be the lowest concentration at which staining of RSV-infected cotton rat lungs was maximal. Thus 25 and 2.5 μg/mL concentrations were used.

RSV-infected (2-3+ staining) and uninfected cotton rat lung tissue were used as the positive and negative control tissues. The negative control in the study was human IgG_1 kappa myeloma protein (the molecular structure of this protein to MEDI-493 is not known).

Findings: (for both adult & neonatal tissues)
Concentrations of 25 μg/mL of biotinylated MEDI-493 showed weak (1+) to moderate (2+) staining in epithelial tissue, vascular smooth muscle, and cardiac muscle. At this concentration, strong staining (3+) was noted in the corneal epithelium, large intestine smooth muscle, skin, spleen, Hassall's corpuscles of the thymus, and urinary bladder smooth muscle. Through titration assays on various tissues, staining occurred at concentrations higher than 10 μg/mL. MEDI-493 concentrations of 2.5 μg/mL were reactive with renal tubular epithelium (1-2+), ductal epithelium of the salivary gland (1+), skin epidermis (<1+), and Hassall's corpuscles of the thymus (1+). Binding in all tissues at both concentrations of MEDI-493, appeared to be intracellular. In order to elucidate results further, the following was done:

Table 8.2 Summary of Competitive Inhibition Cross-Reactivity Binding Assays with MEDI-493

Study #	Test Material or Inhibitor	RSV-Infected Rat Lung	Human Tissues
1	Biotinylated MEDI-493	Positive	Positive
2	Biotinylated MEDI-493 + F Protein	Reduced	Negative
3	Murine 1129	Positive	Negative
4	Biotinylated MEDI-493 + Murine 1129	Negative	Positive
5	Biotinylated MEDI-493 + Nonbiotinylated MEDI-493	Negative	Positive

#2. Done to block the CDR region to determine the specificity of binding. Binding was inhibited by the specific antigen, but the report notes that the F-protein-antibody complex may have been hindered sterically from attaching to the tissues (based on other competitive inhibition studies performed).

#3. Done to determine if the identical CDR region of murine MAb 1129 counterpart would bind to human tissues. Results indicate that MEDI-493 binding is due to nonspecific binding through the human framework regions or through the biotinylation of the molecule.

#4. Done because MAb 1129 is a specific competitive inhibitor of MEDI-493. Results indicate that binding to the human tissues was nonspecific.

#5. Done to determine whether the binding of MEDI-493 was due to an artifact created by biotinylation. Results showed that specific binding to RSV-infected rat lung was inhibited, but binding to human tissues remained. [The sponsor concludes that the human tissue binding was due to the biotin on the MEDI-493. The non-biotinylated MEDI-493 could not compete with the biotin-directed binding of biotinylated MEDI-493.]

In addition, non-specific binding was not altered by increasing the salt concentration or changing the pH. The use of Tris buffer enhanced attachment of the control antibody to renal tubules, supporting non-specific attachment of the biotinylated MEDI-493.

Comment:

- In the monkey PK study, the plasma concentrations of an IV injection of 10 mg/kg MEDI-493 ranged from about 200 to 20 μg/mL. No alterations in clinical pathology data were noted, but the animals were not killed and evaluated histologically.

5. Cross-Reactivity of Biotinylated Humanized MAb MEDI-493 with Cynomolgus Monkey Tissues

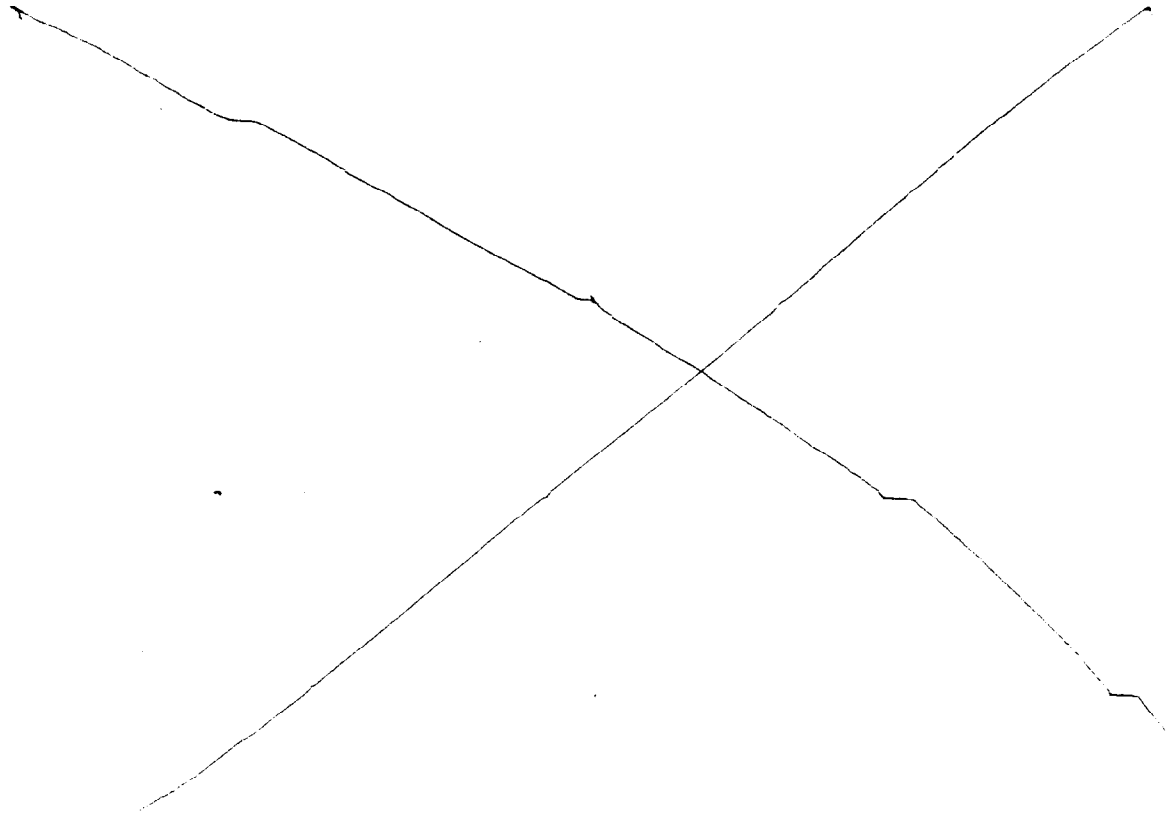
Methods:

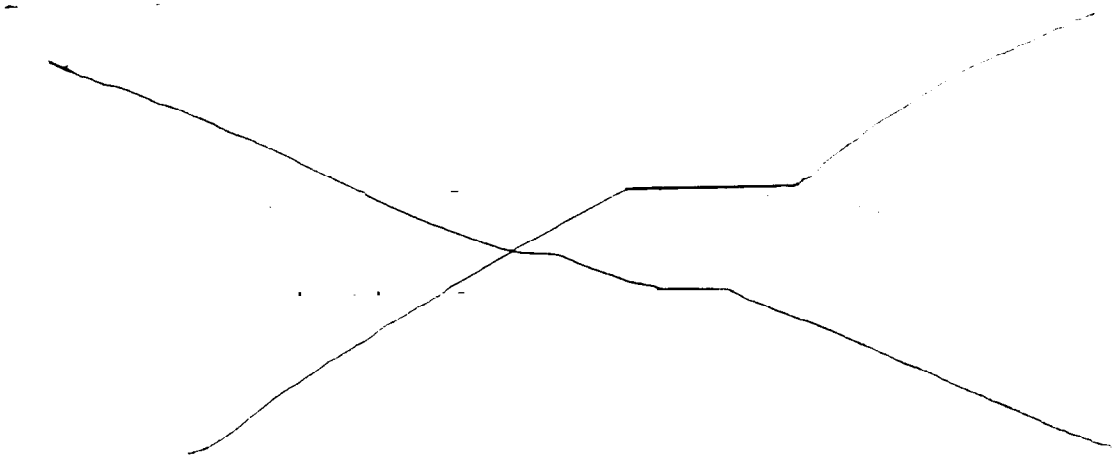
Biotinylated MEDI-493 was incubated with frozen normal cyno monkey tissue specimens (kidney, liver, skin, heart, small intestine) for measurement of cross-reactivity. MEDI-493 concentrations of 2.5 and 25 μg/mL were used.

RSV-infected (2-3+ staining) and cotton rat lung tissue was used as the positive control tissue. The negative control in the study was human IgG_1 kappa myeloma protein (the molecular structure of this protein to MEDI-493 is not known).

Findings:

Concentrations of 25 μg/mL of biotinylated MEDI-493 showed weak (1+) to moderate (2+) staining in epidermis, vascular smooth muscle, cardiac muscle, and hair follicular epithelium (skin). MEDI-493 concentrations of 2.5 μg/mL (2+) and 25 μg/mL (3+) were reactive with renal tubular epithelium. Binding in all tissues appeared to be cytoplasmic/intracellular. The binding was consistent to the pattern noted with the human tissues, i.e., attributable to the presence of biotin on the molecule.





CONCLUSION:

The proposed clinical indication [per the package insert] for MEDI-493 is for the prevention of serious lower respiratory tract disease caused by RSV in infants and children with a history of premature birth (≤35 weeks gestation) with or without BPD who are under 24 months old at the time of first administration. The sponsor claims that MEDI-493 has proven to be safe and effective in reducing the incidence and days of RSV hospitalization, as well as the severity of the RSV illness in this patient population. The proposed treatment regimen for MEDI-493 [per the package insert] is IM injection (anterolateral aspect of the thigh) of 15 mg/kg/dose, once a month during anticipated periods of RSV prevalence in the community.

RSV is an enveloped single negative-strand RNA virus which belongs to the Paramyxoviridae family and to the genus, Pneumovirus. The RSV genome encodes for at least 10 unique viral polypeptides (9.5 to 160 kD MW). Two glycosylated surface proteins, the F and G proteins, play a role in the infectivity and pathogenesis of the virus. The F protein appears to initiate viral penetration and cell-cell fusion, while the G protein appears to mediate attachment of the virus to host cells. Two major antigenetically distinct subgroups of RSV are group A or B. Evaluation of strains from these groups have shown that the major antigenic differences are on the G protein.

RSV is the major cause of serious lower respiratory tract disease in children, manifested as bronchiolitis and pneumonia. The peak incidence of hospitalization of RSV-associated illness is in infants 2-5 months old. Primary RSV infection occurs before one year of age, with 95% of the children displaying serologic evidence of infection by 2 years, and 100% by adulthood. RSV infection occurs in seasonal outbreaks, peaking during the winter in temperate climates and during the rainy season in warmer climates. Repeated infections are common, but development of neutralizing antibodies results in significant protection. There is presently no vaccine for RSV, however, monthly infusions of 750 mg/kg of RSV-IGIV (also a MedImmune product) is associated with reduced risk of developing severe RSV disease and reduced rates of hospitalization for RSV.

MEDI-493 is specific for the antigenic site A on the highly conserved F protein on the surface of RSV, has potent neutralizing and fusion-inhibiting activity, and is cross reactive to strains from both A and B subtypes of the virus. The MAb is pharmacologically effective in vitro at blocking RSV infection and syncytia formation. MEDI-493 appears to be 50-100 times more potent both in vitro and in vivo compared to RSV-IGIV, thus prophylaxis with MEDI-493 may require smaller doses.

Activity of MEDI-493 was measured both in vitro and in vivo. The number of plaque forming units (pfu) was lower (i.e.,neutralized) for both A and B subtypes of RSV exposed to MEDI-493 levels of 1-1000 μg/mL, compared to RSV-IGIV. Lower concentrations of MEDI-493 were needed to reduce pfus (i.e., higher potency). In addition, 400 ng/mL of MEDI-493 inhibited a panel of 57 clinical isolates for different geographic areas of the USA (isolated between 1987 and 1993).

In vivo, an effective comparable prophylactic dose of MEDI-493 to RSV-IGIV was ≥5 mg/kg - seen as a reduction in lung viral titers in cotton rats infected with human RSV (Long strain). About 250 mg/kg of RSV-IGIV was associated with a 2 log reduction of RSV in lungs of cotton rats. Injection of MEDI-493 also resulted in a 2 log reduction in viral titer, but at a dose that was at least 50-fold lower than RSVIG-IV [~5 mg/kg]. A reduction in RSV titer of ≥100-fold corresponded to a mean serum antibody concentration of 25-40 μg/mL at the time of RSV challenge. MEDI-493 did not enhance infection or lung pathology upon primary or secondary RSV infection.

In the toxicology studies performed, a no effect dose of 30 mg/kg in a primate single IV dose toxicity study was shown. The elimination half life of MEDI-493 in monkeys was ~4-10 days. No

toxic effects were noted in rabbits that were IM or SC injected with a single dose of up to 50 mg/kg of MEDI-493 or in rats IV injected with a single dose up to 840 mg/kg of MEDI-493. The MAb does not appear to cross react with normal human adult or neonatal tissues.

The preclinical data adequately support use of the product MEDI-493, according to the dosage regimen specified by the sponsor.

Mercedes A. Serabian 3/5/98

Mercedes A. Serabian, M.S., D.A.B.T., Toxicologist

Key Words: MEDI-493; Palivizumab; Synagis®; RSV; respiratory syncytial virus; IgG_1; F protein; cotton rats; prophylaxis; pediatrics; BPD

Concurrences:
OTRR/C, P-T/MGreen