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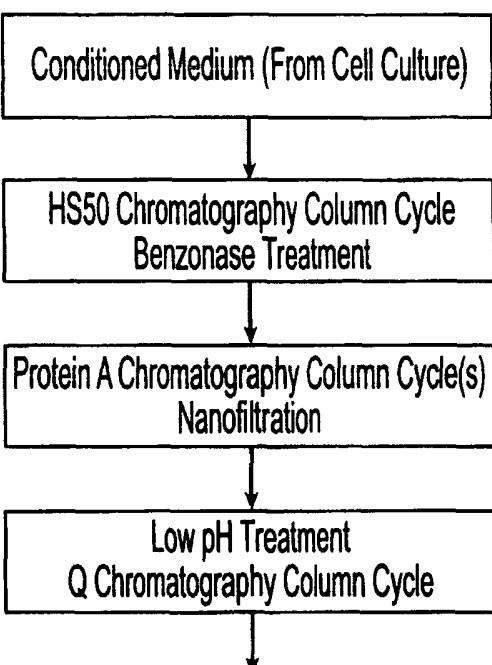
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(54) Title: STABILIZED ANTI-RESPIRATORY SYNCYTIAL VIRUS (RSV) ANTIBODY FORMULATIONS



**(57) Abstract:** The present invention provides liquid formulations of antibodies or fragments thereof that immunospecifically bind to a respiratory syncytial virus (RSV) antigen, which formulations exhibit stability, low to undetectable levels of aggregation, and very little to no loss of the biological activities of the antibodies or antibody fragments, even during long periods of storage. In particular, the present invention provides liquid formulations of antibodies or fragments thereof that immunospecifically bind to a RSV antigen, which formulations are substantially free of surfactant, inorganic salts, and/or other common excipients. Furthermore, the invention provides methods of preventing, treating or ameliorating one or more symptoms associated with RSV infection utilizing the liquid formulations of the present invention.

WO 03/106644 A2



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## **STABILIZED ANTI-RESPIRATORY SYNCYTIAL VIRUS (RSV) ANTIBODY FORMULATIONS**

This application is entitled to and claims priority benefit to U.S. provisional application Serial No. 60/388,920 filed June 14, 2002, which is incorporated herein by reference in its entirety.

### **1. INTRODUCTION**

The present invention relates to liquid formulations of antibodies or fragments thereof that immunospecifically bind to a respiratory syncytial virus (RSV) antigen, which formulations exhibit stability, low to undetectable levels of antibody fragmentation, low to undetectable levels of aggregation, and very little to no loss of the biological activity (e.g., therapeutic efficacy) of the antibodies or antibody fragments, even during long periods of storage. In particular, the present invention relates to liquid formulations of antibodies or fragments thereof that immunospecifically bind to a RSV antigen, which formulations are substantially free of surfactant and/or inorganic salts. The present invention also relates to methods of preventing, treating, managing or ameliorating a RSV infection or one or more symptoms thereof utilizing liquid formulations of antibodies or fragments thereof that immunospecifically bind to a RSV antigen.

### **2. BACKGROUND OF THE INVENTION**

Respiratory syncytial virus (RSV) is the leading cause of serious lower respiratory tract disease in infants and children (Feigen *et al.*, eds., 1987, *In: Textbook of Pediatric Infectious Diseases*, WB Saunders, Philadelphia at pages 1653-1675; New Vaccine Development, Establishing Priorities, Vol. 1, 1985, National Academy Press, Washington DC at pages 397-409; and Ruuskanen *et al.*, 1993, *Curr. Probl. Pediatr.* 23:50-79). The yearly epidemic nature of RSV infection is evident worldwide, but the incidence and severity of RSV disease in a given season vary by region (Hall, C.B., 1993, *Contemp. Pediatr.* 10:92-110). In temperate regions of the northern hemisphere, it usually begins in late fall and ends in late spring (Hall, C.B., 1995, *In: Mandell G.L., Bennett J.E., Dolin R., eds., 1995, Principles and Practice of Infectious Diseases*. 4th ed., Churchill Livingstone, New York at pages 1501-1519). It is estimated that RSV illness results in 90,000

hospitalizations and causes 4,500 deaths annually in the United States. Primary RSV infection occurs most often in children from 6 weeks to 2 years of age and uncommonly in the first 4 weeks of life during nosocomial epidemics (Hall *et al.*, 1979, *New Engl. J. Med.* 300:393-396). RSV is estimated to cause as much as 75% of all childhood bronchiolitis and up to 40% of all pediatric pneumonias (Cunningham, C.K. *et al.*, 1991, *Pediatrics* 88:527-532). Children at increased risk from RSV infection include preterm infants (Hall *et al.*, 1979, *New Engl. J. Med.* 300:393-396) and children with bronchopulmonary dysplasia (Groothuis *et al.*, 1988, *Pediatrics* 82:199-203), congenital heart disease (MacDonald *et al.*, *New Engl. J. Med.* 307:397-400), congenital or acquired immunodeficiency (Ogra *et al.*, 1988, *Pediatr. Infect. Dis. J.* 7:246-249; and Pohl *et al.*, 1992, *J. Infect. Dis.* 165:166-169), and cystic fibrosis (Abman *et al.*, 1988, *J. Pediatr.* 113:826-830). The fatality rate in infants with heart or lung disease who are hospitalized with RSV infection is 3%-4% (Navas *et al.*, 1992, *J. Pediatr.* 121:348-354).

RSV infects adults as well as infants and children. In healthy adults, RSV causes predominantly upper respiratory tract disease. It has recently become evident that some adults, especially the elderly, have symptomatic RSV infections more frequently than had been previously reported (Evans, A.S., eds., 1989, *Viral Infections of Humans. Epidemiology and Control*, 3<sup>rd</sup> ed., Plenum Medical Book, New York at pages 525-544).

Several epidemics also have been reported among nursing home patients and institutionalized young adults (Falsey, A.R., 1991, *Infect. Control Hosp. Epidemiol.* 12:602-608; and Garvie *et al.*, 1980, *Br. Med. J.* 281:1253-1254). Finally, RSV may cause serious disease in immunosuppressed persons, particularly bone marrow transplant patients (Hertz *et al.*, 1989, *Medicine* 68:269-281).

Treatment options for established RSV disease are limited. Severe RSV disease of the lower respiratory tract often requires considerable supportive care, including administration of humidified oxygen and respiratory assistance (Fields *et al.*, eds, 1990, *Fields Virology*, 2<sup>nd</sup> ed., Vol. 1, Raven Press, New York at pages 1045-1072). The only drug approved for treatment of infection is the antiviral agent ribavirin (American Academy of Pediatrics Committee on Infectious Diseases, 1993, *Pediatrics* 92:501-504). It has been shown to be effective in the treatment of RSV pneumonia and bronchiolitis, modifying the course of severe RSV disease in immunocompetent children (Smith *et al.*, 1991, *New Engl. J. Med.* 325:24-29). However, ribavirin has a number of limitations including high cost,

need for prolonged aerosol administration and potential risk to pregnant women as well as to exposed health care personnel. The American Academy of Pediatrics Committee on Infectious Diseases revised their recommendation for use of ribavirin. The current recommendation is that the decision to use ribavirin should be based on the particular clinical circumstances and physician's experience (American Academy of Pediatrics. *Summaries of Infectious Diseases*. In: Pickering L.K., ed., *2000 Red Book: Report of the Committee on Infectious Diseases*. 25th ed., Elk Grove Village, IL, American Academy of Pediatrics, 2000, pp. 483-487).

While a vaccine might prevent RSV infection, no vaccine is yet licensed for this indication. A major obstacle to vaccine development is safety. A formalin-inactivated vaccine, though immunogenic, unexpectedly caused a higher and more severe incidence of lower respiratory tract disease due to RSV in immunized infants than in infants immunized with a similarly prepared trivalent parainfluenza vaccine (Kim *et al.*, 1969, *Am. J. Epidemiol.* 89:422-434; and Kapikian *et al.*, 1969, *Am. J. Epidemiol.* 89:405-421). Several candidate RSV vaccines have been abandoned and others are under development (Murphy *et al.*, 1994, *Virus Res.* 32:13-36), but even if safety issues are resolved, vaccine efficacy must also be improved. A number of problems remain to be solved. Immunization would be required in the immediate neonatal period since the peak incidence of lower respiratory tract disease occurs at 2-5 months of age. The immaturity of the neonatal immune response together with high titers of maternally acquired RSV antibody may be expected to reduce vaccine immunogenicity in the neonatal period (Murphy *et al.*, 1988, *J. Virol.* 62:3907-3910; and Murphy *et al.*, 1991, *Vaccine* 9:185-189). Finally, primary RSV infection and disease do not protect well against subsequent RSV disease (Henderson *et al.*, 1979, *New Engl. J. Med.* 300:530-534).

Currently, the only approved approach to prophylaxis of RSV disease is passive immunization. Initial evidence suggesting a protective role for IgG was obtained from observations involving maternal antibody in ferrets (Prince, G.A., Ph.D. diss., University of California, Los Angeles, 1975) and humans (Lambrecht *et al.*, 1976, *J. Infect. Dis.* 134:211-217; and Glezen *et al.*, 1981, *J. Pediatr.* 98:708-715). Hemming *et al.* (Morell *et al.*, eds., 1986, *Clinical Use of Intravenous Immunoglobulins*, Academic Press, London at pages 285-294) recognized the possible utility of RSV antibody in the treatment or prevention of RSV infection during studies involving the pharmacokinetics of an intravenous immune

globulin (IVIG) in newborns suspected of having neonatal sepsis. They noted that one infant, whose respiratory secretions yielded RSV, recovered rapidly after IVIG infusion. Subsequent analysis of the IVIG lot revealed an unusually high titer of RSV neutralizing antibody. This same group of investigators then examined the ability of hyperimmune serum or immune globulin, enriched for RSV neutralizing antibody, to protect cotton rats and primates against RSV infection (Prince *et al.*, 1985, *Virus Res.* 3:193-206; Prince *et al.*, 1990, *J. Virol.* 64:3091-3092; Hemming *et al.*, 1985, *J. Infect. Dis.* 152:1083-1087; Prince *et al.*, 1983, *Infect. Immun.* 42:81-87; and Prince *et al.*, 1985, *J. Virol.* 55:517-520). Results of these studies suggested that RSV neutralizing antibody given prophylactically inhibited respiratory tract replication of RSV in cotton rats. When given therapeutically, RSV antibody reduced pulmonary viral replication both in cotton rats and in a nonhuman primate model. Furthermore, passive infusion of immune serum or immune globulin did not produce enhanced pulmonary pathology in cotton rats subsequently challenged with RSV.

A humanized antibody directed to an epitope in the A antigenic site of the F protein of RSV, SYNAGIS®, comprising variable heavy (VH) complementarity determining regions (CDRs) having the amino acid sequence of SEQ ID NO:7 and variable light (VL) CDRs having the amino acid sequence of SEQ ID NO:8, is approved for intramuscular administration to pediatric patients for prevention of serious lower respiratory tract disease caused by RSV at recommended monthly doses of 15 mg/kg of body weight throughout the RSV season (November through April in the northern hemisphere). SYNAGIS® is a composite of human (95%) and murine (5%) antibody sequences. See, Johnson *et al.*, 1997, *J. Infect. Diseases* 176:1215-1224 and U.S. Patent No. 5,824,307, the entire contents of which are incorporated herein by reference. The human heavy chain sequence was derived from the constant domains of human IgG<sub>1</sub> and the variable framework regions of the VH genes of Cor (Press *et al.*, 1970, *Biochem. J.* 117:641-660) and Cess (Takashi *et al.*, 1984, *Proc. Natl. Acad. Sci. USA* 81:194-198). The human light chain sequence was derived from the constant domain of C<sub>k</sub> and the variable framework regions of the VL gene K104 with J<sub>k</sub>-4 (Bentley *et al.*, 1980, *Nature* 288:5194-5198). The murine sequences were derived from a murine monoclonal antibody, Mab 1129 (Beeler *et al.*, 1989, *J. Virology* 63:2941-2950), in a process which involved the grafting of the murine complementarity determining regions into the human antibody frameworks.

SYNAGIS® has high specific activity against RSV *in vitro* (approximately 50-100 times that of RespiGam®) and is known to neutralize a broad range of RSV isolates. Since it is not derived from human plasma, prophylactic treatment with SYNAGIS® does not carry potential risk of transmission of blood borne pathogens.

SYNAGIS® was initially formulated as a liquid for IV use, at a concentration of 10 mg/ml SYNAGIS® in phosphate buffered saline. A lyophilized formulation of SYNAGIS®, which allows a higher concentration (100 mg/ml after reconstitution, in 50 mM histidine and 3.2 mM glycine buffer with 6% (w/v) mannitol at pH 6.0) of the antibody than this initial liquid formulation, was produced later to allow intramuscular use. The lyophilized formulation of SYNAGIS® is prepared by lyophilizing SYNAGIS® at 54 mg/ml in an aqueous solution containing 25 mM histidine, 1.6 mM glycine, and 3% (w/v) mannitol at pH 6.0. The initial liquid formulation in PBS and the lyophilized formulation of SYNAGIS® have been tested in phase I clinical studies in healthy adults. The lyophilized formulation was tested in phase I through phase IV studies in pediatric patients.

SYNAGIS®, at doses of 15 mg/kg to 30 mg/kg for adults is found to be well tolerated, and 15 mg/kg for children is found to be safe and efficacious for RSV prophylaxis. The lyophilized formulation was approved in 1998 by the FDA for use in the prevention of serious lower respiratory tract disease caused by RSV in children at high risk of RSV disease.

However, the lyophilized formulation has a number of limitations, including a prolonged process for lyophilization and resulting high cost for manufacturing. In addition, the lyophilized formulation has to be reconstituted aseptically and accurately by healthcare practitioners prior to administering to patients. The reconstitution step itself requires certain specific procedures: (1) a sterile diluent (*i.e.*, water or 5% dextrose in water for intravenous administration and water for intramuscular administration) is added to the vial containing lyophilized SYNAGIS®, slowly and aseptically, and the vial must be swirled very gently for 30 seconds to avoid foaming; (2) the reconstituted SYNAGIS® needs to stand at room temperature for a minimum of 20 minutes until the solution clarifies; and (3) the reconstituted preparation must be administered within six (6) hours after the reconstitution. Such reconstitution procedure is cumbersome and the time limitation after the reconstitution can cause a great inconvenience in administering the formulation to patients, leading to

significant waste, if not reconstituted properly or if the reconstituted dose is not used within six (6) hours and must be discarded.

Thus, a need exists for a liquid formulation of anti-RSV antibodies, in general, at a concentration comparable to or higher than the reconstituted lyophilized formulation so that there is no need to reconstitute the formulation prior to administration. This allows healthcare practitioners much quicker and easier administration of anti-RSV antibodies to a patient.

Prior liquid antibody preparations have short shelf lives and may lose biological activity of the antibodies resulting from chemical and physical instabilities during the storage. Chemical instability may be caused by deamidation, racemization, hydrolysis, oxidation, beta elimination or disulfide exchange, and physical instability may be caused by antibody denaturation, aggregation, precipitation or adsorption. Among those, aggregation, deamidation and oxidation are known to be the most common causes of the antibody degradation (Wang *et al.*, 1988, *J. of Parenteral Science & Technology* 42(Suppl):S4-S26; Cleland *et al.*, 1993, *Critical Reviews in Therapeutic Drug Carrier Systems* 10(4):307-377). Thus, there is a need for a stable liquid formulation of an anti-RSV antibody effective to prevent RSV infection.

### 3. SUMMARY OF INVENTION

The present invention is based, in part, on the development of high concentration liquid formulations of antibodies or fragments thereof that immunospecifically bind to a RSV antigen, which formulations exhibit, in the absence of surfactant, inorganic salts, and/or other excipients, stability and low to undetectable levels of antibody fragmentation and/or aggregation, and very little to no loss of biological activities of the antibody or antibody fragment during manufacture, preparation, transportation, and storage. In particular, the present invention provides liquid formulation of antibodies or fragments thereof immunospecifically bind to a RSV antigen, which antibodies are highly potent, have an improved pharmacokinetic profile and, thus, have an overall improved therapeutic profile, compared to SYNAGIS®. The liquid formulations of the present invention facilitate the administration of antibodies or fragments thereof that immunospecifically bind to a RSV antigen for the prevention, treatment, management and/or amelioration of a RSV infection, one or more symptoms thereof, and other respiratory disorders that is associated with, potentiated by or potentiates a RSV infection. In particular, the liquid formulations of

the present invention enable a healthcare professional to quickly administer a sterile dosage of antibodies or fragments thereof that immunospecifically bind to a RSV antigen without having to accurately and aseptically reconstitute the antibody or antibody fragment prior to administration as required for the lyophilized dosage form. Such liquid formulations can be manufactured more easily and cost effectively than lyophilized formulations since liquid formulations do not require a prolonged drying step, such as lyophilization, freeze-drying, etc. The liquid formulations are made by a process in which the antibody being formulated is in an aqueous phase throughout the purification and formulation process. Preferably, the liquid formulations are made by a process that does not include a drying step, for example, but not by way of limitation, a lyophilization, freeze-drying, spray-drying, or air-drying step.

The present invention provides liquid formulations of anti-RSV antibodies or fragments thereof substantially free of surfactant, inorganic salts, sugars, and/or other common excipients, said formulations comprising histidine and a concentration of about 15 mg/ml or higher of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen. Optionally, the formulation may further comprise glycine. Alternatively, the formulation of the present invention may further comprise other common excipients, such as saccharides, polyols and amino acids, including, but not limited to, arginine, lysine, and methionine. The present invention also provides liquid formulations substantially free of surfactant, inorganic salts, sugars, and/or other commonly-known excipients, with pH ranges of about 5.0 to about 7.0, preferably about 5.5 to 6.5, more preferably about 5.8 to about 6.2, and most preferably about 6.0, said formulations comprising histidine and a concentration of about 15 mg/ml or higher of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen.

The present invention encompasses stable liquid formulations of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen, which formulations exhibit low to undetectable levels of antibody aggregation and/or fragmentation with very little to no loss of the biological activities of the antibody or antibody fragment during manufacture, preparation, transportation, and long periods of storage. The present invention also encompasses stable liquid formulations of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen and have increased *in vivo* half-lives relative to known antibodies such as, *e.g.*, SYNAGIS®, said formulations exhibiting low to

undetectable levels of antibody aggregation and/or fragmentation and very little to no loss of biological activities of the antibodies or antibody fragments. The present invention also encompasses stable liquid formulations of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen, said antibody or antibody fragment comprising a variable heavy (VH) and/or variable light (VL) domain having the amino acid sequence of any VH and/or VL domain listed in Table 1, *infra*, and said formulations exhibiting low to undetectable levels of antibody aggregation and/or fragmentation, and very little to no loss of the biological activities of the antibodies or antibody fragments. The present invention further encompasses stable liquid formulations of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen, said antibody or antibody fragment comprising one or more VH complementarity determining regions (CDRs) and/or one or more VL CDRs having the amino acid sequence of one or more VH CDRs and/or VL CDRS listed in Table 1 and/or Table 2, *infra*, and said formulations exhibiting low to undetectable levels of antibody aggregation and/or fragmentation, and very little to no loss of the biological activities of the antibodies or antibody fragments.

Table 1. Antibodies and Fragments Thereof

Antibody Name	VH Domain	VH CDR1	VH CDR2	VH CDR3	VL Domain	VL CDR1	VL CDR2	VL CDR3
*SYNAGIS	SEQ ID NO:7	TSGMSVG (SEQ ID NO:1)	DIWWBDDKK DYNPSLKS (SEQ ID NO:2)	SMITNWYFDV (SEQ ID NO:3)	SEQ ID NO:8	KCQLS <sub>H</sub> VGYM (SEQ ID NO:4)	DTSKLAS (SEQ ID NO:5)	FQGSGYPFT (SEQ ID NO:6)
AFFF	SEQ ID NO:9	TAGMSVG (SEQ ID NO:10)	DIWWDDKK DYNPSLKS (SEQ ID NO:2)	SMITNFYFDV (SEQ ID NO:12)	SEQ ID NO:13	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQESGYPF (SEQ ID NO:16)
p12f2	SEQ ID NO:17	TPGMSVG (SEQ ID NO:18)	DIWWDDKK HYNPSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:21	SLSSRVGYMH (SEQ ID NO:22)	DTFYLSS (SEQ ID NO:23)	FQGSGYPFT (SEQ ID NO:6)
p12f4	SEQ ID NO:24	TPGMSVG (SEQ ID NO:18)	DIWWDGKK HYNPSLKD (SEQ ID NO:25)	DMIFENFYFDV (SEQ ID NO:20)	SEQ ID NO:26	SLSSRVGYMH (SEQ ID NO:22)	DTRGLPS (SEQ ID NO:27)	FQGSGYPFT (SEQ ID NO:6)
p11d4	SEQ ID NO:28	TPGMSVG (SEQ ID NO:18)	DIWWDGKK HYNPSLKD (SEQ ID NO:25)	DMIFENWYFDV (SEQ ID NO:29)	SEQ ID NO:30	SPSSRVGYMH (SEQ ID NO:31)	DTMRLAS (SEQ ID NO:32)	FQGSGYPFT (SEQ ID NO:6)
Al <sub>e</sub> 109	SEQ ID NO:33	TAGMSVG (SEQ ID NO:10)	DIWWDGKK HYNPSLKD (SEQ ID NO:25)	DMIFENWYFDV (SEQ ID NO:29)	SEQ ID NO:34	SLSSRVGYMH (SEQ ID NO:22)	DTFKLSS (SEQ ID NO:35)	FQGSGYPFT (SEQ ID NO:6)

Antibody Name	VH Domain	VH CDR1	VH CDR2	VH CDR3	VL Domain	VL CDR1	VL CDR2	VL CDR3
A12a6	SEQ ID NO:36	<u>T</u> AGMSVG (SEQ ID NO:10)	DIWW <u>D</u> GKK DYNP <u>L</u> KD (SEQ ID NO:37)	<u>D</u> M <u>I</u> F <u>N</u> FYFDV (SEQ ID NO:20)	SEQ ID NO:38	<u>S</u> ASS <u>R</u> VGYMH (SEQ ID NO:39)	<u>D</u> TE <u>K</u> L <u>S</u> S (SEQ ID NO:35)	FQGSGYPFT (SEQ ID NO:6)
A13c4	SEQ ID NO:40	<u>T</u> AGMSVG (SEQ ID NO:10)	DIWW <u>D</u> GKK <u>S</u> YN <u>P</u> SL <u>K</u> D (SEQ ID NO:41)	<u>D</u> M <u>I</u> F <u>N</u> FYFDV (SEQ ID NO:20)	SEQ ID NO:42	<u>S</u> LS <u>S</u> R <u>V</u> GYMH (SEQ ID NO:22)	<u>D</u> TM <u>Y</u> Q <u>S</u> S (SEQ ID NO:43)	FQGSGYPFT (SEQ ID NO:6)
A17d4	SEQ ID NO:44	<u>T</u> AGMSVG (SEQ ID NO:10)	DIWW <u>D</u> DKKK YN <u>P</u> SL <u>K</u> D (SEQ ID NO:45)	<u>D</u> M <u>I</u> F <u>N</u> FYFDV (SEQ ID NO:20)	SEQ ID NO:46	<u>L</u> P <u>S</u> SS <u>R</u> VGYMH (SEQ ID NO:47)	<u>D</u> TM <u>Y</u> Q <u>S</u> S (SEQ ID NO:43)	FQGSGYPFT (SEQ ID NO:6)
A4B4	SEQ ID NO:48	<u>T</u> AGMSVG (SEQ ID NO:10)	DIWW <u>D</u> DKKK H <u>N</u> P <u>S</u> L <u>K</u> D (SEQ ID NO:19)	<u>D</u> M <u>I</u> F <u>N</u> FYFDV (SEQ ID NO:20)	SEQ ID NO:49	<u>S</u> ASS <u>R</u> VGYMH (SEQ ID NO:39)	<u>D</u> TE <u>F</u> L <u>D</u> S (SEQ ID NO:50)	FQGSGYPFT (SEQ ID NO:6)
A8C7	SEQ ID NO:51	<u>T</u> AGMSVG (SEQ ID NO:10)	DIWW <u>D</u> DKKK YN <u>P</u> SL <u>K</u> D (SEQ ID NO:45)	<u>D</u> M <u>I</u> F <u>N</u> WYFDV (SEQ ID NO:29)	SEQ ID NO:52	<u>S</u> P <u>S</u> SS <u>R</u> VGYMH (SEQ ID NO:31)	<u>D</u> TR <u>Y</u> Q <u>S</u> S (SEQ ID NO:53)	FQGSGYPFT (SEQ ID NO:6)
1X-493L1FR	SEQ ID NO:7	TSGMSVG (SEQ ID NO:1)	DIWW <u>D</u> DKKK DYN <u>P</u> SL <u>K</u> S (SEQ ID NO:2)	SMIT <u>N</u> WYFDV (SEQ ID NO:3)	SEQ ID NO:54	<u>S</u> ASS <u>S</u> VGYMH (SEQ ID NO:14)	DTS <u>K</u> L <u>A</u> S (SEQ ID NO:5)	FQGSGYPFT (SEQ ID NO:6)

Antibody Name	VH Domain	VH CDR1	VH CDR2	VH CDR3	VL Domain	VL CDR1	VL CDR2	VL CDR3
H3-3F4	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDDKK DYNPSLKS (SEQ ID NO:2)	<u>DMIFENWYFDV</u> (SEQ ID NO:29)	SEQ ID NO:56	<u>SASSSVGYMH</u> (SEQ ID NO:14)	<u>DTFKLAS</u> (SEQ ID NO:15)	FQSGGYPFT (SEQ ID NO:6)
M3H9	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDDKK DYNPSLKS (SEQ ID NO:2)	<u>DMIFENWYFDV</u> (SEQ ID NO:29)	SEQ ID NO:56	<u>SASSSVGYMH</u> (SEQ ID NO:14)	<u>DTYKQTS</u> (SEQ ID NO:57)	FQSGGYPFT (SEQ ID NO:6)
Y10H6	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDDKK DYNPSLKS (SEQ ID NO:2)	<u>DMIFENWYFDV</u> (SEQ ID NO:29)	SEQ ID NO:58	<u>SASSSVGYMH</u> (SEQ ID NO:14)	<u>DTRYLSS</u> (SEQ ID NO:59)	FQSGGYPFT (SEQ ID NO:6)
DG	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKK DYNPSLKS (SEQ ID NO:2)	<u>DMITNFYFDV</u> (SEQ ID NO:79)	SEQ ID NO:56	<u>SASSSVGYMH</u> (SEQ ID NO:14)	<u>DTFKLAS</u> (SEQ ID NO:15)	FQSGGYPFT (SEQ ID NO:6)
AFF(1)	SEQ ID NO:9	TAGMSVG (SEQ ID NO:10)	DIWWDDKK DYNPSLKS (SEQ ID NO:2)	<u>SMITNFYFDV</u> (SEQ ID NO:12)	SEQ ID NO:60	<u>SASSSVGYMH</u> (SEQ ID NO:14)	<u>DTFKLAS</u> (SEQ ID NO:15)	FQGSEFYPFT (SEQ ID NO:61)
6H8	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKK DYNPSLKS (SEQ ID NO:2)	<u>DMITNFYFDV</u> (SEQ ID NO:79)	SEQ ID NO:62	<u>SASSSVGYMH</u> (SEQ ID NO:14)	<u>DTFKLTS</u> (SEQ ID NO:63)	FQGSGGYPFT (SEQ ID NO:6)
L1-7E5	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKK DYNPSLKS (SEQ ID NO:2)	<u>DMITNFYFDV</u> (SEQ ID NO:79)	SEQ ID NO:64	<u>SASSRVGYMH</u> (SEQ ID NO:39)	<u>DTFKLAS</u> (SEQ ID NO:15)	FQGSGGYPFT (SEQ ID NO:6)
L215B10	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKK DYNPSLKS (SEQ ID NO:2)	<u>DMITNFYFDV</u> (SEQ ID NO:79)	SEQ ID NO:65	<u>SASSSVGYMH</u> (SEQ ID NO:14)	<u>DTFRLAS</u> (SEQ ID NO:66)	FQGSGGYPFT (SEQ ID NO:6)

Antibody Name	VH Domain	VH CDR1	VH CDR2	VH CDR3	VL Domain	VL CDR1	VL CDR2	VL CDR3
A13A11	SEQ ID NO:67	<u>T</u> AGMSVG (SEQ ID NO:10)	<u>D</u> IWWDDKK <u>H</u> YNPSLKD (SEQ ID NO:19)	<u>D</u> M <u>I</u> FNWYFDV (SEQ ID NO:29)	SEQ ID NO:68	<u>S</u> P <u>S</u> RVGYMH (SEQ ID NO:31)	<u>D</u> T <u>Y</u> RHSS (SEQ ID NO:69)	FQGSGYPFT (SEQ ID NO:6)
A1H5	SEQ ID NO:70	<u>T</u> AGMSVG (SEQ ID NO:10)	<u>D</u> IWWDGKK <u>H</u> YNPSLKD (SEQ ID NO:25)	<u>D</u> M <u>I</u> FNWYFDV (SEQ ID NO:29)	SEQ ID NO:71	<u>S</u> L <u>SS</u> RVGYMH (SEQ ID NO:72)	<u>D</u> TF <u>F</u> HR <u>S</u> (SEQ ID NO:73)	FQGSGYPFT (SEQ ID NO:6)
A4B4(1)	SEQ ID NO:48	<u>T</u> AGMSVG (SEQ ID NO:10)	<u>D</u> IWWDDKK <u>H</u> YNPSLKD (SEQ ID NO:19)	<u>D</u> M <u>I</u> FN <u>F</u> YFDV (SEQ ID NO:20)	SEQ ID NO:74	<u>S</u> A <u>S</u> RVGYMH (SEQ ID NO:39)	<u>D</u> T <u>L</u> LD <u>S</u> (SEQ ID NO:75)	FQGSGYPFT (SEQ ID NO:6)
A4B4L1FR-S28R	SEQ ID NO:48	<u>T</u> AGMSVG (SEQ ID NO:10)	<u>D</u> IWWDDKK <u>H</u> YNPSLKD (SEQ ID NO:19)	<u>D</u> M <u>I</u> FN <u>F</u> YFDV (SEQ ID NO:20)	SEQ ID NO:11	<u>S</u> A <u>S</u> RVGYMH (SEQ ID NO:39)	<u>D</u> TS <u>K</u> LAS (SEQ ID NO:5)	FQGSGYPFT (SEQ ID NO:6)
A4B4-F52S	SEQ ID NO:48	<u>T</u> AGMSVG (SEQ ID NO:10)	<u>D</u> IWWDDKK <u>H</u> YNPSLKD (SEQ ID NO:19)	<u>D</u> M <u>I</u> FN <u>F</u> YFDV (SEQ ID NO:20)	SEQ ID NO:76	<u>S</u> A <u>S</u> RVGYMH (SEQ ID NO:39)	<u>D</u> TS <u>E</u> LD <u>S</u> (SEQ ID NO:77)	FQGSGYPFT (SEQ ID NO:6)

\*Bold faced residues are preferred residues for substitution to obtain antibodies with improved affinity for a RSV antigen.

Underlined residues are the amino acid residues which are distinct from those residues in SYNAGIS®.

Table 2. CDR Sequences

VH CDR1	VH CDR2	VH CDR3	VL CDR1	VL CDR2	VL CDR3
T <u>G</u> MSVG (SEQ ID NO:1)	DIWWD <u>D</u> KK <u>D</u> YNPSL KS (SEQ ID NO:2)	<u>S</u> MT <u>T</u> NW <u>Y</u> FDV (SEQ ID NO:3)	<u>K</u> C <u>Q</u> <u>L</u> S <u>V</u> GYMH (SEQ ID NO:4)	DT <u>S</u> KL <u>A</u> S (SEQ ID NO:5)	FQ <u>G</u> S <u>G</u> YPFT (SEQ ID NO:6)
T <u>G</u> MSVG (SEQ ID NO:18)	DIWWD <u>D</u> KK <u>H</u> YNPSL KD (SEQ ID NO:19)	<u>D</u> MT <u>T</u> N <u>F</u> YFDV (SEQ ID NO:208)	<u>K</u> C <u>Q</u> <u>S</u> <u>V</u> GYMH (SEQ ID NO:80)	DT <u>S</u> Y <u>L</u> <u>A</u> S (SEQ ID NO:81)	FQ <u>E</u> S <u>G</u> YPFT (SEQ ID NO:209)
T <u>G</u> MSVG (SEQ ID NO:10)	DIWWD <u>D</u> KK <u>H</u> YNPSL KS (SEQ ID NO:82)	<u>D</u> MT <u>T</u> N <u>W</u> YFDV (SEQ ID NO:83)	<u>K</u> C <u>Q</u> <u>S</u> <u>R</u> VGYMH (SEQ ID NO:84)	DT <u>S</u> Y <u>L</u> <u>S</u> <u>S</u> (SEQ ID NO:85)	FQ <u>G</u> S <u>F</u> YPFT (SEQ ID NO:61)
	DIWWD <u>D</u> KK <u>D</u> YNPSL KD (SEQ ID NO:86)	<u>D</u> MT <u>F</u> N <u>W</u> YFDV (SEQ ID NO:29)	<u>K</u> C <u>Q</u> <u>L</u> R <u>V</u> GYMH (SEQ ID NO:87)	DT <u>K</u> <u>K</u> <u>L</u> <u>S</u> (SEQ ID NO:88)	
	DIWWD <u>D</u> KK <u>H</u> YNPSL KS (SEQ ID NO:91)	<u>D</u> MT <u>F</u> N <u>F</u> YFDV (SEQ ID NO:20)	<u>K</u> L <u>Q</u> <u>L</u> S <u>V</u> GYMH (SEQ ID NO:89)	DT <u>F</u> <u>Y</u> <u>L</u> <u>S</u> (SEQ ID NO:90)	
	DIWWD <u>D</u> KK <u>D</u> YNPSL KD (SEQ ID NO:93)	<u>S</u> MT <u>T</u> N <u>F</u> YFDV (SEQ ID NO:12)	<u>K</u> L <u>Q</u> <u>S</u> <u>S</u> VGYMH (SEQ ID NO:92)	DT <u>E</u> <u>K</u> <u>L</u> <u>A</u> S (SEQ ID NO:15)	
	DIWWD <u>D</u> GKK <u>H</u> YNPSL KD (SEQ ID NO:25)	<u>S</u> MT <u>F</u> N <u>W</u> YFDV (SEQ ID NO:94)	<u>K</u> L <u>Q</u> <u>S</u> <u>R</u> VGYMH (SEQ ID NO:95)	DT <u>E</u> <u>K</u> <u>L</u> <u>S</u> <u>S</u> (SEQ ID NO:96)	

	DIWW <u>DGKKD</u> YNPSL <u>KS</u> (SEQ ID NO:100)	<u>SMIFENFYFDV</u> (SEQ ID NO:97)	<u>KLQLR</u> VGymh (SEQ ID NO:98)	<u>DTEFLAS</u> (SEQ ID NO:99)
	DIWW <u>DGKKD</u> YNPSL <u>KD</u> (SEQ ID NO:103)	<u>KLSLSVGymh</u> (SEQ ID NO:101)	<u>DTSKLPS</u> (SEQ ID NO:102)	
	DIWW <u>DGKKH</u> YNPSL <u>KS</u> (SEQ ID NO:106)	<u>KLSSSVGymh</u> (SEQ ID NO:104)	<u>DTSGLAS</u> (SEQ ID NO:105)	
	DIWW <u>DGKKSY</u> NP <u>SL</u> <u>KS</u> (SEQ ID NO:109)	<u>KLSR</u> VGymh **(SEQ ID NO:107)	<u>DTSGLPS</u> (SEQ ID NO:108)	
	DIWW <u>DDGKKSY</u> NP <u>SL</u> <u>KD</u> (SEQ ID NO:111)	<u>KLSLR</u> VGymh (SEQ ID NO:110)	<u>DTRGLPS</u> (SEQ ID NO:27)	
	DIWW <u>DGKKSY</u> NP <u>SL</u> <u>KS</u> (SEQ ID NO:114)	<u>KCSLSVGymh</u> (SEQ ID NO:112)	<u>DTRKLAS</u> (SEQ ID NO:113)	
	DIWW <u>DGKKSY</u> NP <u>SL</u> <u>KD</u> (SEQ ID NO:41)	<u>KCSSSVGymh</u> (SEQ ID NO:115)	<u>DTRGLAS</u> (SEQ ID NO:116)	
		<u>KCSSR</u> VGymh (SEQ ID NO:117)	<u>DTRKLPs</u> (SEQ ID NO:118)	
		<u>KCSLR</u> VGymh (SEQ ID NO:119)	<u>DTMRLAS</u> (SEQ ID NO:32)	

	<u>SLSLSVGYMH</u> (SEQ ID NO:120)	<u>DTMKLAS</u> (SEQ ID NO:121)	
	<u>SLSSSVGYMH</u> (SEQ ID NO:122)	<u>DTSRILAS</u> (SEQ ID NO:123)	
	<u>SLSSRVGYMH</u> (SEQ ID NO:22)	<u>DTSILLAS</u> (SEQ ID NO:124)	
	<u>SLSLRVGYMH</u> (SEQ ID NO:125)	<u>DTSILLDS</u> (SEQ ID NO:126)	
	<u>SCQLSVGYMH</u> (SEQ ID NO:127)	<u>DTSKLDS</u> (SEQ ID NO:128)	
	<u>SCOSSVGYMH</u> (SEQ ID NO:129)	<u>DTLLLDS</u> (SEQ ID NO:75)	
	<u>SCOSRVGYMH</u> (SEQ ID NO:130)	<u>DTLKLDs</u> (SEQ ID NO:131)	
	<u>SCQLRVGYMH</u> (SEQ ID NO:132)	<u>DTLILLAS</u> (SEQ ID NO:133)	
	<u>SLQLSVGYMH</u> (SEQ ID NO:134)	<u>DTLKLAS</u> (SEQ ID NO:135)	
	<u>SLQSSVGYMH</u> (SEQ ID NO:136)	<u>DTSKLSS</u> (SEQ ID NO:137)	
	<u>SLOSRVGYMH</u> (SEQ ID NO:138)	<u>DTSKQAS</u> (SEQ ID NO:139)	

	<u><b>SLOQLRVGYMH</b></u> (SEQ ID NO:140)	<u><b>DTSKQSS</b></u> (SEQ ID NO:141)	
	<u><b>SCSLSVGYMH</b></u> (SEQ ID NO:142)	<u><b>DTSYLAS</b></u> (SEQ ID NO:143)	
	<u><b>SCSSSVGYMH</b></u> (SEQ ID NO:144)	<u><b>DTSYLS</b></u> (SEQ ID NO:145)	
	<u><b>SCSSSRVGYMH</b></u> (SEQ ID NO:146)	<u><b>DTSYQAS</b></u> (SEQ ID NO:147)	
	<u><b>SCSLLRVGYMH</b></u> (SEQ ID NO:148)	<u><b>DTSYQSS</b></u> (SEQ ID NO:149)	
	<u><b>KPSSRVGYMH</b></u> (SEQ ID NO:150)	<u><b>DTMYQAS</b></u> (SEQ ID NO:151)	
	<u><b>KPSLRVGYMH</b></u> (SEQ ID NO:152)	<u><b>DTMYQSS</b></u> (SEQ ID NO:43)	
	<u><b>KPSSSVGYMH</b></u> (SEQ ID NO:153)	<u><b>DTMKQAS</b></u> (SEQ ID NO:154)	
	<u><b>KPSLSVGYMH</b></u> (SEQ ID NO:155)	<u><b>DTMKQSS</b></u> (SEQ ID NO:156)	
	<u><b>KPQSRVGYMH</b></u> (SEQ ID NO:157)	<u><b>DTMYLAS</b></u> (SEQ ID NO:158)	
	<u><b>KPOLRVGYMH</b></u> (SEQ ID NO:159)	<u><b>DTMYLSS</b></u> (SEQ ID NO:160)	

	<b>KPQSSVGYMH</b> (SEQ ID NO:161)	<b>DTMKLAS</b> (SEQ ID NO:162)
	<b>KPQLSVGYMH</b> (SEQ ID NO:163)	<b>DTMKLSS</b> (SEQ ID NO:164)
	<b>SPSSSRVGYMH</b> (SEQ ID NO:31)	<b>DTSKLSS</b> (SEQ ID NO:165)
	<b>SPSILRVGYMH</b> (SEQ ID NO:166)	<b>DTRYQAS</b> (SEQ ID NO:167)
	<b>SPSSSSVGYMH</b> (SEQ ID NO:168)	<b>DTRYQSS</b> (SEQ ID NO:53)
	<b>SPSILSVGYMH</b> (SEQ ID NO:169)	<b>DTRKQAS</b> (SEQ ID NO:170)
	<b>SPOSRSVGYMH</b> (SEQ ID NO:171)	<b>DTRKQSS</b> (SEQ ID NO:172)
	<b>SPQLLRVGYMH</b> (SEQ ID NO:173)	<b>DTRKLAS</b> (SEQ ID NO:174)
	<b>SPQSSSVGYMH</b> (SEQ ID NO:175)	<b>DTRKLSS</b> (SEQ ID NO:176)
	<b>SPQLSVGYMH</b> (SEQ ID NO:177)	<b>DTRYLAS</b> (SEQ ID NO:178)
	<b>KAQOSRVGYMH</b> (SEQ ID NO:179)	<b>DTRYLSS</b> (SEQ ID NO:59)

	<u>KAQLR</u> VGYMH (SEQ ID NO:180)	DT <u>FIELD</u> (SEQ ID NO:50)	
	<u>KAQSS</u> VGYMH (SEQ ID NO:181)	DT <u>FIELD</u> (SEQ ID NO:77)	
	<u>KAQLS</u> VGYMH (SEQ ID NO:182)		
	<u>KASSR</u> VGYMH (SEQ ID NO:183)		
	<u>KASLR</u> VGYMH (SEQ ID NO:184)		
	<u>KASSS</u> VGYMH (SEQ ID NO:185)		
	<u>KASLS</u> VGYMH (SEQ ID NO:186)		
	<u>SASSR</u> VGYMH (SEQ ID NO:39)		
	<u>SASLR</u> VGYMH (SEQ ID NO:187)		
	<u>SASSS</u> VGYMH (SEQ ID NO:14)		
	<u>SASLS</u> VGYMH (SEQ ID NO:188)		

	<u>SAQS</u> RVGYMH (SEQ ID NO:189)	
	<u>SAQ</u> <u>L</u> RVGYMH (SEQ ID NO:190)	
	<u>SAQSS</u> VGYMH (SEQ ID NO:191)	
	<u>LPSSR</u> VGYMH (SEQ ID NO:47)	
	<u>LPSI</u> SVGYMH (SEQ ID NO:192)	
	<u>LPSSS</u> VGYMH (SEQ ID NO:193)	
	<u>LPSI</u> <u>L</u> RVGYMH (SEQ ID NO:194)	
	<u>LCSSR</u> VGYMH (SEQ ID NO:195)	
	<u>LCSL</u> SVGYMH (SEQ ID NO:196)	
	<u>LCSSS</u> VGYMH (SEQ ID NO:197)	
	<u>LCSL</u> <u>R</u> VGYMH (SEQ ID NO:198)	

	<b><u>LPQSR</u></b> VGYMH (SEQ ID NO:199)	
	<b><u>LPQLS</u></b> VGYMH (SEQ ID NO:200)	
	<b><u>LPQSS</u></b> VGYMH (SEQ ID NO:201)	
	<b><u>LPQLR</u></b> VGYMH (SEQ ID NO:202)	
	<b><u>LCQSR</u></b> VGYMH (SEQ ID NO:203)	
	<b><u>LCQLS</u></b> VGYMH (SEQ ID NO:204)	
	<b><u>LCQSS</u></b> VGYMH (SEQ ID NO:205)	
	<b><u>LCQLR</u></b> VGYMH (SEQ ID NO:206)	
	<b><u>SAQLS</u></b> VGYMH (SEQ ID NO:207)	

Bold faced and underlined residues are the amino acid residues which are distinct from those residues in SYNAGIS®.

The present invention encompasses liquid formulations of antibodies or fragments thereof that immunospecifically bind to a RSV antigen, said formulations having stability at 38 - 42°C as assessed by high performance size exclusion chromatography (HPSEC). The present invention encompasses liquid formulations of an antibody or fragment thereof that immunospecifically binds to a RSV antigen, said formulations having stability, as assessed by HPSEC, at the temperature ranges of 38°C-42°C for at least 60 days (in specific embodiments, not more than 120 days), of 20°C-24°C for at least 1 year, and of 2°C-8°C for at least 3 years. The present invention also encompasses liquid formulations of antibodies or fragments thereof that immunospecifically bind to a RSV antigen, said formulations having low to undetectable levels of antibody aggregation as measured by HPSEC, and further, exhibit very little to no loss of the biological activity(ies) of the antibodies or antibody fragments of the formulation compared to the reference antibodies as measured by antibody binding assays such as, *e.g.*, ELISAs.

The present invention provides methods for preparing liquid formulations of an antibody or fragment thereof that immunospecifically binds to a RSV antigen, said methods comprising concentrating a fraction containing the purified antibody or antibody fragment to a final concentration about 15 mg/ml, about 20 mg/ml, about 30 mg/ml, about 40 mg/ml, about 50 mg/ml, about 60 mg/ml, about 70 mg/ml, about 80 mg/ml, about 90 mg/ml, about 100 mg/ml, about 200 mg/ml, about 250 mg/ml, or about 300 mg/ml using a semi-permeable membrane with an appropriate molecular weight (mw) cutoff (*e.g.*, a 30 kD cutoff for whole antibody molecules and F(ab')<sub>2</sub> fragments, and a 10 kD cutoff for antibody fragments such as a Fab fragments and diafiltering the concentrated antibody or antibody fragment fraction into the formulation buffer using the same membrane. The formulation buffer of the present invention comprises histidine at a concentration ranging from about 1 mM to about 100 mM, about 10 mM to about 50 mM, about 20 mM to about 30 mM, or about 23 mM to about 27 mM, and is most preferably about 25 mM. To obtain an appropriate pH for a particular antibody or antibody fragment, it is preferable that histidine (and glycine, if added) is first dissolved in water to obtain a buffer solution with higher pH than the desired pH and then the pH is brought down to the desired level by the addition of HCl. This way, the formation of inorganic salts (*e.g.*, the formation of NaCl when, *e.g.*, histidine hydrochloride is used as the source of histidine and the pH is raised to the desired level by the addition of NaOH) can be avoided.

The liquid formulations of the present invention are prepared by maintaining the antibodies in an aqueous solution at any time during the preparation. In other words, the liquid formulations are prepared without involving any step of drying the antibodies or the formulations themselves by, for example, lyophilization, vacuum drying, etc.

The liquid formulations of the present invention may be sterilized by sterile filtration using a 0.2 or 0.22 micron filter. Sterilized liquid formulations of the present invention may be administered to a subject to prevent, treat, manage or ameliorate a RSV infection or one or more symptoms thereof, or other respiratory conditions associated with, potentiated by, or potentiates a RSV infection.

The present invention also provides kits comprising the liquid formulations of antibodies or fragments thereof that immunospecifically bind to a RSV antigen for use by, *e.g.*, a healthcare professional. The present invention further provides methods of preventing, treating, managing or ameliorating a RSV infection or one or more symptoms thereof, or other respiratory conditions associated with, potentiated by, or potentiates a RSV infection by administering the liquid formulations of the present invention. The liquid formulations of the present invention can also be used to diagnose, detect or monitor a RSV infection.

### 3.1 Terminology

All liquid formulations of antibodies and/or fragments thereof that immunospecifically bind to a RSV antigen described above are herein collectively referred to as “liquid formulations of the invention,” “antibody liquid formulations of the invention,” “liquid formulations of antibodies or fragments thereof that immunospecifically bind to a RSV antigen,” or “liquid formulations of anti-RSV antibodies.”

As used herein, the term “analogue” in the context of proteinaceous agent (*e.g.*, proteins, polypeptides, peptides, and antibodies) refers to a proteinaceous agent that possesses a similar or identical function as a second proteinaceous agent but does not necessarily comprise a similar or identical amino acid sequence of the second proteinaceous agent, or possess a similar or identical structure of the second proteinaceous agent. The analogues herein referred to do not include SYNAGIS®. In a specific embodiment, antibody analogues immunospecifically bind to the same epitope as the original antibodies from which the analogues were derived. In an alternative embodiment, antibody analogues

immunospecifically bind to different epitopes than the original antibodies from which the analogues were derived. In another embodiment, antibody analogues compete with antibodies for binding to the epitope that SYNAGIS® binds to or compete for binding to an epitope with SYNAGIS®. A proteinaceous agent that has a similar amino acid sequence refers to a second proteinaceous agent that satisfies at least one of the following: (a) a proteinaceous agent having an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the amino acid sequence of a second proteinaceous agent; (b) a proteinaceous agent encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence encoding a second proteinaceous agent of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, or at least 150 contiguous amino acid residues; and (c) a proteinaceous agent encoded by a nucleotide sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleotide sequence encoding a second proteinaceous agent. A proteinaceous agent with similar structure to a second proteinaceous agent refers to a proteinaceous agent that has a similar secondary, tertiary or quaternary structure to the second proteinaceous agent. The structure of a proteinaceous agent can be determined by methods known to those skilled in the art, including but not limited to, peptide sequencing, X ray crystallography, nuclear magnetic resonance, circular dichroism, and crystallographic electron microscopy.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then

compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length.

The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2264 2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873 5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present invention. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, Nucleic Acids Res. 25:3389 3402. Alternatively, PSI BLAST can be used to perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, the NCBI website). Another preferred, non limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11 17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

As used herein, the term “analogue” in the context of a non-proteinaceous analog refers to a second organic or inorganic molecule which possess a similar or identical function as a first organic or inorganic molecule and is structurally similar to the first organic or inorganic molecule.

The term “antibody fragment” as used herein refers to a fragment of an antibody that immunospecifically binds to a RSV antigen. Antibody fragments may be generated by any technique known to one of skill in the art. For example, Fab and F(ab')<sub>2</sub> fragments may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the complete light chain, and the variable region, the CH1 region and the hinge region of the heavy chain. Antibody fragments can be also produced by recombinant DNA technologies. Antibody fragments may be one or more complementarity determining regions (CDRs) of antibodies.

The terms “antibody” and “antibodies” as used herein refer to monoclonal antibodies, bispecific antibodies, multispecific antibodies, human antibodies, humanized antibodies, chimeric antibodies, camelised antibodies, single domain antibodies, single-chain Fvs (scFv), single chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass.

As used herein, the term “cytokine receptor modulator” refers to an agent which modulates the phosphorylation of a cytokine receptor, the activation of a signal transduction pathway associated with a cytokine receptor, and/or the expression of a particular protein such as a cytokine. Such an agent may directly or indirectly modulate the phosphorylation of a cytokine receptor, the activation of a signal transduction pathway associated with a cytokine receptor, and/or the expression of a particular protein such as a cytokine. Thus, examples of cytokine receptor modulators include, but are not limited to, cytokines, fragments of cytokines, fusion proteins and antibodies that immunospecifically binds to a cytokine receptor or a fragment thereof. Further, examples of cytokine receptor modulators

include, but are not limited to, peptides, polypeptides (*e.g.*, soluble cytokine receptors), fusion proteins and antibodies that immunospecifically binds to a cytokine or a fragment thereof.

As used herein, the term “derivative” in the context of proteinaceous agent (*e.g.*, proteins, polypeptides, peptides, and antibodies) refers to a proteinaceous agent that comprises an amino acid sequence which has been altered by the introduction of amino acid residue substitutions, deletions, and/or additions. The term “derivative” as used herein also refers to a proteinaceous agent which has been modified, *i.e.*, by the covalent attachment of any type of molecule to the proteinaceous agent. For example, but not by way of limitation, an antibody may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of a proteinaceous agent may be produced by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of a proteinaceous agent may contain one or more non-classical amino acids. A derivative of a proteinaceous agent possesses a similar or identical function as the proteinaceous agent from which it was derived.

As used herein, the term “derivative” in the context of a non-proteinaceous derivative refers to a second organic or inorganic molecule that is formed based upon the structure of a first organic or inorganic molecule. A derivative of an organic molecule includes, but is not limited to, a molecule modified, *e.g.*, by the addition or deletion of a hydroxyl, methyl, ethyl, carboxyl or amine group. An organic molecule may also be esterified, alkylated and/or phosphorylated.

The term “epitope” as used herein refers to a fragment of a RSV polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. An epitope having immunogenic activity is a fragment of a RSV polypeptide that elicits an antibody response in an animal. An epitope having antigenic activity is a fragment of a RSV polypeptide to which an antibody immunospecifically binds as determined by any method well known in the art, for example, by the immunoassays described herein. Antigenic epitopes need not necessarily be immunogenic.

The term “excipients” as used herein refers to inert substances which are commonly used as a diluent, vehicle, preservatives, binders, or stabilizing agent for drugs and includes, but not limited to, proteins (*e.g.*, serum albumin, etc.), amino acids (*e.g.*, aspartic acid, glutamic acid, lysine, arginine, glycine, histidine, etc.), fatty acids and phospholipids (*e.g.*, alkyl sulfonates, caprylate, etc.), surfactants (*e.g.*, SDS, polysorbate, nonionic surfactant, etc.), saccharides (*e.g.*, sucrose, maltose, trehalose, etc.) and polyols (*e.g.*, mannitol, sorbitol, etc.). Also see Remington’s Pharmaceutical Sciences (by Joseph P. Remington, 18th ed., Mack Publishing Co., Easton, PA), which is hereby incorporated in its entirety.

The term “fragment” as used herein refers to a peptide, polypeptide, or protein (including an antibody) comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least contiguous 80 amino acid residues, at least contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least contiguous 200 amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of another polypeptide or protein. In a specific embodiment, a fragment of a protein or polypeptide retains at least one function of the protein or polypeptide. In another embodiment, a fragment of a protein or polypeptide retains at least two, three or four functions of the protein or polypeptide. Preferably a fragment of an antibody that immunospecifically binds to a RSV antigen retains the ability to bind to a RSV antigen.

The term “fusion protein” as used refers to a polypeptide or protein that comprises an amino acid sequence of a first protein, polypeptide or fragment, analogue or derivative thereof, and an amino acid sequence of a heterologous protein or polypeptide (*i.e.*, a second protein, polypeptide or fragment, analogue or derivative thereof different than the first protein or fragment, analogue or derivative thereof). In one embodiment, a fusion protein comprises a prophylactic or therapeutic agent fused to a heterologous protein, polypeptide or peptide. In accordance with this embodiment, the heterologous protein, polypeptide or peptide may or may not be a different type of prophylactic or therapeutic agent.

The terms "high concentration" and "concentrated antibody" as used herein refer to a concentration of 50 mg/ml or higher, preferably 95 mg/ml or higher of an antibody or fragment thereof that immunospecifically binds to a RSV antigen, in an antibody formulation.

The term "host cell" as used herein includes a subject cell transfected or transformed with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

The term "hybridizes under stringent conditions" as used herein describes conditions for hybridization and washing under which nucleotide sequences at least 30% (preferably at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%) identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. In one, non limiting example stringent hybridization conditions are hybridization at 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.1XSSC, 0.2% SDS at about 68°C. In a preferred, non-limiting example stringent hybridization conditions are hybridization in 6XSSC at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C (i.e., one or more washes at 50°C, 55°C, 60°C or 65°C). It is understood that the nucleic acids of the invention do not include nucleic acid molecules that hybridize under these conditions solely to a nucleotide sequence consisting of only A or T nucleotides.

The term "human infant" as used herein refers to a human less than 24 months, preferably less than 16 months, less than 12 months, less than 6 months, less than 3 months, less than 2 months, or less than 1 month of age.

The term "human infant born prematurely" as used herein refers to a human born at less than 40 weeks gestational age, preferably less than 35 weeks gestational age, who is less than 6 months old, preferably less than 3 months old, more preferably less than 2 months old, and most preferably less than 1 month old.

As used herein, the term “immunospecifically bind to a RSV antigen”, “anti-RSV antibodies” and analogous terms refer to antibodies or fragments thereof that specifically bind to a RSV antigen and do not specifically bind to other polypeptides. An antibody or fragment thereof that immunospecifically binds to a RSV antigen may bind to other peptides or polypeptides with lower affinity as determined by, *e.g.*, immunoassays, BIAcore, isothermal titration calorimetry, or other assays known in the art. An antibody or a fragment thereof that immunospecifically binds to a RSV antigen may be cross-reactive with related antigens. Preferably, an antibody or a fragment thereof that immunospecifically binds to a RSV antigen does not cross-react with other antigens. An antibody or a fragment thereof that immunospecifically binds to a RSV antigen can be identified, for example, by immunoassays, BIAcore, or other techniques known to those of skill in the art. An antibody or a fragment thereof binds specifically to a RSV antigen when it binds to a RSV antigen with higher affinity than to any cross-reactive antigen as determined using experimental techniques, such as radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (ELISAs). See, *e.g.*, Paul, ed., 1989, Fundamental Immunology Second Edition, Raven Press, New York at pages 332-336 for a discussion regarding antibody specificity.

The term “immunomodulatory agent” and variations thereof including, but not limited to, immunomodulatory agents, as used herein refer to an agent that modulates a host’s immune system. In certain embodiments, an immunomodulatory agent is an immunosuppressant agent. In certain other embodiments, an immunomodulatory agent is an immunostimulatory agent. In accordance with the invention, an immunomodulatory agent used in the combination therapies of the invention does not include an anti-RSV antibody or fragment thereof. Immunomodulatory agents include, but are not limited to, small molecules, peptides, polypeptides, proteins, fusion proteins, antibodies, inorganic molecules, mimetic agents, and organic molecules.

The term “in combination” as used herein refers to the use of more than one therapies (*e.g.*, prophylactic and/or therapeutic agents). The use of the term “in combination” does not restrict the order in which therapies (*e.g.*, prophylactic and/or therapeutic agents) are administered to a subject with a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection. A first therapy (*e.g.*, a prophylactic or therapeutic agent) can be administered prior to (*e.g.*, 5 minutes, 15

minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy (e.g., a prophylactic or therapeutic agent) to a subject with a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection.

The term “inorganic salt” as used herein refers to any compounds containing no carbon that result from replacement of part or all of the acid hydrogen or an acid by a metal or a group acting like a metal and are often used as a tonicity adjusting compound in pharmaceutical compositions and preparations of biological materials. The most common inorganic salts are NaCl, KCl, NaH<sub>2</sub>PO<sub>4</sub>, etc.

As used herein, the term “isolated” in the context of a proteinaceous agent (e.g., a peptide, polypeptide, fusion protein, or antibody) refers to a proteinaceous agent which is substantially free of cellular material or contaminating proteins from the cell or tissue source from which it is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language “substantially free of cellular material” includes preparations of a proteinaceous agent in which the proteinaceous agent is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a proteinaceous agent that is substantially free of cellular material includes preparations of a proteinaceous agent having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein, polypeptide, peptide, or antibody (also referred to as a “contaminating protein”). When the proteinaceous agent is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When the proteinaceous agent is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the proteinaceous agent. Accordingly, such preparations of a proteinaceous agent have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the

proteinaceous agent of interest. In a preferred embodiment, an antibody of the invention is isolated.

As used herein, the term “isolated” in the context of nucleic acid molecules refers to a nucleic acid molecule which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an “isolated” nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In a preferred embodiment, a nucleic acid molecule encoding an antibody of the invention is isolated.

The phrase “low to undetectable levels of aggregation” as used herein refers to samples containing no more than 5%, no more than 4%, no more than 3%, no more than 2%, no more than 1% and most preferably no more than 0.5% aggregation by weight of protein as measured by high performance size exclusion chromatography (HPSEC).

The term “low to undetectable levels of fragmentation” as used herein refers to samples containing equal to or more than 80%, 85%, 90%, 95%, 98% or 99% of the total protein, for example, in a single peak as determined by HPSEC, or in two peaks (heavy- and light-chains) by reduced Capillary Gel Electrophoresis (rCGE), representing the non-degraded antibody or a non-degraded fragment thereof, and containing no other single peaks having more than 5%, more than 4%, more than 3%, more than 2%, more than 1%, or more than 0.5% of the total protein in each. The term “reduced Capillary Gel Electrophoresis” as used herein refers to capillary gel electrophoresis under reducing conditions sufficient to reduce disulfide bonds in an antibody or fragment thereof.

As used herein, the terms “manage”, “managing” and “management” refer to the beneficial effects that a subject derives from a therapy (*e.g.*, a prophylactic or therapeutic agent), which does not result in a cure of the infection. In certain embodiments, a subject is administered one or more therapies (*e.g.*, prophylactic or therapeutic agents) to “manage” a infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection, so as to prevent the progression or worsening of the infection.

As used herein, the term “mast cell modulator” refers to an agent which modulates the activation of a mast cell, mast cell degranulation, and/or expression of a particular protein such as a cytokine. Such an agent may directly or indirectly modulate the activation

of a mast cell, degranulation of the mast cell, and/or the expression of a particular protein such as a cytokine. Non-limiting examples of mast cell modulators include, but are not limited to, small molecules, peptides, polypeptides, proteins, nucleic acids (e.g., DNA and RNA nucleotides including, but not limited to, antisense nucleotide sequences, triple helices, RNAi, and nucleotide sequences encoding biologically active proteins, polypeptides, or peptides), fusion proteins, antibodies, synthetic or natural inorganic molecules, synthetic or natural organic molecule, or mimetic agents which inhibit and/or reduce the expression, function, and/or activity of a stem cell factor, a mast cell protease, a cytokine (such as IL-3, IL-4, and IL-9), a cytokine receptor (such as IL-3R, IL-4R, and IL-9R), and a stem cell receptor. Other non-limiting examples of mast cell modulators include, but are not limited to small molecules, peptides, polypeptides, proteins, nucleic acids (e.g., DNA and RNA nucleotides including, but not limited to, antisense nucleotide sequences, triple helices, RNAi, and nucleotide sequences encoding biologically active proteins, polypeptides, or peptides), fusion proteins, antibodies, synthetic or natural inorganic molecules, synthetic or natural organic molecule, or mimetic agents which inhibit and/or reduce the expression, function and/or activity of IgE. In certain embodiments, a mast cell modulator is an agent that prevents or reduces the activation of additional mast cells following degranulation of mast cells. In other embodiments, a mast cell modulator is an agent that inhibits or reduces mast cell degranulation.

The terms “non-responsive” and “refractory” as used herein describe patients treated with a currently available therapy (such as but not limited to, a prophylactic or therapeutic agent) for a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection, which is not clinically adequate to relieve one or more symptoms associated with the infection. Typically, such patients suffer from severe, persistently active infection and require additional therapy to ameliorate the symptoms associated with their infection or respiratory condition.

As used herein, the terms “nucleic acids” and “nucleotide sequences” include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), combinations of DNA and RNA molecules or hybrid DNA/RNA molecules, and analogues of DNA or RNA molecules. Such analogues can be generated using, for example, nucleotide analogues, which include, but are not limited to, inosine or tritylated bases. Such analogues can also comprise DNA or RNA molecules comprising modified backbones that lend beneficial

attributes to the molecules such as, for example, nuclease resistance or an increased ability to cross cellular membranes. The nucleic acids or nucleotide sequences can be single-stranded, double-stranded, may contain both single-stranded and double-stranded portions, and may contain triple-stranded portions, but preferably is double-stranded DNA.

The term “pharmaceutically acceptable” as used herein means being approved by a regulatory agency of the Federal or a state government, or listed in the U.S. Pharmacopia, European Pharmacopia or other generally recognized pharmacopia for use in animals, and more particularly in humans.

The term “polyol” as used herein refers to a sugar that contains many -OH groups compared to a normal saccharide.

The terms “prophylactic agent” and “prophylactic agents” as used refer to any agent(s) which can be used in the prevention of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection. In certain embodiments, the term “prophylactic agent” refers to an antibody or fragment thereof that immunospecifically binds to a RSV antigen. In accordance with these embodiments, the antibody or antibody fragment may be a component of a liquid formulation of the invention. In certain other embodiments, the term “prophylactic agent” does not refer to an antibody or fragment thereof that immunospecifically binds to a RSV antigen or a formulation comprising such an antibody or antibody fragment. In certain other embodiments, the term “prophylactic agent” does not refer to SYNAGIS® or a antigen-binding fragment thereof.

The terms “prevent”, “preventing” and “prevention” as used herein refer to the prevention of the recurrence, onset, or development of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection in a subject resulting from the administration of a therapy (*e.g.*, a prophylactic or therapeutic agent), or combination therapies (*e.g.*, the administration of a combination of prophylactic agents).

The phrase “prophylactically effective amount” as used herein refers to the amount of a therapy (*e.g.*, a prophylactic agent (*e.g.*, an antibody or fragment thereof that immunospecifically binds to a RSV antigen or the amount of a liquid formulation of the invention comprising said antibody or antibody fragment)), which is sufficient to result in the prevention of the development, recurrence, onset, or progression of a RSV infection,

one or more symptoms thereof, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection, or to enhance or improve the prophylactic effect(s) of another therapy (*e.g.*, a prophylactic agent). In a specific embodiment, a prophylactically effective amount of a prophylactic agent reduces one or more of the following steps of a RSV life cycle: the docking of the virus particle to a cell, the introduction of viral genetic information into a cell, the expression of viral proteins, the production of new virus particles and the release of virus particles from a cell by at least 5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%. In another specific embodiment, a prophylactically effective amount of a prophylactic agent reduces the replication, multiplication or spread of a virus by at least 5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% compared to the same in the absence of the agent or the presence of a negative control (*e.g.*, control IgG or phosphate buffered saline (PBS)).

The term “RSV antigen” as used herein refers to a RSV protein, polypeptide, peptide or fragment thereof to which an antibody or antibody fragment immunospecifically binds. A RSV antigen also refers to a derivative of a RSV protein, polypeptide, peptide or a fragment thereof to which an antibody or antibody fragment immunospecifically binds.

The term “saccharide” as used herein refers to a class of molecules that are derivatives of polyhydric alcohols. Saccharides are commonly referred to as carbohydrates and may contain different amounts of sugar (saccharide) units, *e.g.*, monosaccharides, disaccharides and polysaccharides.

The phrase “side effects” as used herein encompasses unwanted and adverse effects of a prophylactic or therapeutic agent. Adverse effects are always unwanted, but unwanted effects are not necessarily adverse. An adverse effect from a prophylactic or therapeutic agent might be harmful or uncomfortable or risky.

The term “small molecule” and analogous terms include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogues, polynucleotides, polynucleotide analogues, nucleotides, nucleotide analogues, organic or inorganic

compounds (*i.e.*, including heterorganic and/or ganometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

The terms “stability” and “stable” as used herein in the context of a liquid formulation comprising an antibody or fragment thereof that immunospecifically binds to a RSV antigen refer to the resistance of the antibody or antibody fragment in the formulation to thermal and chemical unfolding, aggregation, degradation or fragmentation under given manufacture, preparation, transportation and storage conditions. The “stable” formulations of the invention retain biological activity equal to or more than 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% under given manufacture, preparation, transportation and storage conditions. The stability of the antibody or antibody fragment can be assessed by degrees of aggregation, degradation or fragmentation by methods known to those skilled in the art, including but not limited to reduced Capillary Gel Electrophoresis (rCGE), Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and HPSEC, compared to a reference, that is, a commercially available lyophilized SYNAGIS® reconstituted to 100 mg/ml in 50 mM histidine/3.2 mM glycine buffer with 6% mannitol at pH 6.0. The reference regularly gives a single peak ( $\geq 97\%$  area) by HPSEC. The overall stability of a formulation comprising an antibody or fragment thereof that immunospecifically binds to a RSV antigen can be assessed by various immunological assays including, for example, ELISA and radioimmunoassay using the specific epitope of RSV.

As used herein, the term “SYNAGIS® standard reference” or analogous terms refer to commercially available lyophilized SYNAGIS®, as described in the Physicians’ Desk Reference, 56<sup>th</sup> edition, 2002. Reconstituted SYNAGIS® may contain, *e.g.*, the following excipients: 47 mM histidine, 3.0 mM glycine and 5.6% manitol and the active ingredient, the antibody, at a concentration of 100 milligrams per ml solution.

As used herein, the terms “subject” and “patient” are used interchangeably. As used herein, the terms “subject” and “subjects” refer to an animal, preferably a mammal including a non-primate (*e.g.*, a cow, pig, horse, cat, dog, rat, and mouse) and a non-primate

(*e.g.*, a monkey such as a cynomolgous monkey and a human), and more preferably a human.

The term “substantially free of surfactant” as used herein refers to a formulation of an antibody or fragment thereof that immunospecifically binds to a RSV antigen, said formulation containing less than 0.0005%, less than 0.0003%, or less than 0.0001% of surfactants and/or less than 0.0005%, less than 0.0003%, or less than 0.0001% of surfactants.

The term “substantially free of salt” as used herein refers to a formulation of an antibody or fragment thereof that immunospecifically binds to a RSV antigen, said formulation containing less than 0.0005%, less than 0.0003%, or less than 0.0001% of inorganic salts.

The term “surfactant” as used herein refers to organic substances having amphipathic structures; namely, they are composed of groups of opposing solubility tendencies, typically an oil-soluble hydrocarbon chain and a water-soluble ionic group. Surfactants can be classified, depending on the charge of the surface-active moiety, into anionic, cationic, and nonionic surfactants. Surfactants are often used as wetting, emulsifying, solubilizing, and dispersing agents for various pharmaceutical compositions and preparations of biological materials.

The term “synergistic” as used herein refers to a combination of therapies (*e.g.*, use of prophylactic or therapeutic agents) which is more effective than the additive effects of any two or more single therapy. For example, a synergistic effect of a combination of prophylactic or therapeutic agents permits the use of lower dosages of one or more of the agents and/or less frequent administration of said agents to a subject with a RSV infection. The ability to utilize lower dosages of prophylactic or therapeutic therapies and/or to administer said therapies less frequently reduces the toxicity associated with the administration of said therapies to a subject without reducing the efficacy of said therapies in the prevention, management or treatment of a RSV infection. In addition, a synergistic effect can result in improved efficacy of therapies in the prevention or treatment of a RSV infection. Finally, synergistic effect of a combination of therapies (*e.g.*, prophylactic or therapeutic agents) may avoid or reduce adverse or unwanted side effects associated with the use of any single therapy.

The terms “therapeutic agent” and “therapeutic agents” as used herein refer to any agent(s) which can be used in the treatment, management or amelioration a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection. In certain embodiments, the term “therapeutic agent” refers to an antibody or fragment thereof that immunospecifically binds to a RSV antigen. In accordance with these embodiments, the antibody or antibody fragment may be a component of a liquid formulation of the invention. In certain other embodiments, the term “therapeutic agent” does not refer to an antibody or fragment thereof that immunospecifically binds to a RSV antigen or a liquid formulation comprising such an antibody or antibody fragment. In certain other embodiments, the term “therapeutic agent” does not refer to SYNAGIS® or an antigen-binding fragment thereof.

The term “therapeutically effective amount” as used herein refers to the amount of a therapy (*e.g.*, a therapeutic agent (*e.g.*, an antibody or a fragment thereof, which immunospecifically binds to a RSV antigen or a liquid formulation of the invention comprising said antibody or antibody fragment)), which is sufficient to (i) reduce the severity, and/or duration of a RSV infection, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection; (ii) ameliorate one or more symptoms associated with a RSV infection, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection; (iii) prevent the advancement of a RSV infection, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection; (iv) cause regression of a RSV infection, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection; or (v) enhance or improve the therapeutic effect(s) of another therapy (*e.g.*, another therapeutic agent). With respect to the treatment of a RSV infection, a therapeutically effective amount refers to the amount of a therapeutic agent sufficient to reduce or inhibit the replication of a virus, inhibit or reduce the infection of cell with the virus, inhibit or reduce the production of the viral particles, inhibit or reduce the release of viral particles, inhibit or reduce the spread of the virus to other tissues or subjects, or ameliorate one or more symptoms associated with the infection. In a specific embodiment, a therapeutically effective amount of a therapeutic agent reduces one or more of the following steps of a RSV life cycle: the docking of the virus particle to a cell, the introduction of viral genetic information into a cell, the expression of viral proteins, the production of new virus particles and the release of virus particles from a cell by at least

5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%. In another specific embodiment, a therapeutically effective amount of a therapeutic agent reduces the replication, multiplication or spread of a virus by at least 5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% compared to the absence of the agent or the presence of a negative control (e.g., a control IgG or PBS).

As used herein, the terms “therapies” and “therapy” can refer to any protocol(s), method(s) and/or agent(s) that can be used in the prevention, treatment, management or amelioration of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection. In certain embodiments, the terms “therapy” and “therapies” refer to hormonal therapy, biological therapy, and/or other therapies useful for the treatment of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection known to medical professionals.

The terms “treat”, “treatment” and “treating” as used herein refer to the reduction or amelioration of the progression, severity, and/or duration of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection resulting from the administration of one or more therapies (including but not limited to, the administration of one or more prophylactic or therapeutic agents, and any other methods that can be used). In specific embodiments, such terms refer to the reduction or inhibition of the replication of a respiratory syncytial virus (RSV), the inhibition or reduction in the spread of a respiratory syncytial virus (RSV) to other tissues or subjects, the inhibition or reduction of infection of a cell with a respiratory syncytial virus (RSV), or the amelioration of one or more symptoms associated with a respiratory syncytial virus (RSV) infection.

As used herein, the term “T cell receptor modulator” refers to an agent which modulates the phosphorylation of a T cell receptor, the activation of a signal transduction pathway associated with a T cell receptor and/or the expression of a particular protein such

as a cytokine. Such an agent may directly or indirectly modulate the phosphorylation of a T cell receptor, the activation of a signal transduction pathway associated with a T cell receptor, and/or the expression of a particular protein such as a cytokine. Examples of T cell receptor modulators include, but are not limited to, peptides, polypeptides, proteins, fusion proteins and antibodies which immunospecifically bind to a T cell receptor or a fragment thereof. Further, examples of T cell receptor modulators include, but are not limited to, proteins, peptides, polypeptides (e.g., soluble T cell receptors), fusion proteins and antibodies that immunospecifically binds to a ligand for a T cell receptor or a fragment thereof.

The term “very little to no loss of the biological activities” as used herein refers to antibody activities, including specific binding abilities of antibodies or antibody fragments to a RSV antigen as measured by various immunological assays, including, but not limited to ELISAs and radioimmunoassays. In one embodiment, the antibodies or antibody fragments of the formulations of the invention retain approximately 50%, preferably 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98% of the ability to immunospecifically bind to a RSV antigen as compared to a reference antibody or antibody fragment (e.g., SYNAGIS®) as measured by an immunological assay known to one of skill in the art or described herein. For example, an ELISA based assay may be used to compare the ability of an antibody or fragment thereof to immunospecifically bind to a RSV antigen to a SYNAGIS® reference standard. In this assay, plates are coated with a RSV antigen and the binding signal of a set concentration of a SYNAGIS® reference standard is compared to the binding signal of the same concentration of a test antibody or antibody fragment.

#### **4. BRIEF DESCRIPTION OF THE FIGURES**

Fig. 1 is a schematic diagram showing the outline for preparing purified antibodies that immunospecifically bind to a RSV antigen.

#### **5. DETAILED DESCRIPTION OF THE INVENTION**

The liquid formulations of the present invention provide a ready-to-use preparation of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen for administering to a subject without having to reconstitute the preparation accurately and aseptically and waiting for a period of time until the solution clarifies before administering the formulation to the subject. It simplifies the procedure of administering the formulation

to a subject for a healthcare professional. Furthermore, due to its high stability during the storage, the formulations of the present invention can contain an antibody or a fragment thereof that immunospecifically binds to a RSV antigen at concentrations in the range of about 15 mg/ml to about 300 mg/ml without causing an adverse effect on the biological activities of the antibody or a fragment thereof due to protein aggregation and/or fragmentation during a prolonged storage. Such stability not only ensures the efficacy of the antibodies or antibody fragments but also reduces possible risks of causing adverse effects on a subject. Furthermore, the use of fewer components in the formulation results in fewer risks of introducing contamination. In addition, the manufacturing process of the liquid formulations of the present invention is simplified and more efficient than the manufacturing process for the lyophilized version because all stages of the manufacturing of the liquid formulations are carried out in an aqueous solution, involving no drying process, such as lyophilization and freeze-drying. Accordingly, it is more cost effective as well.

### **5.1      Liquid Formulations of Anti-RSV Antibodies**

The liquid formulations of the present invention provide antibody formulations which are substantially free of surfactant, inorganic salts, and/or other excipients and yet exhibit high stability during long periods of storage. In a specific embodiment, such antibody formulations are homogeneous. The formulations of the present invention comprise histidine at concentrations between 1 and 100 mM and an antibody or a fragment thereof which immunospecifically binds to a RSV antigen at concentrations of about 15 mg/ml to about 300 mg/ml. In one embodiment, the formulations of the invention do not comprise other ingredients except for water or suitable solvents. In a specific embodiment, the antibody or antibody fragment that immunospecifically binds to a RSV antigen which is included in the liquid formulations of the invention is not SYNAGIS® or a fragment thereof. In an alternative embodiment, at least one of the antibodies or antibody fragments that is included in the liquid formulations of the invention comprises two or more antibodies or antibody fragments that immunospecifically bind to a RSV antigen which is included in the liquid formulations of the invention is SYNAGIS® or a fragment thereof.

In one embodiment, the antibody or antibody fragment that immunospecifically binds to a RSV antigen which is included in the liquid formulations of the invention is an

antibody or antibody fragment comprising a VH domain and/or VL domain listed in Table 1, *supra*. In another embodiment, the antibody or antibody fragment that immunospecifically binds to a RSV antigen which is included in the liquid formulations of the invention is an antibody or antibody fragment comprising one or more VH CDRs and/or one or more VL CDRs in Table 1, *supra*. In another embodiment, the antibody or antibody fragment that immunospecifically binds to a RSV antigen which is included in the liquid formulations of the invention is an antibody or antibody fragment conjugated to another moiety, including, but not limited to, a heterologous polypeptide, another antibody or another fragment, a marker sequence, a diagnostic agent, a therapeutic agent, a radioactive metal ion, a polymer, albumin, and a solid support. In yet another embodiment, liquid formulations of the invention comprise two or more antibodies or antibody fragments that immunospecifically binds to a RSV antigen, wherein at least one of the antibodies or antibody fragments is SYNAGIS® or a fragment thereof.

The concentration of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen which is included in the liquid formulations of the invention is at least 15 mg/ml, at least 20 mg/ml, at least 25 mg/ml, at least 30 mg/ml, at least 35 mg/ml, at least 40 mg/ml, at least 45 mg/ml, at least 50 mg/ml, at least 55 mg/ml, at least 60 mg/ml, at least 65 mg/ml, at least 70 mg/ml, at least 75 mg/ml, at least 80 mg/ml, at least 85 mg/ml, at least 90 mg/ml, at least 95 mg/ml, at least 100 mg/ml, at least 105 mg/ml, at least 110 mg/ml, at least 115 mg/ml, at least 120 mg/ml, at least 125 mg/ml, at least 130 mg/ml, at least 135 mg/ml, at least 140 mg/ml, at least 150 mg/ml, at least 200 mg/ml, at least 250 mg/ml, or at least 300 mg/ml.

The concentration of histidine which is included in the liquid formulations of the invention ranges from about 1 mM to about 100 mM, about 10 mM to about 50 mM, about 20 mM to about 30 mM, or about 23 mM to about 27 mM, and is most preferably about 25 mM. Histidine can be in the form of L-histidine, D-histidine, or a mixture thereof, but L-histidine is the most preferable. Histidine can be also in the form of hydrates. Histidine may be used in a form of pharmaceutically acceptable salt, such as hydrochloride (e.g., monohydrochloride and dihydrochloride), hydrobromide, sulfate, acetate, etc. The purity of histidine should be at least 98%, preferably at least 99%, and most preferably at least 99.5%.

The pH of the formulation should not be equal to the isoelectric point of the particular antibody to be used in the formulation and may range from about 5.0 to about 7, preferably about 5.5 to about 6.5, more preferably about 5.8 to about 6.2, and most preferably about 6.0.

In addition to histidine and an antibody or a fragment thereof that immunospecifically binds to a RSV antigen, the formulations of the present invention may further comprise glycine at a concentration of less than 100 mM, less than 50 mM, less than 3.0 mM, less than 2.0 mM, or less than 1.8 mM, and most preferably 1.6 mM. The amount of glycine in the formulation should not cause a significant buffering effect so that antibody precipitation at its isoelectric point can be avoided. Glycine may be also used in a form of pharmaceutically acceptable salt, such as hydrochloride, hydrobromide, sulfate, acetate, etc. The purity of glycine should be at least 98%, preferably at least 99%, and most preferably 99.5%. In a specific embodiment, glycine is included in the formulations of the present invention.

Optionally, the formulations of the present invention may further comprise other excipients, such as saccharides (*e.g.*, sucrose, mannose, trehalose, etc.) and polyols (*e.g.*, mannitol, sorbitol, etc.). In one embodiment, the other excipient is a saccharide. In a specific embodiment, the saccharide is sucrose, which is at a concentration ranging from between about 1% to about 20%, preferably about 5% to about 15%, and more preferably about 8% to 10%. In another embodiment, the other excipient is a polyol. Preferably, however, the liquid formulations of the present invention do not contain mannitol. In a specific embodiment, the polyol is polysorbate (*e.g.*, Tween 20), which is at a concentration ranging from between about 0.001% to about 1%, preferably, about 0.01 to about 0.1.

The liquid formulations of the present invention exhibit stability at the temperature ranges of 38°C-42°C for at least 60 days and, in some embodiments, not more than 120 days, of 20°C-24°C for at least 1 year, of 2°C-8°C (in particular, at 4°C) for at least 3 years, at least 4 years, or at least 5 years and at -20°C for at least 3 years, at least 4 years, or at least 5 years, as assessed by high performance size exclusion chromatography (HPSEC). Namely, the liquid formulations of the present invention have low to undetectable levels of aggregation and/or fragmentation, as defined herein, after the storage for the defined periods as set forth above. Preferably, no more than 5%, no more than 4%, no more than 3%, no more than 2%, no more than 1%, and most preferably no more than 0.5% of the

antibody or antibody fragment forms an aggregate as measured by HPSEC, after the storage for the defined periods as set forth above. Furthermore, liquid formulations of the present invention exhibit almost no loss in biological activities of the antibody or antibody fragment during the prolonged storage under the condition described above, as assessed by various immunological assays including, but not limited to, enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay to measure the ability of the antibody or antibody fragment to immunospecifically bind to a RSV antigen, and by a C3a/C4a assay to measure the complement activating ability of the antibody. The liquid formulations of the present invention retain after the storage for the above-defined periods more than 80%, more than 85%, more than 90%, more than 95%, more than 98%, more than 99%, or more than 99.5% of the initial biological activities of the formulation prior to the storage.

The liquid formulations of the present invention can be prepared as unit dosage forms. For example, a unit dosage per vial may contain 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml, 10 ml, 15 ml, or 20 ml of different concentrations of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen ranging from about 15 mg/ml to about 300 mg/ml. If necessary, these preparations can be adjusted to a desired concentration by adding a sterile diluent to each vial.

The invention encompasses stable liquid formulations comprising a single antibody or fragment thereof that immunospecifically binds to a RSV antigen, with the proviso that said antibody is not SYNAGIS®. The invention also encompasses stable liquid formulations comprising two or more antibodies or fragments thereof that immunospecifically bind to a RSV antigen. In one embodiment, a stable liquid formulation of the invention comprises two or more antibodies or fragments thereof that immunospecifically bind to a RSV antigen, wherein one of the antibodies or antibody fragments is SYNAGIS® or a fragment thereof. In an alternative embodiment, a stable liquid formulation of the invention comprises two or more antibodies or fragments thereof that immunospecifically bind to a RSV antigen, with the proviso that the antibodies or antibody fragments do not include SYNAGIS® or a fragment thereof.

### **5.1.1 Antibodies Immunospecific for a RSV Antigen**

The invention relates to liquid formulations comprising antibodies that immunospecifically bind to a RSV antigen. In a preferred embodiment, the invention

provides liquid formulations comprising one or more of the antibodies listed in Table 1, *supra*.

The present invention encompasses stable liquid formulations comprising one or more analogues or derivatives of one or more antibodies recited in Table 1, *supra*. Such antibodies or fragments thereof having increased affinity for a RSV antigen will result in increased efficacy for prophylactic or therapeutic uses such that lower serum titers are prophylactically or therapeutically effective, thus allowing administration of lower dosages and/or reduced frequency of administration. Such antibodies or fragments thereof which have increased affinity for a RSV antigen may be obtained by introducing one or more amino acid residue modifications, such as amino acid substitutions, in the light-chain variable domain (VL) and/or heavy-chain variable domain (VH) of the antibodies listed in Table 1, *supra*. Furthermore, antibodies or fragments having improved affinity for a RSV antigen may be obtained by introducing one or more amino acid residue modifications, such as amino acid substitutions, in one or more complementarity determining regions (CDRs) of the VL and/or VH of the antibodies listed in Table 1, *supra*.

Standard techniques known to those of skill in the art can be used to introduce mutations (*e.g.*, additions, deletions, and/or substitutions) in the nucleotide sequence encoding an antibody of the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which results in amino acid substitutions. Preferably, the derivatives include less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the original molecule. In a preferred embodiment, the derivatives have conservative amino acid substitutions made at one or more predicted non-essential amino acid residues (*i.e.*, amino acid residues which are not critical for the antibody to immunospecifically bind to a RSV antigen). A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*,

alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded antibody can be expressed and the activity of the antibody can be determined.

The antibody generated by introducing substitutions in the VH domain, VH CDRs, VL domain and/or VL CDRs of an antibody listed in Table 1 can be tested *in vitro* and *in vivo*, for example, for its ability to bind to a RSV antigen (by, e.g., immunoassays including, but not limited to ELISAs and BIACore), or for its ability to prevent, treat, manage or ameliorate a RSV infection or a symptom thereof.

In a specific embodiment, an antibody that immunospecifically binds to a RSV infection comprises a nucleotide sequence that hybridizes to the nucleotide sequence encoding an antibody listed in Table 1 under stringent conditions, e.g., hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 °C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65 °C, under highly stringent conditions, e.g., hybridization to filter-bound nucleic acid in 6xSSC at about 45 °C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68 °C, or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3).

In a specific embodiment, an antibody that immunospecifically binds to a RSV antigen comprises an amino acid sequence of a VH domain or an amino acid sequence a VL domain encoded by a nucleotide sequence that hybridizes to the nucleotide sequence encoding the VH or VL domains of an antibody listed in Table 1 under stringent conditions, e.g., hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 °C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65 °C, under highly stringent conditions, e.g., hybridization to filter-bound nucleic acid in 6xSSC at about 45 °C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68 °C, or under other stringent hybridization conditions which are known to those of skill in the art

(see, for example, Ausubel, F.M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3).

In another embodiment, an antibody that immunospecifically binds to a RSV antigen comprises an amino acid sequence of a VH CDR and/or an amino acid sequence of a VL CDR encoded by a nucleotide sequence that hybridizes to the nucleotide sequence encoding any one of the VH CDRs or VL CDRs listed in Table 1 or Table 2 under stringent conditions *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 °C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65 °C, under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in 6xSSC at about 45 °C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68 °C, or under other stringent hybridization conditions which are known to those of skill in the art.

In a specific embodiment, an antibody that immunospecifically binds to a RSV antigen comprises an amino acid sequence of a VH domain and/or VL domain that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of a VH domain and/or a VL domain of an antibody listed in Table 1, and/or an amino acid sequence of one or more VL CDRs. The determination of percent identity of two amino acid sequences can be determined by any method known to one skilled in the art, including BLAST protein searches.

In another embodiment, an antibody that immunospecifically binds to a RSV antigen comprises an amino acid sequence of one or more VH CDRs and/or an amino acid sequence of one or more VL CDRs that are at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to any of one of the VH CDRs and/or any of the VL CDRs of an antibody listed in Table 1 or Table 2.

In a specific embodiment, an antibody or fragment thereof that immunospecifically binds to a RSV antigen comprises one or more amino acid residue substitutions of the amino acid residues indicated in bold face and underlining in Table 2 (*see Section 3, supra*). In another specific embodiment, an antibody or a fragment thereof that immunospecifically binds to a RSV antigen comprises a VH domain having an amino acid sequence of any one

of the VH domains listed in Table 1 (*see* Section 3) and/or a VL domain having an amino acid sequence of any one of the VL domains listed in Table 1. In another embodiment, an antibody or a fragment thereof that immunospecifically binds to a RSV antigen comprises one, two or more of the VH CDRs listed in Table 1 and/or Table 2, and/or one or more of the VL CDRs listed in Table 1 and/or Table 2. In yet another embodiment, an antibody or fragment thereof that immunospecifically binds to a RSV antigen comprises a VH CDR1 and a VL CDR1; a VH CDR1 and a VL CDR2; a VH CDR1 and a VL CDR3; a VH CDR2 and a VL CDR1; VH CDR2 and VL CDR2; a VH CDR2 and a VL CDR3; a VH CDR3 and a VH CDR1; a VH CDR3 and a VL CDR2; a VH CDR3 and a VL CDR3; a VH1 CDR1, a VH CDR2 and a VL CDR1; a VH CDR1, a VH CDR2 and a VL CDR2; a VH CDR1, a VH CDR2 and a VL CDR3; a VH CDR2, a VH CDR3 and a VL CDR1, a VH CDR2, a VH CDR3 and a VL CDR2; a VH CDR2, a VH CDR2 and a VL CDR3; a VH CDR1, a VL CDR1 and a VL CDR2; a VH CDR1, a VL CDR1 and a VL CDR3; a VH CDR2, a VL CDR1 and a VL CDR2; a VH CDR2, a VL CDR1 and a VL CDR3; a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR2; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR1, a VL CDR2, and a VL CDR3; a VH CDR1, a VH CDR3, a VL CDR1, a VL CDR2, and a VL CDR3; or any combination thereof of the VH CDRs and VL CDRs listed in Table 1, and/or Table 2, *supra*. Such antibodies and methods for preparing them are disclosed in copending U.S. patent application Serial Nos. 09/724,396 and 09/724,531, both filed November 28, 2000, entitled "Methods of Administering/Dosing Anti-RSV Antibodies for Prophylaxis and Treatment" and by J. Young *et al.*; continuation-in-part applications serial nos. 09/996,288 and 09/996,265, both filed November 28, 2001, also entitled "Methods of Administering/ Dosing Anti-RSV Antibodies for Prophylaxis and Treatment" by Young *et*

*al.*; and continuation-in-part application serial no. 10/403,180, filed March 31, 2003, all of which are hereby incorporated by reference in their entireties. In certain embodiments, the antibodies or fragments thereof that immunospecifically bind to a RSV antigen do not comprise a VH CDR, a VL CDR, the VH domain or the VL domain of SYNAGIS®.

The present invention also encompasses the liquid formulations comprising antibodies that are not those listed in Table 1, which immunospecifically bind to a RSV antigen. In other words, the invention encompasses liquid formulations of any antibodies and fragments thereof which immunospecifically bind to one or more RSV antigens. Further, the invention encompasses liquid formulations comprising one or more of the novel antibodies, fragments and other biological or macromolecules that immunospecifically bind to one or more RSV antigens. These novel agents are disclosed in detail in pending U.S. patent application Serial No. 09/865,499 filed May 25, 2001, which is hereby incorporated by reference in its entirety.

Preferably, the antibodies or fragments thereof contained in the formulations of the invention immunospecifically bind to a RSV antigen regardless of the strain of RSV. Alternatively, the antibodies or fragments thereof may differentially or preferentially bind to RSV antigens from one strain of RSV versus another RSV strain, as assessed, for example, by competitive immunoassays. In a specific embodiment, the antibodies or fragments thereof contained in the formulations of the present invention immunospecifically bind to the RSV F glycoprotein, G glycoprotein or SH protein. In a preferred embodiment, the antibodies or fragments thereof immunospecifically bind to the RSV F glycoprotein. In another preferred embodiment, the antibodies or fragments thereof contained in the formulations of the present invention immunospecifically bind to the A, B, or C antigenic sites of the RSV F glycoprotein. In certain embodiments, such antibodies are not SYNAGIS®.

Antibodies contained in the formulations of the invention include, but are not limited to, monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, chimeric antibodies, single-chain Fvs (scFv), single domain antibodies, single chain antibodies, Fab fragments, F(ab)2 fragments, disulfide-linked Fvs (sdFv), and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. In particular, antibodies of the present invention include immunoglobulin molecules and immunologically active

fragments of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds to a RSV antigen. The immunoglobulin molecules of the invention can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, IgA<sub>1</sub> and IgA<sub>2</sub>) or subclass of immunoglobulin molecule.

The antibodies contained in the formulations of the present invention may be from any animal origin including birds and mammals (*e.g.*, human, murine, donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken). Preferably, the antibodies of the invention are human or humanized monoclonal antibodies. As used herein, “human antibodies” include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from mice that express antibodies from human genes.

The antibodies contained in the formulations of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a RSV protein or polypeptide or may be specific for both a RSV protein or polypeptide as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, *e.g.*, International Publication Nos: WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, *et al.*, *J. Immunol.* 147:60-69(1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny *et al.*, *J. Immunol.* 148:1547-1553 (1992).

The antibodies or fragments thereof contained in the formulations of the present invention may exhibit a high potency as described in copending U.S. patent application Serial Nos. 09/724,396, 60/168,426, 60/186,252. High potency antibodies or fragments thereof can be produced by methods disclosed in copending U.S. patent application Serial Nos. 60/168,426 and 60/186,252, filed January 27, 2000 and March 1, 2000, respectively, both entitled “High Potency Recombinant Antibodies and Methods for Producing Them,” and methods described in U.S. patent application Serial No. 09/724,396, each of which is hereby incorporated by reference by its entirety. For example, high potency antibodies can be produced by genetically engineering appropriate antibody gene sequences and expressing the antibody sequences in a suitable host. The antibodies produced can be screened to identify antibodies having, for example, a high association constant ( $k_{on}$ ) in a BIACore assay. The antibodies or fragments thereof contained in the formulations of the present invention may also exhibit ultra high affinity as described in copending U.S.

application Serial No. 09/771,415, filed January 26, 2001, which is hereby incorporated by reference in its entirety.

The present invention encompasses antibodies that compete with an antibody described herein for binding to a RSV antigen. In a specific embodiment, the present invention encompasses antibodies that compete with SYNAGIS® or an antigen-binding fragment thereof for binding to a RSV antigen. In a particular embodiment, the present invention encompasses antibodies that compete with SYNAGIS® or an antigen-binding fragment thereof for binding to the same epitope of a RSV antigen (in particular RSV F antigen) and does not just sterically inhibit the binding of SYNAGIS® or antigen-binding fragment thereof to its epitope. Techniques well-known in the art (*e.g.*, competitive binding assays) can be used to identify antibodies or fragments thereof that compete with SYNAGIS® or an antigen-binding fragment thereof for binding to its epitope. The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. See U.S. Patent No. 09/996,228 filed November 28, 2001, which is incorporated herein by reference in its entirety. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (*e.g.*,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of the present invention or a fragment thereof for a RSV antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, a RSV antigen is incubated with an antibody of the present invention or a fragment thereof conjugated to a labeled compound (*e.g.*,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second antibody.

In a preferred embodiment, BIACore kinetic analysis is used to determine the binding on and off rates of antibodies or fragments thereof to a RSV antigen. BIACore kinetic analysis comprises analyzing the binding and dissociation of a RSV antigen from chips with immobilized antibodies or fragments thereof on their surface.

Antibodies that immunospecifically bind to a RSV antigen include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation,

pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The present invention also encompasses antibodies that immunospecifically bind to a RSV antigen, said antibodies comprising a framework region known to those of skill in the art. Preferably, the fragment region of an antibody of the invention is human. In a specific embodiment, an antibody that immunospecifically binds to a RSV antigen comprises the framework region of SYNAGIS®.

### **5.1.2 Antibodies Having Increased Half-lives That Immunospecifically Bind to a RSV Antigen**

The present invention encompasses stable liquid formulations comprising one or more antibodies or fragments thereof that immunospecifically bind to a RSV antigen and have improved half-lives compared to other known anti-RSV antibodies, *e.g.*, SYNAGIS®. In particular, the present invention provides liquid formulations comprising one or more antibodies or fragments thereof that immunospecifically bind to a RSV antigen which have a half-life in an animal, preferably a mammal and most preferably a human, of greater than 3 days, greater than 7 days, greater than 10 days, preferably greater than 15 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months. By prolonging the half-lives of antibodies, it is possible to reduce the amount and/or frequency of dosing of the antibodies.

Antibodies that immunospecifically bind to a RSV antigen and have increased half-lives *in vivo* relative to the anti-RSV antibodies may be produced by, for example, introducing modifications (*e.g.*, by amino acid substitution, deletion, or insertion) into the constant domain or FcRn (Fc Receptor-neonate) binding domain of an IgG molecule. This increases the affinity of the constant domain or FcRn binding domain for the FcRn which, in turn, increases the *in vivo* half-life of the IgG molecule. Antibodies and fragments thereof with improved *in vivo* half-lives and methods for preparing them are disclosed in U.S. Patent No. 6,277,375; International Publication Nos. WO 98/23289 and WO 97/3461;

and copending U.S. patent application Serial No. 10/020,354 filed December 12, 2001, which claims priority to U.S. provisional application nos. 60/254,884 filed December 12, 2000 and 60/289,760 filed May 9, 2001, all entitled "Molecules with Extended Half-Lives, Compositions and Uses" and by L. Johnson *et al.*; each of which is incorporated herein by reference in its entirety.

The serum circulation of antibodies (*e.g.*, monoclonal antibodies, single chain antibodies and Fab fragments) *in vivo* may also be prolonged by attaching inert polymer molecules such as high molecular weight polyethyleneglycol (PEG) to the antibodies with or without a multifunctional linker either through site-specific conjugation of the PEG to the N- or C-terminus of the antibodies or via epsilon-amino groups present on lysine residues. Linear or branched polymer derivatization that results in minimal loss of biological activity will be used. The degree of conjugation can be closely monitored by SDS-PAGE and mass spectrometry to ensure proper conjugation of PEG molecules to the antibodies. Unreacted PEG can be separated from antibody-PEG conjugates by size-exclusion or by ion-exchange chromatography. PEG-derivatized antibodies can be tested for binding activity as well as for *in vivo* efficacy using methods known to those of skill in the art, for example, by immunoassays described herein.

Further, antibodies or antibody fragments that immunospecific bind to a RSV antigen can be conjugated to albumin in order to make the antibody or antibody fragment more stable *in vivo* or have a longer half life *in vivo*. The techniques are well known in the art, see *e.g.*, International Publication Nos. WO 93/15199, WO 93/15200, and WO 01/77137; and European Patent No. EP 413, 622, all of which are incorporated herein by reference.

### 5.1.3 Antibody Conjugates

The present invention encompasses the use of liquid formulations of antibodies or fragments thereof that immunospecifically bind to a RSV antigen that conjugated to one or more moieties, including but not limited to, peptides, polypeptides, proteins, fusion proteins, nucleic acid molecules, small molecules, mimetic agents, synthetic drugs, inorganic molecules, and organic molecules.

The present invention encompasses the use of liquid formulations of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to one or

more moieties including, but not limited to, peptides, polypeptides, proteins, fusion proteins, nucleic acid molecules, small molecules, mimetic agents, synthetic drugs, inorganic molecules and organic molecules. The present invention encompasses the use of antibodies or fragments thereof recombinantly fused or chemically conjugated to heterologous protein or polypeptide (or fragment thereof, preferably to a polypeptide of at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids) to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. For example, an antibody may be used to target a heterologous polypeptide to a particular cell type, either *in vitro* or *in vivo*, by fusing or conjugating the antibody to another antibody specific for particular cell surface receptors. An antibody fused or conjugated to a heterologous polypeptide may also be used in *in vitro* immunoassays and purification methods using methods known in the art. See *e.g.*, International publication No. WO 93/21232; European Patent No. EP 439,095; Naramura *et al.*, 1994, *Immunol. Lett.* 39:91-99; U.S. Patent No. 5,474,981; Gillies *et al.*, 1992, *PNAS* 89:1428-1432; and Fell *et al.*, 1991, *J. Immunol.* 146:2446-2452, which are incorporated by reference in their entireties.

The present invention further includes compositions comprising a heterologous protein, peptide or polypeptide fused or conjugated to an antibody fragments. For example, a heterologous polypeptides may be fused or conjugated to a Fab fragment, Fd fragment, Fv fragment, F(ab)2 fragment, a VH domain, a VL domain, a VH CDR, a VL CDR, or fragment thereof. Methods for fusing or conjugating a polypeptide to an antibody fragment are known in the art. See, *e.g.*, U.S. Patent Nos. 5,336,603, 5,622,929, 5,359,046, 5,349,053, 5,447,851, and 5,112,946; European Patent No.s EP 307,434 and EP 367,166; International publication Nos. WO 96/04388 and WO 91/06570; Ashkenazi *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* 88: 10535-10539; Zheng *et al.*, 1995, *J. Immunol.* 154:5590-5600; and Vil *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* 89:11337- 11341 (all references are incorporated herein by reference in their entireties).

Additional fusion proteins may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as “DNA shuffling”). DNA shuffling may be employed to alter the activities of SYNAGIS® or fragments thereof (*e.g.*, an antibody or a fragment thereof with higher affinities and lower dissociation rates). See, generally, U.S. Patent Nos. 5,605,793;

5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten *et al.*, 1997, *Curr. Opinion Biotechnol.* 8:724-33 ; Harayama, 1998, *Trends Biotechnol.* 16(2):76-82; Hansson *et al.*, 1999, *J. Mol. Biol.* 287:265-76; and Lorenzo and Blasco, 1998, *Biotechniques* 24(2):308-313 (each of these patents and publications are hereby incorporated by reference in its entirety). An antibody or a fragment thereof that immunospecifically binds to a RSV antigen, or the nucleic acid encoding an antibody or a fragment thereof that immunospecifically binds to a RSV antigen, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. One or more portions of a polynucleotide encoding an antibody or a fragment thereof that immunospecifically binds to a RSV antigen or a fragment thereof, which portions immunospecifically bind a RSV antigen, may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Moreover, an antibody or a fragment thereof that immunospecifically binds to a RSV antigen or a fragment thereof can be fused to a marker sequence, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz *et al.*, 1989, *Proc. Natl. Acad. Sci. USA* 86:821-824, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, 1984, *Cell* 37:767) and the "flag" tag.

The present invention also encompasses the liquid formulations of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen or a variant thereof conjugated to a diagnostic or detectable agent or any other molecule for which serum half-life is desired to be increased. Such an antibody can be useful for monitoring or prognosing the development or progression of a disease, disorder or infection as part of a clinical testing procedure, such as determining the efficacy of a particular therapy. Such diagnosis and detection can be accomplished by coupling an antibody or a fragment thereof that immunospecifically binds to a RSV antigen or a fragment thereof to a detectable substance including, but not limited to, various enzymes, such as but not limited to, horseradish

peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such as but not limited to, streptavidinbiotin and avidin/biotin; fluorescent materials, such as but not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as but not limited to, luminol; bioluminescent materials, such as but not limited to, luciferase, luciferin, and aequorin; radioactive materials, such as but not limited to iodine ( $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^{3}\text{H}$ ), indium ( $^{115}\text{In}$ ,  $^{113}\text{In}$ ,  $^{112}\text{In}$ ,  $^{111}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ), thallium ( $^{201}\text{Ti}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ),  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{142}\text{Pr}$ ,  $^{105}\text{Rh}$ ,  $^{97}\text{Ru}$ ,  $^{68}\text{Ge}$ ,  $^{57}\text{Co}$ ,  $^{65}\text{Zn}$ ,  $^{85}\text{Sr}$ ,  $^{32}\text{P}$ ,  $^{153}\text{Gd}$ ,  $^{169}\text{Yb}$ ,  $^{51}\text{Cr}$ ,  $^{54}\text{Mn}$ ,  $^{75}\text{Se}$ ,  $^{113}\text{Sn}$ , and  $^{117}\text{Tin}$ ; positron emitting metals using various positron emission tomographies, noradioactive paramagnetic metal ions, and molecules that are radiolabelled or conjugated to specific radioisotopes. The detectable substance may be coupled or conjugated either directly to an antibody or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, e.g., U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as a diagnostics according to the present invention.

The present invention further encompasses uses of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen conjugated to a therapeutic moiety in the liquid formulations of the invention. An antibody or antigen-binding fragment may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytoidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxel, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogues or homologs thereof. Therapeutic moieties include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BCNU) and lomustine (CCNU)), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis dichlorodiamine platinum (II)

(DDP) cisplatin)); anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin); antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)); Auristatin molecules (*e.g.*, auristatin PHE, bryostatin 1, solastatin 10, see Woyke *et al.*, *Antimicrob. Agents Chemother.* 46:3802-8 (2002), Woyke *et al.*, *Antimicrob. Agents Chemother.* 45:3580-4 (2001), Mohammad *et al.*, *Anticancer Drugs* 12:735-40 (2001), Wall *et al.*, *Biochem. Biophys. Res. Commun.* 266:76-80 (1999), Mohammad *et al.*, *Int. J. Oncol.* 15:367-72 (1999), all of which are incorporated herein by reference); anti-mitotic agents (*e.g.*, vincristine and vinblastine); hormones (*e.g.*, glucocorticoids, progestatins, androgens, and estrogens); DNA repair enzyme inhibitors (*e.g.*, etoposide or topotecan); kinase inhibitors (*e.g.*, compound ST1571, imatinib mesylate (Kantarjian *et al.*, *Clin Cancer Res.* 8(7):2167-76 (2002)), and those compounds disclosed in U.S. Pat. Nos. 6,245,759, 6,399,633, 6,383,790, 6,335,156, 6,271,242, 6,242,196, 6,218,410, 6,218,372, 6,057,300, 6,034,053, 5,985,877, 5,958,769, 5,925,376, 5,922,844, 5,911,995, 5,872,223, 5,863,904, 5,840,745, 5,728,868, 5,648,239, and 5,587,459); farnesyl transferase inhibitors (*e.g.*, R115777, BMS 214662, and those disclosed by, for example, U.S. Patent Nos: 6,458,935, 6,451,812, 6,440,974, 6,436,960, 6,432,959, 6,420,387, 6,414,145, 6,410,541, 6,410,539, 6,403,581, 6,399,615, 6,387,905, 6,372,747, 6,369,034, 6,362,188, 6,342,765, 6,342,487, 6,300,501, 6,268,363, 6,265,422, 6,248,756, 6,239,140, 6,232,338, 6,228,865, 6,228,856, 6,225,322, 6,218,406, 6,211,193, 6,187,786, 6,169,096, 6,159,984, 6,143,766, 6,133,303, 6,127,366, 6,124,465, 6,124,295, 6,103,723, 6,093,737, 6,090,948, 6,080,870, 6,077,853, 6,071,935, 6,066,738, 6,063,930, 6,054,466, 6,051,582, 6,051,574, and 6,040,305); topoisomerase inhibitors (*e.g.*, camptothecin, irinotecan, SN 38, topotecan, 9 aminocamptothecin, GG 211 (GI 147211), DX 8951f; IST 622, rubitecan, pyrazoloacridine, XR 5000, saintopin, UCE6, UCE1022, TAN 1518A, TAN 1518B, KT6006, KT6528, ED 110, NB 506, ED 110, NB 506, rebeccamycin, and bulgarein); DNA minor groove binders such as Hoescht dye 33342 and Hoechst dye 33258; nitidine; fagaronine; epiberberine; coralyne; beta lapachone; BC 4 1; and pharmaceutically acceptable salts, solvates, clathrates, and prodrugs thereof (See, *e.g.*, Rothenberg, M.L., *Annals of Oncology* 8:837-855(1997); and Moreau *et al.*, *J. Med. Chem.* 41:1631-1640(1998)). Therapeutic moieties may also be antisense oligonucleotides (*e.g.*, those disclosed in the U.S. Pat. Nos. 6,277,832, 5,998,596, 5,885,834, 5,734,033, and 5,618,709); immunomodulators (*e.g.*, antibodies and cytokines); antibodies (*e.g.*, rituximab (Rituxan®),

calicheamycin (Mylotarg®), ibritumomab tiuxetan (Zevalin®), and tosimumomab (Bexxar®)); and adenosine deaminase inhibitors (*e.g.*, Fludarabine phosphate and 2 Chlorodeoxyadenosine).

Further, an antibody or a fragment thereof that immunospecifically binds to a RSV antigen or a fragment thereof may be conjugated to a therapeutic moiety or drug moiety that modifies a given biological response. Therapeutic moiety or drug moieties are not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, cholera toxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, *e.g.*, TNF- $\alpha$ , TNF- $\beta$ , AIM I (see, International publication No. WO 97/33899), AIM II (see, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, 1994, *J. Immunol.*, 6:1567-1574), and VEGF (see, International publication No. WO 99/23105); or, a biological response modifier such as, for example, a lymphokine (*e.g.*, interferon  $\alpha$ ,  $\beta$ , or  $\gamma$ , interleukin-1 (“IL-1”), interleukin-2 (“IL-2”), interleukin-4 (“IL-4”), interleukin-6 (“IL-6”), interleukin-9 (“IL-9”), interleukin-12 (“IL-12”), granulocyte macrophage colony stimulating factor (“GM-CSF”), and granulocyte colony stimulating factor (“G-CSF”)), a growth factor (*e.g.*, growth hormone (“GH”)).

Moreover, an antibody can be conjugated to therapeutic moieties such as a radioactive metal ion, such as alpha-emitters such as  $^{213}\text{Bi}$  or macrocyclic chelators useful for conjugating radiometal ions, including but not limited to,  $^{131}\text{In}$ ,  $^{131}\text{Lu}$ ,  $^{131}\text{Y}$ ,  $^{131}\text{Ho}$ ,  $^{131}\text{Sm}$ , to polypeptides. In certain embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N’,N”,N”’-tetraacetic acid (DOTA) which can be attached to the antibody via a linker molecule. Such linker molecules are commonly known in the art and described in Denardo *et al.*, 1998, *Clin Cancer Res.* 4(10):2483-90; Peterson *et al.*, 1999, *Bioconjug. Chem.* 10(4):553-7; and Zimmerman *et al.*, 1999, *Nucl. Med. Biol.* 26(8):943-50, each incorporated by reference in their entireties.

Techniques for conjugating therapeutic moieties to antibodies are well known, see, *e.g.*, Arnon *et al.*, “Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy”, in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, “Antibodies For Drug Delivery”, in *Controlled*

Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies 84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, 1982, Immunol. Rev. 62:119-58.

Alternatively, an antibody or a fragment thereof that immunospecifically binds to a RSV antigen or a fragment thereof can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody or a fragment thereof that immunospecifically binds to a RSV antigen or a fragment thereof may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

The therapeutic moiety or drug conjugated to an antibody or a fragment thereof that immunospecifically binds to a RSV antigen or a fragment thereof should be chosen to achieve the desired prophylactic or therapeutic effect(s) for a particular disorder in a subject. A clinician or other medical personnel should consider the following when deciding on which therapeutic moiety or drug to conjugate to an antibody or a fragment thereof that immunospecifically binds to a RSV antigen or a fragment thereof: the nature of the infection, the severity of the infection, and the condition of the subject.

An antibody or a fragment thereof that immunospecifically binds to a RSV antigen or a fragment thereof, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

In a specific embodiment, antibodies of the invention are bispecific T cell engagers (BiTE). Bispecific T cell engagers (BiTE) are bispecific antibodies that can redirect T cells for antigen-specific elimination of targets. A BiTE molecule has an antigen-binding domain that binds to a T cell antigen (e.g., CD3) at one end of the molecule and an antigen binding domain that will bind to an antigen on the target cell. A BiTE molecule was

recently described in International Publication No. WO 99/54440, which is herein incorporated by reference. This publication describes a novel single-chain multifunctional polypeptide that comprises binding sites for the CD19 and CD3 antigens (CD19xCD3). This molecule was derived from two antibodies, one that binds to CD19 on the B cell and an antibody that binds to CD3 on the T cells. The variable regions of these different antibodies are linked by a polypeptide sequence, thus creating a single molecule. Also described, is the linking of the variable heavy chain (VH) and light chain (VL) of a specific binding domain with a flexible linker to create a single chain, bispecific antibody.

In one embodiment, an antibody or a fragment thereof that immunospecifically binds to a RSV antigen comprises a portion of the BiTE molecule. For example, the VH and/or VL (preferably a scFV) of an antibody that binds a RSV antigen can be fused to an anti-CD3 binding portion such as that of the molecule described above, thus creating a BiTE molecule that targets a RSV antigen. In addition to the variable heavy and/or light chain of antibody against a RSV antigen, other molecules that bind a RSV antigen can comprise the BiTE molecule. In another embodiment, the BiTE molecule comprises a molecule that binds to other T cell antigens (other than CD3). For example, antibodies or fragments thereof that immunospecifically bind to T-cell antigens like CD2, CD4, CD8, CD11a, TCR, and CD28 are contemplated to be part of this invention. This list is not meant to be exhaustive but only to illustrate that other molecules that can immunospecifically bind to a T cell antigen can be used as part of a BiTE molecule. These molecules can include the VH and/or VL portion of the antibody or fragment thereof.

The “binding domain” as used in accordance with the present invention denotes a domain comprising a three-dimensional structure capable of specifically binding to an epitope like native antibodies, free scFv fragments or one of their corresponding immunoglobulin chains, preferably the VH chain. Thus, said domain can comprise the VH and/or VL domain of an antibody or an immunoglobulin chain, preferably at least the VH domain or more preferably the VH and VL domain linked by a flexible polypeptide linker (scFv). On the other hand, said binding domain contained in the polypeptide of the invention may comprise at least one complementarity determining region (CDR) of an antibody or immunoglobulin chain recognizing an antigen on the T cell or a cellular antigen. In this respect, it is noted that the binding domain present in the polypeptide of the invention may not only be derived from antibodies but also from other T cell or cellular

antigen binding protein, such as naturally occurring surface receptors or ligands. It is further contemplated that in an embodiment of the invention, said first and or second domain of the above-described polypeptide mimic or correspond to a VH and VL region from a natural antibody. The antibody providing the binding site for the polypeptide of the invention can be, *e.g.*, a monoclonal antibody, polyclonal antibody, chimeric antibody, humanized antibody, bispecific antibody, synthetic antibody, antibody fragment, such as Fab, Fv or scFv fragments etc., or a chemically modified derivative of any of these.

#### **5.1.4 Method of Preparing the Antibody Formulations**

The present invention provides methods for preparing liquid formulations of antibodies, in particular, those listed in Table 2, or derivatives, analogues, or fragments thereof that immunospecifically bind to a RSV antigen. Figure 1 is a schematic diagram showing the outline for preparing purified anti-RSV antibodies. The methods for preparing liquid formulations of the present invention comprise: concentrating a fraction containing the purified antibody or a fragment to a final antibody or fragment concentration of from about 15 mg/ml, about 20 mg/ml, about 30 mg/ml, about 40 mg/ml, about 50 mg/ml, about 60 mg/ml, about 70 mg/ml, about 80 mg/ml, about 90 mg/ml, about 100 mg/ml, about 110 mg/ml, about 125 mg/ml, about 150 mg/ml, about 200 mg/ml, about 250 mg/ml, or about 300 mg/ml using a semipermeable membrane with an appropriate molecular weight (MW) cutoff (*e.g.*, 30 kD cutoff for whole antibody molecules and F(ab')<sub>2</sub> fragments; and 10 kD cutoff for antibody fragments, such as Fab fragments) and difiltrating the concentrated antibody fraction into the formulation buffer using the same membrane. Conditioned medium containing antibody or a fragment thereof that immunospecifically binds to a RSV antigen is subjected to CUNO filtration and the filtered antibody is subjected to HS50 cation exchange chromatography. The fraction from the HS50 cation exchange chromatography is then subjected to rProtein A affinity chromatography followed by low pH treatment. Following low pH treatment, the antibody fraction is subject to super Q 650 anion exchange chromatography and then nanofiltration. The fraction of the antibody obtained after nanofiltration is then subjected to diafiltration to concentrate the antibody fraction into the formulation buffer using the same membrane.

The formulation buffer of the present invention comprises histidine at a concentration ranging from about 1 mM to about 100 mM, about 10 mM to about 50 mM, about 20 mM to about 30 mM, or about 23 mM to about 27 mM. Preferably, the

formulation buffer of the present invention comprises histidine at a concentration of about 25 mM. The formulations may further comprise glycine at a concentration of less than 100 mM, less than 50 mM, less than 3.0 mM, less than 2.0 mM, or less than 1.8 mM.

Preferably, the formulations comprise glycine at a concentration of 1.6 mM. The amount of glycine in the formulation should not cause a significant buffering in order to avoid antibody precipitation at its isoelectric point. The pH of the formulation may range from about 5.0 to about 7.0, preferably about 5.5 to about 6.5, more preferably about 5.8 to about 6.2, and most preferably about 6.0. To obtain an appropriate pH for a particular antibody, it is preferable that histidine (and glycine, if added) is first dissolved in water to obtain a buffer solution with higher pH than the desired pH and then the pH is brought down to the desired level by adding HCl. This way, the formation of inorganic salts (*e.g.*, formation of NaCl when, for example, histidine hydrochloride is used as histidine and pH is raised to a desired level by adding NaOH) can be avoided.

The liquid formulations of the present invention can be prepared as unit dosage forms by preparing a vial containing an aliquot of the liquid formulation for a one-time use. For example, a unit dosage per vial may contain 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml, 10 ml, 15 ml, or 20 ml of different concentrations of an antibody or a fragment thereof that immunospecifically binds to RSV ranging from about 15 mg/ml to about 300 mg/ml. If necessary, these preparations can be adjusted to a desired concentration by adding a sterile diluent to each vial.

The liquid formulations of the present invention may be sterilized by various sterilization methods, including sterile filtration, radiation, etc. In a most preferred embodiment, the difiltrated antibody formulation is filter-sterilized with a presterilized 0.2 or 0.22-micron filter. Sterilized liquid formulations of the present invention may be administered to a subject to prevent, treat, manage or ameliorate a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by, potentiating a RSV infection.

The liquid formulations of the invention can also be used for diagnostic purposes to detect, diagnose, or monitor a RSV infection. In particular, the liquid formulations of the invention comprising antibodies or fragments thereof that immunospecifically bind to a RSV antigen conjugated or fused to a detectable agent or label can be used to detect, diagnose, or monitor a RSV infection.

Although the invention is directed to liquid non-lyophilized formulations, it should be noted for the purpose of equivalents that the formulations of the invention may be lyophilized if desired. Thus, the invention encompasses lyophilized forms of the formulations of the invention although such lyophilized formulations are not necessary and, thus, not preferred.

## 5.2 Methods of Preparing Antibodies

The antibodies that immunospecifically bind to a RSV antigen can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Polyclonal antibodies immunospecific for a RSV antigen can be produced by various procedures well-known in the art. For example, a RSV antigen can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the human antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *corynebacterium parvum*. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. Briefly, mice can be immunized with a non-murine antigen and once an immune response is detected, *e.g.*, antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

The present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with a non-murine antigen with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind to the antigen.

Antibody fragments which recognize specific particular epitopes may be generated by any technique known to those of skill in the art. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain. Further, the antibodies of the present invention can also be generated using various phage display methods known in the art.

In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified from animal cDNA libraries (*e.g.*, human or murine cDNA libraries of affected tissues). The DNA encoding the VH and VL domains are recombined together with an scFv linker by PCR and cloned into a phagemid vector. The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 and the VH and VL domains are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to a particular

antigen can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., 1995, *J. Immunol. Methods* 182:41-50; Ames *et al.*, 1995, *J. Immunol. Methods* 184:177-186; Kettleborough *et al.*, 1994, *Eur. J. Immunol.* 24:952-958; Persic *et al.*, 1997, *Gene* 187:9-18; Burton *et al.*, 1994, *Advances in Immunology* 57:191-280; International application No. PCT/GB91/O1 134; International publication Nos. WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/11236, WO 95/15982, WO 95/20401, and WO97/13844; and U.S. Patent Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.*, as described below. Techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication No. WO 92/22324; Mullinax *et al.*, 1992, *BioTechniques* 12(6):864-869; Sawai *et al.*, 1995, *AJRI* 34:26-34; and Better *et al.*, 1988, *Science* 240:1041-1043 (said references incorporated by reference in their entireties).

To generate whole antibodies, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the VH or VL sequences in scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains can be cloned into vectors expressing a VH constant region, *e.g.*, the human gamma 4 constant region, and the PCR amplified VL domains can be cloned into vectors expressing a VL constant region, *e.g.*, human kappa or lambda constant regions. Preferably, the vectors for expressing the VH or VL domains comprise an EF-1 $\alpha$  promoter, a secretion signal, a cloning site for the variable domain, constant domains, and a selection marker such as neomycin. The VH and VL domains may also be cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected

into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art.

For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use humanized antibodies or chimeric antibodies.

Completely human antibodies and humanized antibodies are particularly desirable for therapeutic treatment of human subjects. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also U.S. Patent Nos. 4,444,887 and 4,716,111; and International publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human

antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see, e.g.*, International publication Nos. WO 98/24893, WO 96/34096, and WO 96/33735; and U.S. Patent Nos. 5,413,923, 5,625,126, 5,633,425, 5,569,825, 5,661,016, 5,545,806, 5,814,318, and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules. Methods for producing chimeric antibodies are known in the art. See *e.g.*, Morrison, 1985, *Science* 229:1202; Oi *et al.*, 1986, *BioTechniques* 4:214; Gillies *et al.*, 1989, *J. Immunol. Methods* 125:191-202; and U.S. Patent Nos. 5,807,715, 4,816,567, 4,816,567, and 6,311,415, which are incorporated herein by reference in their entirety.

A humanized antibody is an antibody or its variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a framework region having substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immunoglobulin. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab')<sub>2</sub>, Fabc, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (*i.e.*, donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG<sub>1</sub>. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG<sub>2</sub> class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired

effector functions is within the ordinary skill in the art. The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, *e.g.*, the donor CDR or the consensus framework may be mutagenized by substitution, insertion or deletion of at least one residue so that the CDR or framework residue at that site does not correspond to either the consensus or the import antibody. Such mutations, however, will not be extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental framework and CDR sequences, more often 90%, and most preferably greater than 95%. A humanized antibody can be produced using variety of techniques known in the art, including but not limited to, CDR-grafting (*see e.g.*, European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089, each of which is incorporated herein in its entirety by reference), veneering or resurfacing (*see e.g.*, European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology 28(4/5):489-498; Studnicka *et al.*, 1994, Protein Engineering 7(6):805-814; and Roguska *et al.*, 1994, PNAS 91:969-973, each of which is incorporated herein by its entirety by reference), chain shuffling (*see e.g.*, U.S. Patent No. 5,565,332, which is incorporated herein in its entirety by reference), and techniques disclosed in, *e.g.*, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, International Publication No. WO 9317105, Tan *et al.*, J. Immunol. 169:1119-25 (2002), Caldas *et al.*, Protein Eng. 13(5):353-60 (2000), Morea *et al.*, Methods 20(3):267-79 (2000), Baca *et al.*, J. Biol. Chem. 272(16):10678-84 (1997), Roguska *et al.*, Protein Eng. 9(10):895-904 (1996), Couto *et al.*, Cancer Res. 55 (23 Supp):5973s - 5977s (1995), Couto *et al.*, Cancer Res. 55(8):1717-22 (1995), Sandhu JS, Gene 150(2):409-10 (1994), and Pedersen *et al.*, J. Mol. Biol. 235(3):959-73 (1994), each of which is incorporated herein in its entirety by reference. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, *e.g.*, Queen *et al.*, U.S. Patent No. 5,585,089; and Riechmann *et al.*, 1988, Nature 332:323, which are incorporated herein by reference in their entireties.)

Further, the antibodies that immunospecifically bind to a RSV antigen can, in turn, be utilized to generate anti-idiotype antibodies that “mimic” an antigen using techniques well known to those skilled in the art. (See, *e.g.*, Greenspan & Bona, 1989, FASEB J. 7(5):437-444; and Nissinoff, 1991, J. Immunol. 147(8):2429-2438).

### 5.2.1 Polynucleotide Sequences Encoding an Antibody

The invention provides polynucleotides comprising a nucleotide sequence encoding an antibody or fragment thereof that immunospecifically binds to an antigen. The invention also encompasses polynucleotides that hybridize under high stringency, intermediate or lower stringency hybridization conditions, *e.g.*, as defined *supra*, to polynucleotides that encode an antibody of the invention.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. The nucleotide sequence of antibodies immunospecific for a desired antigen can be obtained, *e.g.*, from the literature or a database such as GenBank. Nucleotide or alternatively, if the amino acid sequence of an antibody or fragment thereof that immunospecifically binds to a RSV antigen is known, the nucleotide sequences encoding the antibody or a fragment thereof (*e.g.*, a CDR) can be determined using methods well known in the art, *i.e.*, nucleotide codons known to encode particular amino acids are assembled in such a way to generate a nucleic acid that encodes the antibody. Such a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier et al., 1994, BioTechniques 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or

by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.*, eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, one or more of the CDRs is inserted within framework regions using routine recombinant DNA techniques. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, *e.g.*, Chothia et al., 1998, *J. Mol. Biol.* 278: 457-479 for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds to a particular antigen. Preferably, as discussed *supra*, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

### 5.2.2 Recombinant Expression of an Antibody

Recombinant expression of an antibody of the invention, derivative, analogue or fragment thereof, (*e.g.*, a heavy or light chain of an antibody of the invention or a portion thereof or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof

(preferably, but not necessarily, containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well-known in the art. See, e.g., U.S. Patent No. 6,331,415, which is incorporated herein by reference in its entirety. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, a heavy or light chain of an antibody, a heavy or light chain variable domain of an antibody or a portion thereof, or a heavy or light chain CDR, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., International Publication No. WO 86/05807 and WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy, the entire light chain, or both the entire heavy and light chains.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention or fragments thereof, or a heavy or light chain thereof, or portion thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention (see, e.g., U.S. Patent No. 5,807,715). Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention *in situ*. These include but are not limited to microorganisms such as bacteria

(e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, NS0, and 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., 1986, Gene 45:101; and Cockett et al., 1990, Bio/Technology 8:2). In a specific embodiment, the expression of nucleotide sequences encoding antibodies which immunospecifically bind to a RSV antigen is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such an antibody is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO 12:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione 5-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by

adsorption and binding to matrix glutathione agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (*e.g.*, see Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:355-359). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, *e.g.*, Bittner et al., 1987, Methods in Enzymol. 153:51-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed.

To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, W138, BT483, Hs578T, HTB2, BT2O and T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7O3O and HsS78Bst cells.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler et al., 1977, Cell 11:223), hypoxanthineguanine phosphoribosyltransferase (Szybalska & Szybalski, 1992, Proc. Natl. Acad. Sci. USA 89:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, Cell 22:8-17) genes can be employed in tk-, hprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler et al., 1980, Natl. Acad. Sci. USA 77:357; O'Hare et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); *neo*, which confers resistance to the aminoglycoside G-418 (Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62: 191-217; May, 1993, TIB TECH 11(5):155-2 15); and *hygro*, which confers resistance to hygromycin

(Santerre et al., 1984, Gene 30:147). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli *et al.* (eds.), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., 1981, J. Mol. Biol. 150:1, which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., 1983, Mol. Cell. Biol. 3:257).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, Nature 322:52; and Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2 197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by recombinant expression, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the antibodies of the present invention or fragments

thereof may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

### 5.3 Methods of Monitoring the Stability And Aggregation of Antibody Formulations

There are various methods available for assessing the stability of the liquid formulations of the present invention, based on the physical and chemical structures of the proteins (e.g., antibodies or fragments thereof) as well as on their biological activities. For example, to study denaturation of proteins, methods such as charge-transfer absorption, thermal analysis, fluorescence spectroscopy, circular dichroism, NMR, and HPSEC, are available. *See, for example, Wang et al., 1988, J. of Parenteral Science & Technology 42(Suppl):S4-S26.*

The rCGE and HPSEC are the most common and simplest methods to assess the formation of protein aggregates, protein degradation, and protein fragmentation. Accordingly, the stability of the liquid formulations of the present invention may be assessed by these methods.

For example, the stability of the liquid formulations of the present invention may be evaluated by HPSEC or rCGE, wherein the percent area of the peaks represents the non-degraded antibody or non-degraded antibody fragments. In particular, approximately 250  $\mu$ g of the antibody or antibody fragment that immunospecifically binds to a RSV antigen (approximately 25  $\mu$ l of a liquid formulation comprising 10 mg/ml said antibody or antibody fragment) is injected onto a TosoH Biosep TSK G3000SW<sub>XL</sub> column (7.8 mm x 30 cm) fitted with a TSK SW x1 guard column (6.0 mm CX 4.0 cm). The antibody or antibody fragment is eluted isocratically with 0.1 M disodium phosphate containing 0.1 M sodium sulfate and 0.05% sodium azide, at a flow rate of 0.8 to 1.0 ml/min. Eluted protein is detected using UV absorbance at 280 nm. SYNAGIS<sup>®</sup> reference standard is run in the assay as a control, and the results are reported as the area percent of the product monomer peak compared to all other peaks excluding the included volume peak observed at approximately 12 to 14 minutes. Peaks eluting earlier than the monomer peak are recorded as percent aggregate.

The liquid formulations of the present invention exhibit low to undetectable levels of aggregation as measured by HPSEC or rCGE, that is, no more than 5%, no more than 4%, no more than 3%, no more than 2%, no more than 1%, and most preferably no more

than 0.5% aggregate by weight protein, and low to undetectable levels of fragmentation, that is, 80% or higher, 85% or higher, 90% or higher, 95% or higher, 98% or higher, or 99% or higher, or 99.5% or higher of the total peak area in the peak(s) representing intact antibodies or fragments thereof. In the case of SDS-PAGE, the density or the radioactivity of each band stained or labeled with radioisotope can be measured and the % density or % radioactivity of the band representing non-degraded antibodies or fragments thereof can be obtained.

The stability of the liquid formulations of the present invention can be also assessed by any assays which measures the biological activity of the antibody or fragments thereof in the formulation. The biological activities of antibodies include, but are not limited to, antigen-binding activity, complement-activation activity, Fc-receptor binding activity, and so forth. Antigen-binding activity of the antibodies can be measured by any method known to those skilled in the art, including but not limited to ELISA, radioimmunoassay, Western blot, and the like. Complement-activation activity can be measured by a C3a/C4a assay in the system where the antibody which immunospecifically binds to a RSV antigen is reacted in the presence of the complement components with the cells expressing the RSV antigen. *Also see Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).* An ELISA based assay, *e.g.*, may be used to compare the ability of an antibody or fragment thereof to immunospecifically bind to a RSV antigen to a SYNAGIS® reference standard. In this assay, plates are coated with a RSV antigen and the binding signal of a set concentration of a SYNAGIS® reference standard is compared to the binding signal of the same concentration of a test antibody or antibody fragment.

The purity of the liquid antibody formulations of the invention may be measured by any method well-known to one of skill in the art such as, *e.g.*, HPSEC. The sterility of the liquid antibody formulations may be assessed as follows: sterile soybean-casein digest medium and fluid thioglycollate medium are inoculated with a test liquid antibody formulation by filtering the liquid antibody formulation through a sterile filter having a nominal porosity of 0.45  $\mu$ m. When using the Sterisure™ or Steritest™ method, each filter device is aseptically filled with approximately 100 ml of sterile soybean-casein digest medium or fluid thioglycollate medium. When using the conventional method, the challenged filter is aseptically transferred to 100 ml of sterile soybean-casein digest medium

or fluid thioglycollate medium. The media are incubated at appropriate temperatures and observed three times over a 14 day period for evidence of bacterial or fungal growth.

#### **5.4 Prophylactic And Therapeutic Utility Of The Antibody Formulations**

The present invention is also directed to antibody-based therapies which involve administering to a subject, preferably a human, the liquid antibody formulations of the present invention for preventing, treating, managing or ameliorating a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection. The liquid formulations of the invention comprise an antibody or a fragment thereof at concentrations of from about 15 mg/ml to about 300 mg/ml in a solution containing histidine, which antibody or a fragment thereof immunospecifically binds to a RSV antigen. The liquid formulations of the invention may comprise a single antibody or fragment thereof that immunospecifically binds to a RSV antigen, with the proviso that said antibody or antibody fragment is not SYNAGIS® or a fragment thereof. The liquid formulations of the invention may also comprise two or more antibodies or fragments thereof that immunospecifically bind to a RSV antigen. In a specific embodiment, one of the antibodies or antibody fragments included in such liquid formulations is SYNAGIS® or a fragment thereof. In an alternative embodiment, one of the antibodies or antibody fragments included in such liquid formulations is not SYNAGIS® or a fragment thereof.

The liquid formulations of the invention may comprise antibodies or fragments thereof that immunospecifically bind to a RSV antigen and exhibiting improved *in vivo* half-lives compared to known antibodies that immunospecifically bind to a RSV antigen (e.g., unmodified SYNAGIS®).

Antibodies or fragments thereof in the liquid formulations of the present invention may function as antagonists of a RSV infection and can be administered to a subject, preferably a human, to treat, prevent, manage or ameliorate a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection. For example, antibodies or fragments thereof which disrupt or prevent the interaction between a RSV antigen and its host cell receptor may be administered to a subject, preferably a human, in the liquid formulations of the present invention to treat,

prevent, manage or ameliorate a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by, or potentiating a RSV infection.

In a specific embodiment, a liquid formulation of the present invention comprises an antibody or a fragment thereof that prevents RSV from binding to its host cell receptor by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to RSV binding to its host cell receptor in the absence of said antibodies or antibody fragments or the presence of an negative control (*e.g.*, an unrelated IgG antibody or phosphate buffered saline). In another embodiment, a liquid formulation of the present invention comprises a combination of antibodies, a combination of antibody fragments, or a combination of antibodies and antibody fragments that prevents RSV from binding to its host cell receptor by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to RSV binding to its host cell receptor in the absence of said antibodies and/or antibody fragments or the presence of an negative control (*e.g.*, an unrelated IgG antibody or phosphate buffered saline). In a preferred embodiment, one of antibodies in the combination of antibodies and/or antibody fragments in the liquid formulations of the present invention, is one of the antibodies listed in Table 1, not including SYNAGIS® or a fragment thereof, which immunospecifically binds to a RSV. In another embodiment, one of the antibodies in the combination of antibodies and/or antibody fragments in the liquid formulations of the present invention is SYNAGIS® or a fragment thereof.

Antibodies or fragments thereof which do not prevent RSV from binding its host cell receptor but inhibit or downregulate RSV replication can also be administered in the liquid formulations of the invention to a subject to treat, prevent, manage or ameliorate a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection. The ability of an antibody or a fragment thereof to inhibit or downregulate RSV replication may be determined by techniques described herein or otherwise known in the art. For example, the inhibition or downregulation of RSV replication can be determined by detecting the RSV titer in the lungs of a subject, preferably a human.

In a specific embodiment, a liquid formulation of the present invention comprises an antibody or a fragment thereof that inhibits or downregulates RSV replication by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to RSV replication in absence of said antibodies or antibody fragments or the presence of an negative control (e.g., an unrelated IgG antibody or phosphate buffered saline). In another embodiment, a liquid formulation of the present invention comprises a combination of antibodies, a combination of antibody fragments, or a combination of antibodies and antibody fragments that inhibit or downregulate RSV replication by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to RSV replication in absence of said antibodies and/or antibody fragments or the presence of an negative control (e.g., an unrelated IgG antibody or phosphate buffered saline). In another embodiment, the liquid formulations of the invention are administered to a subject in an institution or group home (e.g., a nursing home or orphanage).

The liquid formulations of the present invention may be used locally or systemically in the body of a subject in need thereof as prophylactic or therapeutic agent. The formulations of the present invention may also be advantageously utilized in combination with other agents (see Section 5.5) locally or systemically in the body of a subject in need thereof to prevent or treat a RSV infection or a respiratory condition that is associated with, potentiated by or potentiates a RSV infection.

Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human or humanized antibodies, fragments derivatives, or analogues, are administered to a human patient for therapy or prophylaxis.

In a specific embodiment, a liquid formulation of the invention is administered in combination with a IL-9 antagonist to a subject in need thereof to prevent, treat, manage or ameliorating wheezing associated with a RSV infection. In certain cases, wheezing precedes the onset or development of a RSV infection. In a specific embodiment, the invention provides methods of preventing, treating, ameliorating, or managing wheezing associated with a RSV infection, said methods comprising administering to a subject in

need thereof an effective amount of one or more IL-9 antagonists in combination with a liquid formulation of the invention. In other embodiments, the invention provides methods of preventing the onset and/or development of asthma (which may be associated with, potentiated by or potentiates a RSV infection) in subjects with wheezing, said method comprising administering to said subject an effective amount of one or more IL-9 antagonists in combination with an effective amount of a liquid formulation of the invention.

In certain embodiments, a liquid formulation of the invention and one or more other therapies (e.g., one or more other prophylactic or therapeutic agents) useful for prevention, treatment, management or amelioration of a RSV infection are administered in a cycle of less than about 3 weeks, about once every two weeks, about once every 10 days or about once every week. One cycle can comprise the administration of a therapy (e.g., a therapeutic or prophylactic agent) by infusion over about 90 minutes every cycle, about 1 hour every cycle, about 45 minutes every cycle. Each cycle can comprise at least 1 week of rest, at least 2 weeks of rest, at least 3 weeks of rest. The number of cycles administered is from about 1 to about 12 cycles, more typically from about 2 to about 10 cycles, and more typically from about 2 to about 8 cycles.

It is preferred to use high affinity and/or potent *in vivo* inhibiting antibodies and/or neutralizing antibodies that immunospecifically bind to a RSV antigen (for prevention, treatment, management or amelioration of a RSV infection or a symptom thereof). It is also preferred to use antibodies that have improved *in vivo* half-lives compared to known antibodies that immunospecifically binds to a RSV antigen, for example, SYNAGIS®. Such antibodies or fragments thereof will preferably have an affinity for the RSV F glycoprotein and/or fragments of the F glycoprotein.

In one embodiment, the liquid formulations of the present invention are administered to a subject, preferably a human, to treat, prevent or ameliorate one or more symptoms associated with RSV infection. In another embodiment, the liquid formulations of the invention are administered to a human with cystic fibrosis, bronchopulmonary dysplasia, congenital heart disease, congenital immunodeficiency or acquired immunodeficiency, or to a human who has had a bone marrow transplant to treat, prevent or ameliorate one or more symptoms associated with RSV infection. In another embodiment, the liquid formulations of the invention are administered to a human infant, preferably a

human infant born prematurely or a human infant at risk of hospitalization for RSV infection to treat, prevent or ameliorate one or more symptoms associated with RSV infection. In another embodiment, the liquid formulations of the invention are administered to an elderly person to prevent, treat, or ameliorate one or more symptoms associated with RSV infection. In yet another embodiment, the liquid formulations of the invention are administered to a subject in an institution or group home (e.g., a hospital, nursing home, or orphanage).

### **5.5 Agent Useful in Combination with the Antibody Formulations**

The present invention provides methods for preventing, managing, treating, or ameliorating a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection comprising administering to a subject in need thereof one or more antibody liquid formulations of the invention alone or in combination with one or more therapies (e.g., one or more prophylactic or therapeutic agents) other than the antibody liquid formulations of the invention. The present invention provides methods for preventing, managing, treating, or ameliorating a RSV infection, one or more symptoms thereof, or a respiratory condition (e.g., airway hyperresponsiveness, asthma, etc.) that is associated with, potentiated by or potentiating a RSV infection comprising administering to a subject in need thereof one or more liquid formulations of the invention alone or in combination with one or more therapies (e.g., one or more prophylactic or therapeutic agents) other than an antibody liquid formulation of the invention. The present invention also provides compositions comprising a liquid formulation of an antibody or a fragment thereof that immunospecifically bind to a RSV antigen and one or more prophylactic or therapeutic agents other than a liquid antibody formulation of the invention and methods of preventing, managing, treating, or ameliorating a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection utilizing said compositions. Therapeutic or prophylactic agents include, but are not limited to, small molecules, synthetic drugs, peptides, polypeptides, proteins, nucleic acids (e.g., DNA and RNA nucleotides including, but not limited to, antisense nucleotide sequences, triple helices, RNA interference (RNAi), and nucleotide sequences encoding biologically active proteins, polypeptides or peptides)

antibodies, synthetic or natural inorganic molecules, mimetic agents, and synthetic or natural organic molecules.

Any therapy which is known to be useful, or which has been used or is currently being used for the prevention, management, treatment, or amelioration of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection can be used in combination with an antibody liquid formulation in accordance with the invention described herein. See, *e.g.*, Gilman *et al.*, *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, 10th ed., McGraw-Hill, New York, 2001; *The Merck Manual of Diagnosis and Therapy*, Berkow, M.D. *et al.* (eds.), 17th Ed., Merck Sharp & Dohme Research Laboratories, Rahway, NJ, 1999; *Cecil Textbook of Medicine*, 20th Ed., Bennett and Plum (eds.), W.B. Saunders, Philadelphia, 1996, for information regarding therapies (*e.g.*, prophylactic or therapeutic agents) which have been or are currently being used for preventing, treating, managing, or ameliorating a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection or one or more symptoms thereof. Examples of such agents include, but are not limited to, immunomodulatory agents, anti-inflammatory agents (*e.g.*, adrenocorticoids, corticosteroids (*e.g.*, beclomethasone, budesonide, flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, prednisone, hydrocortisone), glucocorticoids, steroids, non-steroidal anti-inflammatory drugs (*e.g.*, aspirin, ibuprofen, diclofenac, and COX-2 inhibitors), pain relievers, leukotriene antagonists (*e.g.*, montelukast, methyl xanthines, zafirlukast, and zileuton), beta2-agonists (*e.g.*, albuterol, biterol, fenoterol, isoetharic, metaproterenol, pirbuterol, salbutamol, terbutalin formoterol, salmeterol, and salbutamol terbutaline), anticholinergic agents (*e.g.*, ipratropium bromide and oxitropium bromide), sulphosalazine, penicillamine, dapsone, antihistamines, anti-malarial agents (*e.g.*, hydroxychloroquine)), anti-viral agents, and antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, erythromycin, penicillin, mithramycin, and anthramycin (AMC)).

In specific embodiments, a liquid formulation of the invention is used in combination with a monoclonal or chimeric antibody, or with a lymphokine or hematopoietic growth factor (such as, *e.g.*, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL12, and interferon  $\alpha$ ,  $\beta$ , and  $\gamma$ ), which, for example, serves to increase the number or activity of effector cells which interact with the antibody. A liquid formulation of the present invention may also be advantageously utilized in combination with other monoclonal or

chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, *e.g.*, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL12, and interferon  $\alpha$ ,  $\beta$ , and  $\gamma$ ), which, for example, serve to increase the immune response. The liquid formulations of the present invention may also be advantageously utilized in combination with one or more drugs used to treat RSV infection such as, for example anti-viral agents. Further, the liquid formulations of the present invention may be used in combination with one or more of the following drugs: NIH-351 (Gemini Technologies), recombinant RSV vaccine (MedImmune Vaccines, Inc. U.S. Application Nos. 60/358,934 filed February 21, 2002, 10/373,567 filed February 21, 2003, 10/371,099 filed February 21, 2003, 10/371,122 filed February 21, 2003, 10/371,264 filed February 21, 2003, 60/466,181 filed April 25, 2003 and 60/465,811 filed April 25, 2003, all of which are incorporated herein by reference), RSVf-2 (Intracel), F-50042 (Pierre Fabre), T-786 (Trimeris), VP-36676 (ViroPharma), RFI-641 (American Home Products), VP-14637 (ViroPharma), PFP-1 and PFP-2 (American Home Products), RSV vaccine (Avant Immunotherapeutics), and F-50077 (Pierre Fabre).

### 5.5.1 Immunomodulatory Agents

Any immunomodulatory agent well-known to one of skilled in the art may be used in the methods and compositions of the invention. Immunomodulatory agents can affect one or more or all aspects of the immune response in a subject. Aspects of the immune response include, but are not limited to, the inflammatory response, the complement cascade, leukocyte and lymphocyte differentiation, proliferation, and/or effector function, monocyte and/or basophil counts, and the cellular communication among cells of the immune system. In certain embodiments of the invention, an immunomodulatory agent modulates one aspect of the immune response. In other embodiments, an immunomodulatory agent modulates more than one aspect of the immune response. In a preferred embodiment of the invention, the administration of an immunomodulatory agent to a subject inhibits or reduces one or more aspects of the subject's immune response capabilities. In an alternative embodiment of the invention, the immunomodulatory agent enhances one or more aspects of a subject's immune response. In certain embodiments, an immunomodulatory agent is not an anti-inflammatory agent. In other embodiments, an immunomodulatory agent is an agent other than a chemotherapeutic agent.

Examples of immunomodulatory agents include, but are not limited to, proteinaceous agents such as cytokines, peptide mimetics, and antibodies (*e.g.*, human,

humanized, chimeric, monoclonal, polyclonal, Fvs, ScFvs, Fab or F(ab)<sub>2</sub> fragments or epitope binding fragments), nucleic acid molecules (*e.g.*, antisense nucleic acid molecules and triple helices), small molecules, organic compounds, and inorganic compounds. In particular, immunomodulatory agents include, but are not limited to, methotrexate, leflunomide, cyclophosphamide, cytoxin, Immuran, cyclosporine A, minocycline, azathioprine, antibiotics (*e.g.*, FK506 (tacrolimus)), methylprednisolone (MP), corticosteroids, steroids, mycophenolate mofetil, rapamycin (sirolimus), mizoribine, deoxyspergualin, brequinar, malononitroloamides (*e.g.*, leflunamide), T cell receptor modulators, cytokine receptor modulators, and modulators mast cell modulators.

Examples of T cell receptor modulators include, but are not limited to, anti-T cell receptor antibodies (*e.g.*, anti-CD4 antibodies (*e.g.*, cM-T412 (Boeringer), IDEC-CE9.1® (IDEC and SKB), mAB 4162W94, Orthoclone and OKTcdr4a (Janssen-Cilag)), anti-CD3 antibodies (*e.g.*, Nuvion (Product Design Labs), OKT3 (Johnson & Johnson), or Rituxan (IDEC)), anti-CD5 antibodies (*e.g.*, an anti-CD5 ricin-linked immunoconjugate), anti-CD7 antibodies (*e.g.*, CHH-380 (Novartis)), anti-CD8 antibodies, anti-CD40 ligand monoclonal antibodies (*e.g.*, IDEC-131 (IDEC)), anti-CD52 antibodies (*e.g.*, CAMPATH 1H (Ilex)), anti-CD2 antibodies (*e.g.*, MEDI-507 (MedImmune, Inc., International Publication Nos. WO 02/098370 and WO 02/069904), anti-CD11a antibodies (*e.g.*, Xanlim (Genentech)), and anti-B7 antibodies (*e.g.*, IDEC-114) (IDEC))), CTLA4-immunoglobulin, and LFA-3TIP (Biogen, International Publication No. WO 93/08656 and U.S. Patent No. 6,162,432).

Examples of cytokine receptor modulators include, but are not limited to, soluble cytokine receptors (*e.g.*, the extracellular domain of a TNF- $\alpha$  receptor or a fragment thereof, the extracellular domain of an IL-1 $\beta$  receptor or a fragment thereof, and the extracellular domain of an IL-6 receptor or a fragment thereof), cytokines or fragments thereof (*e.g.*, interleukin IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-23, TNF- $\alpha$ , TNF- $\beta$ , interferon (IFN)- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , and GM-CSF), anti-cytokine receptor antibodies (*e.g.*, anti-IFN receptor antibodies, anti-IL-2 receptor antibodies (*e.g.*, Zenapax (Protein Design Labs)), anti-IL-3 receptor antibodies, anti-IL-4 receptor antibodies, anti-IL-6 receptor antibodies, anti-IL-10 receptor antibodies, anti-IL-12 receptor antibodies, anti-IL-13 receptor antibodies, anti-IL-15 receptor antibodies, and anti-IL-23 receptor antibodies), anti-cytokine antibodies (*e.g.*, anti-IFN antibodies, anti-TNF- $\alpha$  antibodies, anti-IL-1 $\beta$  antibodies, anti-IL-3 antibodies, anti-IL-6 antibodies, anti-IL-8

antibodies (*e.g.*, ABX-IL-8 (Abgenix)), anti-IL-12 antibodies, anti-IL-13 antibodies, anti-IL-15 antibodies, and anti-IL-23 antibodies).

In a specific embodiment, a cytokine receptor modulator is IL-3, IL-4, IL-10, or a fragment thereof. In another embodiment, a cytokine receptor modulator is an anti-IL-1 $\beta$  antibody, anti-IL-6 antibody, anti-IL-12 receptor antibody, or anti-TNF- $\alpha$  antibody. In another embodiment, a cytokine receptor modulator is the extracellular domain of a TNF- $\alpha$  receptor or a fragment thereof. In certain embodiments, a cytokine receptor modulator is not a TNF- $\alpha$  antagonist.

In one embodiment, a cytokine receptor modulator is a mast cell modulator. In an alternative embodiment, a cytokine receptor modulator is not a mast cell modulator.

Examples of mast cell modulators include, but are not limited to stem cell factor (c-kit receptor ligand) inhibitor (*e.g.*, mAb 7H6, mAb 8H7a, pAb 1337, FK506, CsA, dexamethasone, and fluconcinonide), c-kit receptor inhibitor (*e.g.*, STI 571 (formerly known as CGP 57148B)), mast cell protease inhibitor (*e.g.*, GW-45, GW-58, wortmannin, LY 294002, calphostin C, cytochalasin D, genistein, KT5926, staurosporine, and lactoferrin), relaxin (“RLX”), IgE antagonist (*e.g.*, antibodies rhuMAb-E25 omalizumab, HMK-12 and 6HD5, and mAB Hu-901), IL-3 antagonist, IL-4 antagonists, IL-10 antagonists, and TGF-beta.

An immunomodulatory agent may be selected to interfere with the interactions between the T helper subsets (TH1 or TH2) and B cells to inhibit neutralizing antibody formation. Antibodies that interfere with or block the interactions necessary for the activation of B cells by TH (T helper) cells, and thus block the production of neutralizing antibodies, are useful as immunomodulatory agents in the methods of the invention. For example, B cell activation by T cells requires certain interactions to occur (Durie *et al.*, Immunol. Today, 15(9):406-410 (1994)), such as the binding of CD40 ligand on the T helper cell to the CD40 antigen on the B cell, and the binding of the CD28 and/or CTLA4 ligands on the T cell to the B7 antigen on the B cell. Without both interactions, the B cell cannot be activated to induce production of the neutralizing antibody.

The CD40 ligand (CD40L)-CD40 interaction is a desirable point to block the immune response because of its broad activity in both T helper cell activation and function as well as the absence of redundancy in its signaling pathway. Thus, in a specific embodiment of the invention, the interaction of CD40L with CD40 is transiently blocked at

the time of administration of one or more of the immunomodulatory agents. This can be accomplished by treating with an agent which blocks the CD40 ligand on the TH cell and interferes with the normal binding of CD40 ligand on the T helper cell with the CD40 antigen on the B cell. An antibody to CD40 ligand (anti-CD40L) (available from Bristol-Myers Squibb Co; see, *e.g.*, European patent application 555,880, published Aug. 18, 1993) or a soluble CD40 molecule can be selected and used as an immunomodulatory agent in accordance with the methods of the invention.

An immunomodulatory agent may be selected to inhibit the interaction between TH1 cells and cytotoxic T lymphocytes ("CTLs") to reduce the occurrence of CTL-mediated killing. An immunomodulatory agent may be selected to alter (*e.g.*, inhibit or suppress) the proliferation, differentiation, activity and/or function of the CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells. For example, antibodies specific for T cells can be used as immunomodulatory agents to deplete, or alter the proliferation, differentiation, activity and/or function of CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells.

In one embodiment of the invention, an immunomodulatory agent that reduces or depletes T cells, preferably memory T cells, is administered to a subject with a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection in accordance with the methods of the invention. See, *e.g.*, U.S. Pat. No. 4,658,019. In another embodiment of the invention, an immunomodulatory agent that inactivates CD8<sup>+</sup> T cells is administered to a subject with a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection in accordance with the methods of the invention. In a specific embodiment, anti-CD8 antibodies are used to reduce or deplete CD8<sup>+</sup> T cells.

In another embodiment, an immunomodulatory agent which reduces or inhibits one or more biological activities (*e.g.*, the differentiation, proliferation, and/or effector functions) of TH0, TH1, and/or TH2 subsets of CD4<sup>+</sup> T helper cells is administered to a subject with a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection in accordance with the methods of the invention. One example of such an immunomodulatory agent is IL-4. IL-4 enhances antigen-specific activity of TH2 cells at the expense of the TH1 cell function (see, *e.g.*, Yokota *et al*, 1986 Proc. Natl. Acad. Sci., USA, 83:5894-5898; and U.S. Pat. No. 5,017,691). Other examples of immunomodulatory agents that affect the biological activity (*e.g.*, proliferation,

differentiation, and/or effector functions) of T-helper cells (in particular, TH1 and/or TH2 cells) include, but are not limited to, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-15, IL-23, and interferon (IFN)- $\gamma$ .

In another embodiment, an immunomodulatory agent administered to a subject with a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection in accordance with the methods of the invention is a cytokine that prevents antigen presentation. In a specific embodiment, an immunomodulatory agent used in the methods of the invention is IL-10. IL-10 also reduces or inhibits macrophage action which involves bacterial elimination.

An immunomodulatory agent may be selected to reduce or inhibit the activation, degranulation, proliferation, and/or infiltration of mast cells. In certain embodiments, the immunomodulatory agent interferes with the interactions between mast cells and mast cell activating agents, including, but not limited to stem cell factors (c-kit ligands), IgE, IL-4, environmental irritants, and infectious agents. In a specific embodiment, the immunomodulatory agent reduces or inhibits the response of mast cells to environmental irritants such as, but not limited to, pollen, dust mites, tobacco smoke, and/or pet dander. In another specific embodiment, the immunomodulatory agent reduces or inhibits the response of mast cells to infectious agents, such as viruses, bacteria, and fungi. Examples of mast cell modulators that reduce or inhibit the activation, degranulation, proliferation, and/or infiltration of mast cells include, but are not limited to, stem cell factor (c-kit receptor ligand) inhibitors (e.g., mAb 7H6, mAb 8H7a, and pAb 1337 (see Mendiaz *et al.*, 1996, Eur J Biochem 293(3):842-849), FK506 and CsA (Ito *et al.*, 1999 Arch Dermatol Res 291(5):275-283), dexamthasone and fluconcinonide (see Finooto *et al.* J Clin Invest 1997 99(7):1721-1728)), c-kit receptor inhibitors (e.g., STI 571 (formerly known as CGP 57148B) (see Heinrich *et al.*, 2000 Blood 96(3):925-932)), mast cell protease inhibitors (e.g., GW-45 and GW-58 (see Temkin *et al.*, 2002 J Immunol 169(5):2662-2669), wortmannin, LY 294002, calphostin C, and cytochalasin D (see Vosseller *et al.*, 1997, Mol Biol Cell 1997:909-922), genistein, KT5926, and staurosporine (see Nagai *et al.* 1995, Biochem Biophys Res Commun 208(2):576-581), and lactoferrin (see He *et al.*, 2003 Biochem Pharmacol 65(6):1007-1015)), relaxin ("RLX") (see Bani *et al.*, 2002 Int Immunopharmacol 2(8):1195-1294), IgE antagonists (e.g., antibodies rhuMAb-E25 omalizumab (see Finn *et al.*, 2003 J Allergy Clin Immuno 111(2):278-284; Corren *et al.*,

2003 J Allergy Clin Immuno 111(1):87-90; Busse and Neaville, 2001 Curr Opin Allergy Clin Immuno 1(1):105-108; and Tang and Powell, 2001, Eur J Pediatr 160(12): 696-704), HMK-12 and 6HD5 (see Miyajima *et al.*, 2202 Int Arch Allergy Immuno 128(1):24-32), and mAB Hu-901 (see van Neerven *et al.*, 2001 Int Arch Allergy Immuno 124(1-3):400), IL-3 antagonist, IL-4 antagonists, IL-10 antagonists, and TGF-beta (see Metcalfe *et al.*, 1995, Exp Dermatol 4(4 Pt 2):227-230).

In a preferred embodiment, proteins, polypeptides or peptides (including antibodies) that are utilized as immunomodulatory agents are derived from the same species as the recipient of the proteins, polypeptides or peptides so as to reduce the likelihood of an immune response to those proteins, polypeptides or peptides. In another preferred embodiment, when the subject is a human, the proteins, polypeptides, or peptides that are utilized as immunomodulatory agents are human or humanized.

In accordance with the invention, one or more immunomodulatory agents are administered to a subject with a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection prior to, subsequent to, or concomitantly with a liquid formulation of an antibody or a fragment thereof that immunospecifically bind to a RSV antigen. Preferably, one or more immunomodulatory agents are administered in combination with a liquid formulation of an antibody or a fragment thereof that immunospecifically bind to a RSV antigen to a subject with a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection to reduce or inhibit one or more aspects of the immune response as deemed necessary by one skilled in the art. Any technique well-known to one skilled in the art can be used to measure one or more aspects of the immune response in a particular subject, and thereby determine when it is necessary to administer an immunomodulatory agent to said subject. In a preferred embodiment, a mean absolute lymphocyte count of approximately 500 cells/mm<sup>3</sup>, preferably 600 cells/mm<sup>3</sup>, 650 cells/mm<sup>3</sup>, 700 cells/mm<sup>3</sup>, 750 cells/mm<sup>3</sup>, 800 cells/mm<sup>3</sup>, 900 cells/mm<sup>3</sup>, 1000 cells/mm<sup>3</sup>, 1100 cells/mm<sup>3</sup>, or 1200 cells/mm<sup>3</sup> is maintained in a subject. In another preferred embodiment, a subject with a respiratory condition associated with, potentiated by or potentiating a RSV infection is not administered an immunomodulatory agent if their absolute lymphocyte count is 500 cells/mm<sup>3</sup> or less, 550 cells/mm<sup>3</sup> or less, 600 cells/mm<sup>3</sup> or less, 650 cells/mm<sup>3</sup> or less, 700 cells/mm<sup>3</sup> or less, 750 cells/mm<sup>3</sup> or less, or 800 cells/mm<sup>3</sup> or less.

In a specific embodiment, one or more immunomodulatory agents are administered in combination with a liquid formulation of an antibody or a fragment thereof that immunospecifically bind to a RSV antigen to a subject with a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection so as to transiently reduce or inhibit one or more aspects of the immune response. Such a transient inhibition or reduction of one or more aspects of the immune system can last for hours, days, weeks, or months. Preferably, the transient inhibition or reduction in one or more aspects of the immune response lasts for a few hours (e.g., 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, 14 hours, 16 hours, 18 hours, 24 hours, 36 hours, or 48 hours), a few days (e.g., 3 days, 4 days, 5 days, 6 days, 7 days, or 14 days), or a few weeks (e.g., 3 weeks, 4 weeks, 5 weeks or 6 weeks). The transient reduction or inhibition of one or more aspects of the immune response enhances the prophylactic and/or therapeutic effect(s) of a liquid formulation of SYNAGIS® or an antigenic fragment thereof.

Nucleic acid molecules encoding proteins, polypeptides, or peptides with immunomodulatory activity or proteins, polypeptides, or peptides with immunomodulatory activity can be administered to a subject with a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection in accordance with the methods of the invention. Further, nucleic acid molecules encoding derivatives, analogues, or fragments of proteins, polypeptides, or peptides with immunomodulatory activity, or derivatives, analogues, or fragments of proteins, polypeptides, or peptides with immunomodulatory activity can be administered to a subject with a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection in accordance with the methods of the invention. Preferably, such derivatives, analogues, and fragments retain the immunomodulatory activity of the full-length, wild-type protein, polypeptide, or peptide.

Preferably, agents that are commercially available and known to function as immunomodulatory agents are used in the methods of the invention. The immunomodulatory activity of an agent can be determined *in vitro* and/or *in vivo* by any technique well-known to one skilled in the art, including, e.g., by CTL assays, proliferation assays, and immunoassays (e.g. ELISAs) for the expression of particular proteins such as co-stimulatory molecules and cytokines.

#### 5.1.1.1. Interleukin-9 Antagonists

In a specific embodiment, a liquid formulation of the invention is used in combination with a interleukin-9 (IL-9) antagonists to prevent or treat a RSV infection or a respiratory condition that associated with, potentiated by or potentiates a RSV infection. The terms “IL-9 antagonist” or “IL-9 antagonists” as used herein, refer to any agent that blocks, inhibits, reduces, or neutralizes the function, activity and/or expression of an IL-9 protein, polypeptide or peptide. An IL-9 antagonist may inhibit a pathologic cellular or humoral phenotype associated with or resulting from IL-9 expression and/or activity (*e.g.*, decreased secretion of mucin, the differentiation of IL-9 expressing cells into a mucin-secreting cell, the secretion of inflammatory agents, the proliferation, migration, and increase in volume of cells (*e.g.*, immune and smooth muscle cells), the secretion of extracellular matrix molecules or matrix metalloproteinases and/or the binding of IL-9 to the IL-9 receptor (“IL-9R”)). IL-9 antagonists are disclosed in U.S. Application NOS. 60/462,307 filed April 11, 2003, and 60/462,259 filed April 11, 2003, both of which are incorporated herein by reference.

IL-9 antagonists include, but are not limited to, proteinaceous agents (*e.g.*, proteins, polypeptides, peptides, fusion proteins, antibodies, and antibody fragments), nucleic acid molecules (*e.g.*, IL-9 antisense nucleic acid molecules, triple helices, RNAi, or nucleic acid molecules encoding proteinaceous agents), organic molecules, inorganic molecules, small organic molecules, drugs, and small inorganic molecules that block, inhibit, reduce or neutralize a function, an activity and/or the expression of an IL-9 polypeptide, the function, an activity, and/or expression of the IL-9R or a subunit thereof, and/or the binding of an IL-9 polypeptide to the IL-9R or a subunit thereof. In various embodiments, an IL-9 antagonist reduces the function, activity, and/or expression of another molecule other than an IL-9 polypeptide or the IL-9R or a subunit thereof. In other embodiments, an IL-9 antagonist reduces the function, activity, and/or expression of an IL-9 polypeptide, the function, activity, and/or expression of the IL-9R or a subunit thereof, and/or the binding of an IL-9 polypeptide to the IL-9R or a subunit thereof. In particular embodiments, an IL-9 antagonist reduces the function, activity and/or expression of an IL-9 polypeptide, the function, activity, and/or expression of the IL-9R or a subunit thereof, and/or the binding of an IL-9 polypeptide to the IL-9R or a subunit thereof by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at

least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% relative to a control such as PBS.

In a preferred embodiment, a liquid formulation of the invention is administered in combination with a IL-9 antagonist to a subject in need thereof to prevent, treat, manage or ameliorating wheezing associated with a RSV infection. In certain cases, wheezing precedes the onset or development of a RSV infection. In a specific embodiment, the invention provides methods of preventing, treating, ameliorating, or managing wheezing associated with a RSV infection, said methods comprising administering to a subject in need thereof an effective amount of one or more IL-9 antagonists in combination with a liquid formulation of the invention. In other embodiments, the invention provides methods of preventing the onset and/or development of asthma (which may be associated with, potentiated by or potentiates a RSV infection) in subjects with wheezing, said method comprising administering to said subject an effective amount of one or more IL-9 antagonists in combination with an effective amount of a liquid formulation of the invention.

### 5.5.2 Anti-inflammatory Agents

Any anti-inflammatory agent, including agents useful in therapies for inflammatory disorders, well-known to one of skilled in the art can be used in the compositions and methods of the invention. Non-limiting examples of anti-inflammatory agents include non-steroidal anti-inflammatory drugs (NSAIDs), steroidal anti-inflammatory drugs, anticholinergics (*e.g.*, atropine sulfate, atropine methylnitrate, and ipratropium bromide (ATROVENT<sup>TM</sup>)), beta2-agonists (*e.g.*, albuterol (VENTOLIN<sup>TM</sup> and PROVENTIL<sup>TM</sup>), bitolterol (TORNALATE<sup>TM</sup>), levalbuterol (XOPONEX<sup>TM</sup>), metaproterenol (ALUPENT<sup>TM</sup>), pirbuterol (MAXAIR<sup>TM</sup>), terbutaline (BRETHAIRE<sup>TM</sup> and BRETHINE<sup>TM</sup>), albuterol (PROVENTIL<sup>TM</sup>, REPETABS<sup>TM</sup>, and VOLMAX<sup>TM</sup>), formoterol (FORADIL AEROLIZER<sup>TM</sup>), and salmeterol (SEREVENT<sup>TM</sup> and SEREVENT DISKUS<sup>TM</sup>)), and methylxanthines (*e.g.*, theophylline (UNIPHYL<sup>TM</sup>, THEO-DUR<sup>TM</sup>, SLO-BID<sup>TM</sup>, AND TEHO-42<sup>TM</sup>)). Examples of NSAIDs include, but are not limited to, aspirin, ibuprofen, celecoxib (CELEBREX<sup>TM</sup>), diclofenac (VOLTAREN<sup>TM</sup>), etodolac (LODINE<sup>TM</sup>), fenoprofen (NALFON<sup>TM</sup>), indomethacin (INDOCIN<sup>TM</sup>), ketoralac (TORADOL<sup>TM</sup>), oxaprozin (DAYPRO<sup>TM</sup>), nabumetone (RELAFENT<sup>TM</sup>), sulindac (CLINORIL<sup>TM</sup>),

tolmentin (TOLECTINTM), rofecoxib (VIOXXTM), naproxen (ALEVETM, NAPROSYNTM), ketoprofen (ACTRONTM) and nabumetone (RELAFENTM). Such NSAIDs function by inhibiting a cyclooxygenase enzyme (e.g., COX-1 and/or COX-2). Examples of steroidal anti-inflammatory drugs include, but are not limited to, glucocorticoids, dexamethasone (DECADRONTM), corticosteroids (e.g., methylprednisolone (MEDROLTM)), cortisone, hydrocortisone, prednisone (PREDNISONETM and DELTASONETM), prednisolone (PRELONETM and PEDIAPREDTM), triamcinolone, azulfidine, and inhibitors of eicosanoids (e.g., prostaglandins, thromboxanes, and leukotrienes (see Table 2, *infra*, for non-limiting examples of leukotriene and typical dosages of such agents)).

In certain embodiments, the anti-inflammatory agent is an agent useful in the prevention, management, treatment, and/or amelioration of asthma or one or more symptoms thereof. Non-limiting examples of such agents include adrenergic stimulants (e.g., catecholamines (e.g., epinephrine, isoproterenol, and isoetharine), resorcinols (e.g., metaproterenol, terbutaline, and fenoterol), and saligenins (e.g., salbutamol)), adrenocorticoids, glucocorticoids, corticosteroids (e.g., beclomethasone, budesonide, flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, and prednisone), other steroids, beta2-agonists (e.g., albuterol, bitolterol, fenoterol, isoetharine, metaproterenol, pirbuterol, salbutamol, terbutaline, formoterol, salmeterol, and albutamol terbutaline), anti-cholinergics (e.g., ipratropium bromide and oxitropium bromide), IL-4 antagonists (including antibodies), IL-5 antagonists (including antibodies), IL-13 antagonists (including antibodies), PDE4-inhibitor, NF-Kappa- $\beta$  inhibitor, VLA-4 inhibitor, CpG, anti-CD23, selectin antagonists (TBC 1269), mast cell protease inhibitors (e.g., tryptase kinase inhibitors (e.g., GW-45, GW-58, and genisteine), phosphatidylinositide-3' (PI3)-kinase inhibitors (e.g., calphostin C), and other kinase inhibitors (e.g., staurosporine) (see Temkin *et al.*, 2002 J Immunol 169(5):2662-2669; Vosseller *et al.*, 1997 Mol. Biol. Cell 8(5):909-922; and Nagai *et al.*, 1995 Biochem Biophys Res Commun 208(2):576-581)), a C3 receptor antagonists (including antibodies), immunosuppressant agents (e.g., methotrexate and gold salts), mast cell modulator (e.g., cromolyn sodium (INTALTM) and nedocromil sodium (TILADETM)), and mucolytic agents (e.g., acetylcysteine)). In a specific embodiment, the anti-inflammatory agent is a leukotriene inhibitor (e.g., montelukast (SINGULAIRTM), zafirlukast (ACCOLATETM), pranlukast (ONONTM), or zileuton (ZYFLOTM)).

In certain embodiments, the anti-inflammatory agent is an agent useful in preventing, treating, managing, or ameliorating allergies or one or more symptoms thereof. Non-limiting examples of such agents include antimediator drugs (e.g., antihistamine, *see* Table 4 for non-limiting examples of antihistamine and typical dosages of such agents), corticosteroids, decongestants, sympathomimetic drugs (e.g.,  $\alpha$ -adrenergic and  $\beta$ -adrenergic drugs), TNX901 (Leung *et al.*, 2003, *N Engl J Med* 348(11):986-993), IgE antagonists (e.g., antibodies rhuMAb-E25 omalizumab (see Finn *et al.*, 2003 *J Allergy Clin Immuno* 111(2):278-284; Corren *et al.*, 2003 *J Allergy Clin Immuno* 111(1):87-90; Busse and Neaville, 2001 *Curr Opin Allergy Clin Immuno* 1(1):105-108; and Tang and Powell, 2001, *Eur J Pediatr* 160(12): 696-704), HMK-12 and 6HD5 (see Miyajima *et al.*, 2202 *Int Arch Allergy Immuno* 128(1):24-32), and mAB Hu-901 (see van Neerven *et al.*, 2001 *Int Arch Allergy Immuno* 124(1-3):400), theophylline and its derivatives, glucocorticoids, and immunotherapies (e.g., repeated long-term injection of allergen, short course desensitization, and venom immunotherapy).

Anti-inflammatory therapies and their dosages, routes of administration, and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (57th ed., 2003) and *The Merk Manual* (17th ed., 1999).

### 5.5.3 Anti-Viral Agents

Any anti-viral agent well-known to one of skilled in the art for the treatment, prevention, management, or amelioration of a RSV infection or a respiratory condition associated with, potentiated by or potentiating a RSV infection can be used in the compositions and methods of the invention. Non-limiting examples of anti-viral agents include proteins, polypeptides, peptides, fusion proteins antibodies, nucleic acid molecules, organic molecules, inorganic molecules, and small molecules that inhibit and/or reduce the attachment of a virus to its receptor, the internalization of a virus into a cell, the replication of a virus, or release of virus from a cell. In particular, anti-viral agents include, but are not limited to, nucleoside analogues (e.g., zidovudine, acyclovir, gancyclovir, vidarabine, idoxuridine, trifluridine, and ribavirin), foscarnet, amantadine, rimantadine, saquinavir, indinavir, ritonavir, alpha-interferons and other interferons, and AZT.

In a specific embodiment, the anti-viral agent is an antibody that is immunospecific for a non-RSV viral antigen. As used herein, the term "viral antigen" includes, but is not

limited to, any viral peptide, polypeptide and protein (e.g., influenza virus neuraminidase, influenza virus hemagglutinin, and herpes simplex virus glycoprotein (e.g., gB, gC, gD, and gE)) that is capable of eliciting an immune response. Antibodies useful in this invention for prevention, management, treatment, and/or amelioration of a non-RSV viral infectious disease that may potentiate or potentiated by a RSV infection include, but are not limited to, antibodies against antigens of pathogenic viruses, including as examples and not by limitation: adenoviridae (e.g., mastadenovirus and aviadenovirus), herpesviridae (e.g., herpes simplex virus 1, herpes simplex virus 2, herpes simplex virus 5, and herpes simplex virus 6), leviviridae (e.g., levivirus, enterobacteria phage MS2, allovirus), poxviridae (e.g., chordopoxvirinae, parapoxvirus, avipoxvirus, capripoxvirus, leporiipoxvirus, suipoxvirus, molluscipoxvirus, and entomopoxvirinae), papovaviridae (e.g., polyomavirus and papillomavirus), paramyxoviridae (e.g., paramyxovirus, parainfluenza virus 1, mumps virus (e.g., measles virus), rubulavirus (e.g., mumps virus), pneumonovirinae (e.g., pneumovirus, human respiratory syncytial virus), and metapneumovirus (e.g., avian pneumovirus and human metapneumovirus)), picornaviridae (e.g., enterovirus, rhinovirus, hepatovirus (e.g., human hepatitis A virus), cardiovirus, and aphthovirus), reoviridae (e.g., orthoreovirus, orbivirus, rotavirus, cypovirus, fijivirus, phytoreovirus, and oryzavirus), retroviridae (e.g., mammalian type B retroviruses, mammalian type C retroviruses, avian type C retroviruses, type D retrovirus group, BLV-HTLV retroviruses, lentivirus (e.g. human immunodeficiency virus 1 and human immunodeficiency virus 2), spumavirus), flaviviridae (e.g., hepatitis C virus), hepadnaviridae (e.g., hepatitis B virus), togaviridae (e.g., alphavirus (e.g., sindbis virus) and rubivirus (e.g., rubella virus)), rhabdoviridae (e.g., vesiculovirus, lyssavirus, ephemerovirus, cytorhabdovirus, and nucleorhabdovirus), arenaviridae (e.g., arenavirus, lymphocytic choriomeningitis virus, Ippy virus, and lassa virus), and coronaviridae (e.g., coronavirus and torovirus).

A specific example of antibodies available useful for the prevention, management, treatment, and/or amelioration of a viral infectious disease include, but are not limited to, PRO542 (Progenics), which is a CD4 fusion antibody useful for the treatment of HIV infection.

In a specific embodiment, the anti-viral agent used in the compositions and methods of the invention inhibits or reduces a pulmonary or respiratory virus infection, inhibits or reduces the replication of a virus that causes a pulmonary or respiratory infection, or

inhibits or reduces the spread of a virus that causes a pulmonary or respiratory infection to other cells or subjects. In another specific embodiment, the anti-viral agent used in the compositions and methods of the invention inhibits or reduces infection, inhibits or reduces the replication, or inhibits or reduces the spread to other cells or subjects by RSV, or another virus of which an infection can be potentiated by or potentiating a RSV infection, *e.g.*, hMPV, or PIV. Examples of such agents include, but are not limited to, nucleoside analogues, such as zidovudine, acyclovir, gancyclovir, vidarabine, idoxuridine, trifluridine, and ribavirin, as well as foscarnet, amantadine, rimantadine, saquinavir, indinavir, ritonavir, and the alpha-interferons. See U.S. Provisional Patent Application 60/398,475 filed July 25, 2002, entitled “Methods of Treating and Preventing RSV, HMPV, and PIV Using Anti-RSV, Anti-HMPV, and Anti-PIV Antibodies” and U.S. Patent App. No. 10/371,122 filed February 21, 2003, which are incorporated herein by reference in its entirety.

Anti-viral therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the *Physician’s Desk Reference* (57th ed., 2003). Additional information on respiratory viral infections is available in *Cecil Textbook of Medicine* (18th ed., 1988).

#### **5.5.4 Anti-Bacterial Agents**

Anti-bacterial agents and therapies well-known to one of skilled in the art for the prevention, treatment, management, or amelioration of a respiratory condition associated with, potentiated by or potentiating a RSV infection (*e.g.*, a bacterial respiratory infection) can be used in the compositions and methods of the invention. Non-limiting examples of anti-bacterial agents include proteins, polypeptides, peptides, fusion proteins, antibodies, nucleic acid molecules, organic molecules, inorganic molecules, and small molecules that inhibit and/or reduce a bacterial infection, inhibit and/or reduce the replication of bacteria, or inhibit and/or reduce the spread of bacteria to other cells or subjects. Specific examples of anti-bacterial agents include, but are not limited to, antibiotics such as penicillin, cephalosporin, imipenem, axtremonam, vancomycin, cycloserine, bacitracin, chloramphenicol, erythromycin, clindamycin, tetracycline, streptomycin, tobramycin, gentamicin, amikacin, kanamycin, neomycin, spectinomycin, trimethoprim, norfloxacin, rifampin, polymyxin, amphotericin B, nystatin, ketocanazole, isoniazid, metronidazole, and pentamidine.

In certain embodiments, the anti-bacterial agent is an agent that inhibits or reduces a pulmonary or respiratory bacterial infection, inhibits or reduces the replication of a bacteria that causes a pulmonary or respiratory infection, or inhibits or reduces the spread of a bacteria that causes a pulmonary or respiratory infection to other cells or subjects. In cases in which the pulmonary or respiratory bacterial infection is a mycoplasma infection (e.g., pharyngitis, tracheobronchitis, and pneumonia), the anti-bacterial agent is preferably a tetracycline, erythromycin, or spectinomycin. In cases in which the pulmonary or respiratory bacterial infection is tuberculosis, the anti-bacterial agent is preferably rifampcin, isoniazid, pyrazinamide, ethambutol, and streptomycin. In cases in which the pulmonary or respiratory bacterial infection is pneumonia caused by an aerobic gram negative bacilli (GNB), the anti-bacterial agent is preferably penicillin, first, second, or third generation cephalosporin (e.g., cefaclor, cefadroxil, cephalexin, or cephazolin), erythromycin, clindamycin, an aminoglycoside (e.g., gentamicin, tobramycin, or amikacine), or a monolactam (e.g., aztreonam). In cases in which the respiratory infection is recurrent aspiration pneumonia, the anti-bacterial agent is preferably penicillin, an aminoglycoside, or a second or third generation cephalosporin.

Anti-bacterial therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (57th ed., 2003), *Cecil Textbook of Medicine* (18th ed., 1988), and *The Merk Manual of Diagnosis and Therapy* (17th ed. 1999).

### 5.5.5 Anti-Fungal Agents

Anti-fungal agents and therapies well known to one of skilled in the art for prevention, management, treatment, and/or amelioration of a respiratory condition associated with, potentiated by or potentiating a RSV infection (e.g., a fungal respiratory infection) can be used in the compositions and methods of the invention. Non-limiting examples of anti-fungal agents include proteins, polypeptides, peptides, fusion proteins, antibodies, nucleic acid molecules, organic molecules, inorganic molecules, and small molecules that inhibit and/or reduce fungal infection, inhibit and/or reduce the replication of fungi, or inhibit and/or reduce the spread of fungi to other subjects. Specific examples of anti-fungal agents include, but are not limited to, azole drugs (e.g., miconazole, ketoconazole (NIZORAL<sup>®</sup>), caspofungin acetate (CANCIDAS<sup>®</sup>), imidazole, triazoles (e.g.,

fluconazole (DIFLUCAN<sup>®</sup>), and itraconazole (SPORANOX<sup>®</sup>), polyene (*e.g.*, nystatin, amphotericin B (FUNGIZONE<sup>®</sup>), amphotericin B lipid complex (“ABLC”)(ABELCET<sup>®</sup>), amphotericin B colloidal dispersion (“ABCD”)(AMPHOTEC<sup>®</sup>), liposomal amphotericin B (AMBISONE<sup>®</sup>)), potassium iodide (KI), pyrimidine (*e.g.*, flucytosine (ANCOBON<sup>®</sup>)), and voriconazole (VFEND<sup>®</sup>).

In certain embodiments, the anti-fungal agent is an agent that inhibits or reduces a respiratory fungal infection, inhibits or reduces the replication of a fungus that causes a pulmonary or respiratory infection, or inhibits or reduces the spread of a fungus that causes a pulmonary or respiratory infection to other subjects. In cases in which the pulmonary or respiratory fungal infection is *Blastomyces dermatitidis*, the anti-fungal agent is preferably itraconazole, amphotericin B, fluconazole, or ketoconazole. In cases in which the pulmonary or respiratory fungal infection is pulmonary aspergilloma, the anti-fungal agent is preferably amphotericin B, liposomal amphotericin B, itraconazole, or fluconazole. In cases in which the pulmonary or respiratory fungal infection is histoplasmosis, the anti-fungal agent is preferably amphotericin B, itraconazole, fluconazole, or ketoconazole. In cases in which the pulmonary or respiratory fungal infection is coccidioidomycosis, the anti-fungal agent is preferably fluconazole or amphotericin B. In cases in which the pulmonary or respiratory fungal infection is cryptococcosis, the anti-fungal agent is preferably amphotericin B, fluconazole, or combination of the two agents. In cases in which the pulmonary or respiratory fungal infection is chromomycosis, the anti-fungal agent is preferably itraconazole, fluconazole, or flucytosine. In cases in which the pulmonary or respiratory fungal infection is mucormycosis, the anti-fungal agent is preferably amphotericin B or liposomal amphotericin B. In cases in which the pulmonary or respiratory fungal infection is pseudoallescheriasis, the anti-fungal agent is preferably itraconazole or miconazole.

Anti-fungal therapies and their dosages, routes of administration, and recommended usage are known in the art and have been described in such literature as Dodds *et al.*, 2000 *Pharmacotherapy* 20(11) 1335-1355, the *Physician’s Desk Reference* (57th ed., 2003) and the *Merk Manual of Diagnosis and Therapy* (17th ed., 1999).

### 5.6 Methods of Administering the Antibody Formulations

The invention provides methods of treatment, prophylaxis, and amelioration of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection by administrating to a subject of an effective amount of liquid formulations of the invention. The subject is preferably a mammal such as non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats etc.) and a primate (*e.g.*, monkey such as a cynomolgous monkey and a human). In a preferred embodiment, the subject is a human. In another preferred embodiment, the subject is a human infant or a human infant born prematurely.

Various delivery systems are known and can be used to administer a liquid formulation of the present invention. Methods of administering antibody liquid formulations of the present invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural administration, topical administration, pulmonary administration, and mucosal administration (*e.g.*, intranasal and oral routes). In a specific embodiment, liquid formulations of the present invention are administered intramuscularly, intravenously, or subcutaneously and, preferably, intramuscularly. The formulations may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can be employed, *e.g.*, by use of an inhaler or nebulizer.

The invention also provides that a liquid formulation of the present invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of antibody or antibody fragment. Preferably, the liquid formulations of the present invention are in a hermetically sealed container indicating the quantity and concentration of the antibody or antibody fragment. Preferably, the liquid formulation of the present invention is supplied in a hermetically sealed container at least 15 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml, or 300 mg/ml and, most preferably, 105 mg/ml, in a quantity of 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml, 10 ml, 15 ml, or 20 ml and, most preferably, 1.2 ml.

The amount of the liquid formulations of the present invention which will be effective in the treatment, prevention, management or amelioration of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection can be determined by standard clinical techniques. For example, the dosage of a liquid formulation which will be effective in the treatment, prevention, management or amelioration of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection can be determined by administering the formulation to a cotton rat, measuring the RSV titer after challenging the cotton rat with  $10^5$  pfu of RSV and comparing the RSV titer to that obtain for a cotton rat not administered the formulation. Accordingly, a dosage that results in a 2 log decrease or a 99% reduction in RSV titer in the cotton rat challenged with  $10^5$  pfu of RSV relative to the cotton rat challenged with  $10^5$  pfu of RSV but not administered the formulation is the dosage of the formulation that can be administered to a human for the treatment, prevention, management or amelioration of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection. The dosage of a liquid formulation which will be effective in the treatment, prevention, management or amelioration of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection can be determined by administering the formulation to an animal model (e.g., a cotton rat or monkey) and measuring the serum titer of antibodies or fragments thereof that immunospecifically bind to a RSV antigen. Accordingly, a dosage of the formulation that results in a serum titer of at least 1  $\mu$ g/ml, preferably 2  $\mu$ g/ml, 5  $\mu$ g/ml, 10  $\mu$ g/ml, 20  $\mu$ g/ml, 25  $\mu$ g/ml, at least 35  $\mu$ g/ml, at least 40  $\mu$ g/ml, at least 50  $\mu$ g/ml, at least 75  $\mu$ g/ml, at least 100  $\mu$ g/ml, at least 125  $\mu$ g/ml, at least 150  $\mu$ g/ml, at least 200  $\mu$ g/ml, at least 250  $\mu$ g/ml, at least 300  $\mu$ g/ml, at least 350  $\mu$ g/ml, at least 400  $\mu$ g/ml, or at least 450  $\mu$ g/ml can be administered to a human for the treatment, prevention, management or amelioration of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges.

The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the RSV infection, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be

extrapolated from dose-response curves derived from *in vitro* or animal model (e.g., the cotton rat or Cynomolgous monkey) test systems.

For antibodies, proteins, polypeptides, peptides and fusion proteins, the dosage administered to a patient may be about 1 mg/kg to 30 mg/kg of the patients body weight. Preferably, the dosage administered to a patient is between 10 mg/kg and 20 mg/kg of the patients body weight, more preferably 15 mg/kg of the patients body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage, volume and frequency of administration of liquid formulations of the present invention may be reduced by increasing the concentration of an antibody or a fragment thereof in the formulations, increasing affinity and/or avidity of the antibody or a fragment thereof, and/or increasing the half-life of the antibody or a fragment thereof.

Exemplary doses of a small molecule include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram).

In one embodiment, the liquid formulations of the present invention are administered to a mammal, preferably a human, to prevent, treat, manage or ameliorate a RSV infection or one or more symptoms thereof. In another embodiment, the liquid formulations of the invention are administered to a human with cystic fibrosis, bronchopulmonary dysplasia, congenital heart disease, congenital immunodeficiency or acquired immunodeficiency, or to a human who has had a bone marrow transplant to prevent, treat, manage or ameliorate a RSV infection or one or more symptoms thereof. In another embodiment, the liquid formulations of the invention are administered to a human infant, preferably a human infant born prematurely or a human infant at risk of hospitalization for a RSV infection to prevent, treat, manage or ameliorate a RSV infection or one or more symptoms thereof. In another embodiment, the liquid formulations of the invention are administered to an elderly person to prevent, treat, manage or ameliorate a RSV infection or one or more symptoms thereof.

In a specific embodiment, a subject, preferably a human, is administered a stable liquid formulation of the present invention for the treatment, prevention or amelioration of one or more symptoms associated with a RSV infection in an amount effective for decreasing RSV titers. In accordance with this embodiment, an effective amount of the liquid formulations of the present invention reduces the RSV titers in the lung as measured, for example, by the concentration of RSV in sputum samples or a lavage from the lungs from a subject. In another embodiment, a subject, preferably a human, is administered an antibody liquid formulation of the present invention for the treatment, prevention or amelioration of symptoms associated with a RSV infection in an amount effective for inducing an immune response in the subject.

In another embodiment, a subject, preferably a human, is administered a first dose of a liquid formulation of the present invention comprising 30 mg/kg or less, 15 mg/kg or less, 10 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less, or 0.5 mg/kg or less of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen and, preferably has equal to or higher affinity, equal to or higher avidity, and/or equal to or longer half-life than previously known antibodies (e.g., SYNAGIS<sup>®</sup>) for the prevention of a RSV infection in an amount effective to induce a serum titer of at least 1  $\mu$ g/ml, preferably at least 2  $\mu$ g/ml, at least 5  $\mu$ g/ml, at least 10  $\mu$ g/ml, at least 15  $\mu$ g/ml, at least 20  $\mu$ g/ml, at least 25  $\mu$ g/ml, at least 30  $\mu$ g/ml, at least 35  $\mu$ g/ml, at least 40  $\mu$ g/ml 20 days (preferably 25, 30, 35, 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. In a specific embodiment, a liquid formulation of the present invention comprises an antibody having a VH domain of SEQ ID NO:9 and a VL domain of SEQ ID NO:13; a VH domain of SEQ ID NO:17 and a VL domain of SEQ ID NO:21; a VH domain of SEQ ID NO:24 and a VL domain of SEQ ID NO:26; a VH domain of SEQ ID NO:28 and a VL domain of SEQ ID NO:30; a VH domain of SEQ ID NO:33 and a VL domain of SEQ ID NO:34; a VH domain of SEQ ID NO:36 and a VL domain of SEQ ID NO:38; a VH domain of SEQ ID NO:40 and a VL domain of SEQ ID NO:42; a VH domain of SEQ ID NO:44 and a VL domain of SEQ ID NO:46; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:49; a VH domain of SEQ ID NO:51 and a VL domain of SEQ ID NO:52; a VH domain of SEQ ID NO:55 and a VL domain of SEQ ID NO:56; a VH domain of SEQ ID NO:55 and a VL domain of SEQ ID NO:58; a VH domain of SEQ ID NO:78 and a VL domain of

SEQ ID NO:56; a VH domain of SEQ ID NO:9 and a VL domain of SEQ ID NO:60; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:62; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:64; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:65; a VH domain of SEQ ID NO:67 and a VL domain of SEQ ID NO:68 (*see Table 1 and 2, supra*) a VH domain of SEQ ID NO:70 and a VL domain of SEQ ID NO:71; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:74; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:11; or a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:76 (*see Table 1 and 2, supra*).

In another embodiment, a subject, preferably a human, is administered a first dose of a liquid formulation of the present invention comprising 30 mg/kg or less, 15 mg/kg or less, 10 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less or 0.5 mg/kg or less of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen and, preferably, has a higher affinity and/or higher avidity than previously known antibodies (*e.g.*, SYNAGIS<sup>®</sup>) for the treatment or amelioration of a RSV infection or a symptom thereof in an amount effective to induce a serum titer of at least 1  $\mu$ g/ml, preferably at least 2  $\mu$ g/ml, at least 5  $\mu$ g/ml, at least 10  $\mu$ g/ml, at least 15  $\mu$ g/ml, at least 20  $\mu$ g/ml, or at least 25  $\mu$ g/ml 20 days (preferably 25, 30, 35, 40 days) after the administration of the first dose and prior to the administration of subsequent dose. Preferably, the serum titer of said antibodies or antibody fragments is less than 30  $\mu$ g/ml 30 days after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, said antibodies have a VH domain of SEQ ID NO:9 and a VL domain of SEQ ID NO:13; a VH domain of SEQ ID NO:17 and a VL domain of SEQ ID NO:21; a VH domain of SEQ ID NO:24 and a VL domain of SEQ ID NO:26; a VH domain of SEQ ID NO:28 and a VL domain of SEQ ID NO:30; a VH domain of SEQ ID NO:33 and a VL domain of SEQ ID NO:34; a VH domain of SEQ ID NO:36 and a VL domain of SEQ ID NO:38; a VH domain of SEQ ID NO:40 and a VL domain of SEQ ID NO:42; a VH domain of SEQ ID NO:44 and a VL domain of SEQ ID NO:46; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:49; a VH domain of SEQ ID NO:51 and a VL domain of SEQ ID NO:52; a VH domain of SEQ ID NO:7 and a VL domain of SEQ ID NO:54; a VH domain of SEQ ID NO:55 and a VL domain of SEQ ID NO:56; a VH domain of SEQ ID NO:55 and a VL domain of SEQ ID NO:58; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:56; a VH domain of SEQ ID NO:9 and a VL domain of SEQ ID NO:60; a VH domain

of SEQ ID NO:78 and a VL domain of SEQ ID NO:62; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:64; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:65; a VH domain of SEQ ID NO:67 and a VL domain of SEQ ID NO:68 (*see Table 1 and 2, supra*) a VH domain of SEQ ID NO:70 and a VL domain of SEQ ID NO:71; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:74; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:11; or a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:76 (*see Table 1 and 2, supra*)

In another embodiment, a subject, preferably a human, is administered a first dose of a liquid formulation of the present invention comprising 30 mg/kg or less, 15 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less or 0.5 mg/kg or less of an antibody or a fragment thereof which has increased *in vivo* half-life and which immunospecifically binds to a RSV antigen with higher affinity and/or higher avidity than previously known antibodies (*e.g.*, SYNAGIS<sup>®</sup>) for the prevention of a RSV infection in an amount effective to induce a serum titer of at least 1  $\mu$ g/ml, preferably at least 2  $\mu$ g/ml, at least 5  $\mu$ g/ml, at least 10  $\mu$ g/ml, at least 15  $\mu$ g/ml, at least 20  $\mu$ g/ml, or at least 25  $\mu$ g/ml 25 days (preferably 30, 35, or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the serum titer of said antibodies or antibody fragments is less than 30  $\mu$ g/ml 30 days after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the said antibodies have a VH domain of SEQ ID NO:9 and a VL domain of SEQ ID NO:13; a VH domain of SEQ ID NO:17 and a VL domain of SEQ ID NO:21; a VH domain of SEQ ID NO:24 and a VL domain of SEQ ID NO:26; a VH domain of SEQ ID NO:28 and a VL domain of SEQ ID NO:30; a VH domain of SEQ ID NO:33 and a VL domain of SEQ ID NO:34; a VH domain of SEQ ID NO:36 and a VL domain of SEQ ID NO:38; a VH domain of SEQ ID NO:40 and a VL domain of SEQ ID NO:42; a VH domain of SEQ ID NO:44 and a VL domain of SEQ ID NO:46; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:49; a VH domain of SEQ ID NO:51 and a VL domain of SEQ ID NO:52; a VH domain of SEQ ID NO:7 and a VL domain of SEQ ID NO:54; a VH domain of SEQ ID NO:55 and a VL domain of SEQ ID NO:56; a VH domain of SEQ ID NO:55 and a VL domain of SEQ ID NO:58; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:56; a VH domain of SEQ ID NO:9 and a VL domain of SEQ ID NO:60; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:62; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID

NO:64; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:65; a VH domain of SEQ ID NO:67 and a VL domain of SEQ ID NO:68 (*see Table 1 and 2, supra*) a VH domain of SEQ ID NO:70 and a VL domain of SEQ ID NO:71; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:74; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:11; or a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:76 (*see Table 1 and 2, supra*).

In another embodiment, a subject, preferably a human, is administered a first dose of a liquid formulation of the present invention comprising 30 mg/kg or less, 15 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less, or 0.5 mg/kg or less of an antibody or a fragment thereof which has increased *in vivo* half-lives and which immunospecifically bind to a RSV antigen with higher affinity and/or higher avidity than previously known antibodies (e.g., SYNAGIS<sup>®</sup>) for the treatment or amelioration of a RSV infection or one or more symptoms thereof in an amount effective to induce a serum titer of at least 1  $\mu$ g/ml, preferably at least 2  $\mu$ g/ml, at least 5  $\mu$ g/ml, at least 10  $\mu$ g/ml, at least 15  $\mu$ g/ml, at least 20  $\mu$ g/ml, or at least 25  $\mu$ g/ml 25 days (preferably 30, 35, or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the serum titer of said antibodies or antibody fragments is less than 30  $\mu$ g/ml 30 days after the administration of the first dose and prior to the administration of a subsequent dose.

Preferably, the said antibodies have a VH domain of SEQ ID NO:9 and a VL domain of SEQ ID NO:13; a VH domain of SEQ ID NO:17 and a VL domain of SEQ ID NO:21; a VH domain of SEQ ID NO:24 and a VL domain of SEQ ID NO:26; a VH domain of SEQ ID NO:28 and a VL domain of SEQ ID NO:30; a VH domain of SEQ ID NO:33 and a VL domain of SEQ ID NO:34; a VH domain of SEQ ID NO:36 and a VL domain of SEQ ID NO:38; a VH domain of SEQ ID NO:40 and a VL domain of SEQ ID NO:42; a VH domain of SEQ ID NO:44 and a VL domain of SEQ ID NO:46; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:49; a VH domain of SEQ ID NO:51 and a VL domain of SEQ ID NO:52; a VH domain of SEQ ID NO:7 and a VL domain of SEQ ID NO:54; a VH domain of SEQ ID NO:55 and a VL domain of SEQ ID NO:56; a VH domain of SEQ ID NO:55 and a VL domain of SEQ ID NO:58; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:56; a VH domain of SEQ ID NO:9 and a VL domain of SEQ ID NO:60; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:62; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:64; a VH domain of SEQ ID NO:78

and a VL domain of SEQ ID NO:65; a VH domain of SEQ ID NO:67 and a VL domain of SEQ ID NO:68 (*see Table 1 and 2, supra*) a VH domain of SEQ ID NO:70 and a VL domain of SEQ ID NO:71; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:74; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:11; or a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:76 (*see Table 1 and 2, supra*).

In another embodiment, a subject, preferably a human, is administered a first dose of a liquid formulation of the present invention comprising approximately 30 mg/kg or less, 15 mg/kg or less (preferably 10 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less, or 0.5 mg/kg or less) of an antibody or a fragment thereof which has increased *in vivo* half-life for the prevention, treatment or amelioration of a RSV infection or one or more symptoms thereof in an amount effective to induce a serum titer of at least 1  $\mu$ g/ml, preferably at least 30  $\mu$ g/ml, at least 35  $\mu$ g/ml, at least 40  $\mu$ g/ml, or at least 50  $\mu$ g/ml 25 days (preferably 30, 35, or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose.

The present invention encompasses liquid formulations for pulmonary delivery comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens. Preferably, such antibodies and antibody fragments have a higher affinity and/or a higher avidity than previously known antibodies (*e.g.*, SYNAGIS<sup>®</sup>).

In one embodiment, a subject, preferably a human, is administered a first dose of a liquid formulation of the present invention for pulmonary delivery comprising 30 mg/kg or less, 15 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less, 0.5 mg/kg or less, or 0.01 mg/kg or less of an antibody or a fragment thereof that immunospecifically binds to a RSV antigen and, preferably, has higher affinity and/or higher avidity than previously known antibodies (*e.g.*, SYNAGIS<sup>®</sup>) for the prevention, treatment or amelioration of a RSV infection or a symptom thereof in an amount effective to induce a titer of at least 20 ng per mg of lung protein (preferably at least 40 ng/mg, at least 60 ng/mg, at least 80 ng/mg, at least 50 ng/mg, at least 75 ng/mg, at least 100 ng/mg, or at least 150 ng/mg) in an intubation sample or lavage from the lungs of said mammal 20 days (preferably 25, 30, 35, or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the serum titer of said antibodies or antibody fragments is less than 100 ng/ml of protein 30 days after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, said antibodies have a VH domain of SEQ

ID NO:9 and a VL domain of SEQ ID NO:13; a VH domain of SEQ ID NO:17 and a VL domain of SEQ ID NO:21; a VH domain of SEQ ID NO:24 and a VL domain of SEQ ID NO:26; a VH domain of SEQ ID NO:28 and a VL domain of SEQ ID NO:30; a VH domain of SEQ ID NO:33 and a VL domain of SEQ ID NO:34; a VH domain of SEQ ID NO:36 and a VL domain of SEQ ID NO:38; a VH domain of SEQ ID NO:40 and a VL domain of SEQ ID NO:42; a VH domain of SEQ ID NO:44 and a VL domain of SEQ ID NO:46; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:49; a VH domain of SEQ ID NO:51 and a VL domain of SEQ ID NO:52; a VH domain of SEQ ID NO:7 and a VL domain of SEQ ID NO:54; a VH domain of SEQ ID NO:55 and a VL domain of SEQ ID NO:56; a VH domain of SEQ ID NO:55 and a VL domain of SEQ ID NO:58; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:56; a VH domain of SEQ ID NO:9 and a VL domain of SEQ ID NO:60; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:62; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:64; a VH domain of SEQ ID NO:78 and a VL domain of SEQ ID NO:65; a VH domain of SEQ ID NO:67 and a VL domain of SEQ ID NO:68 (*see Table 1 and 2, supra*) a VH domain of SEQ ID NO:70 and a VL domain of SEQ ID NO:71; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:74; a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:11; or a VH domain of SEQ ID NO:48 and a VL domain of SEQ ID NO:76 (*see Table 1 and 2, supra*).

The present invention encompasses liquid formulations of the present invention for pulmonary delivery comprising an antibody or a fragment thereof which has increased *in vivo* half-life and which immunospecifically binds to a RSV antigen and, preferably, has a higher affinity and/or a higher avidity than previously known antibodies (e.g., SYNAGIS®).

In another embodiment, a subject, preferably a human, is administered a first dose of a liquid formulation of the present invention 10 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less, or 0.5 mg/kg or less of an antibody or a fragment thereof for the prevention treatment or amelioration of a RSV infection or one or more symptoms thereof in an amount effective to induce a serum titer of at least 35 µg/ml, at least 40 µg/ml, at least 50 µg/ml, at least 80 µg/ml, at least 100 µg/ml, at least 120 µg/ml, at least 150 µg/ml, at least 200 µg/ml, at least 250 µg/ml, or at least 300 µg/ml 20 days (preferably 25, 30, 35 or 40 days) after the administration of the first dose. In another embodiment, a mammal, preferably a human, is administered a first dose of a liquid formulation of the present

invention comprising approximately 15 mg/kg of an antibody or a fragment thereof for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a serum titer of at least 100  $\mu$ g/ml, at least 125 :g/ml, at least 150  $\mu$ g/ml, at least 200  $\mu$ g/ml, at least 250  $\mu$ g/ml, at least 300  $\mu$ g/ml, at least 350  $\mu$ g/ml, at least 400  $\mu$ g/ml, or at least 450 :g/ml 20 days (preferably 25, 30, 35 or 40 days) after the administration of the first dose. The term "approximately 15 mg/kg" as used herein refers to a range of between 14 mg/kg and 16 mg/kg.

In another embodiment, a subject, preferably a human, is administered a dose of a liquid formulation of the present invention comprising an antibody or a fragment thereof that immunospecifically binds to a RSV antigen for the prevention a RSV infection in an amount effective to induce a prophylactically effective serum titer of 20  $\mu$ g/ml or less, 15  $\mu$ g/ml or less, 10  $\mu$ g/ml or less, 8  $\mu$ g/ml or less, 5  $\mu$ g/ml or less, 3  $\mu$ g/ml or less, 1  $\mu$ g/ml or less, or 0.5  $\mu$ g/ml or less 30 days after the administration of the dose, wherein said prophylactically effective serum titer is the serum titer that reduces the incidence of RSV infection in the human or the serum titer in a cotton rat that results in a RSV titer 5 days after challenge with  $10^5$  pfu RSV that is 99% lower than the RSV titer in the cotton rat 5 days after challenge with  $10^5$  pfu of RSV in a cotton rat not administered the dose prior to challenge. Preferably, the dose of the therapeutic or pharmaceutical composition comprises 15 mg/kg or less, 10 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less, 0.5 mg/kg or less of an antibody or a fragment thereof.

In yet another embodiment, a subject, preferably a human, is administered a dose of a liquid formulation of the present invention comprising an antibody or a fragment thereof that immunospecifically binds to a RSV antigen for the treatment or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a therapeutically effective serum titer of 25  $\mu$ g/ml or less, 20  $\mu$ g/ml or less, 15  $\mu$ g/ml or less, 10  $\mu$ g/ml or less, 8  $\mu$ g/ml or less, 5  $\mu$ g/ml or less, 3  $\mu$ g/ml or less, 1  $\mu$ g/ml or less, or 0.5  $\mu$ g/ml or less 30 days after the administration of the dose, wherein said therapeutically effective serum titer is the serum titer that reduces the severity or length of RSV infection or is the serum titer in a cotton rat that results in a RSV titer in the rat 5 days after challenge with  $10^5$  pfu RSV that is 99% lower than the RSV titer 5 days after challenge with  $10^5$  pfu of RSV in a cotton rat not administered the dose prior to challenge. Preferably, the dose of the liquid formulation of the present invention comprises 15 mg/kg or less, 12 mg/kg or

less, 10 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less, 0.5 mg/kg or less of an antibody or a fragment thereof.

In a specific embodiment, formulations of the present invention are administered once a month just prior to or during the RSV season. In another embodiment, the formulations are administered every two months just prior to or during the RSV season. In yet another embodiment, the stable formulations of the present invention are administered once just prior to or during the RSV season. The term "RSV season" refers to the season when RSV infection is most likely to occur. Typically, the RSV season in the northern hemisphere commences in November and lasts through April.

In one embodiment, approximately 5 mg/kg or less (preferably 1.5 mg/kg or less) of an antibody or fragment thereof, in the liquid formulations of the present invention, which immunospecifically binds to a RSV antigen with a higher avidity and/or higher affinity than previously known antibodies such as, *e.g.*, SYNAGIS®, is administered five times, 3 times, or 1 to 2 times during a RSV season to a mammal, preferably a human. In another embodiment, approximately 1.5 mg/kg of an antibody or a fragment thereof, in the formulations of the present invention, which immunospecifically binds to a RSV antigen with a higher avidity and/or a higher affinity than known antibodies such as, *e.g.*, SYNAGIS®, is administered monthly five times during a RSV season to a mammal, preferably a human, intramuscularly. In another embodiment, 3 mg/kg of an antibody or a fragment thereof in the present formulation which immunospecifically binds to a RSV antigen with a higher avidity and/or a higher affinity than known antibodies such as, *e.g.*, SYNAGIS® is administered monthly three times during a RSV season to a mammal, preferably a human, intramuscularly. In yet another embodiment, 5 mg/kg of an antibody or a fragment thereof in the formulation which immunospecifically binds to a RSV antigen with a higher avidity and/or a higher affinity than known antibodies such as, *e.g.*, SYNAGIS® is administered monthly one to two times during a RSV season to a mammal, preferably a human, intramuscularly.

In a specific embodiment, 15 mg/kg of anti-RSV antibodies or an antigen-binding fragment thereof in the liquid formulation of the present invention is administered to a mammal, preferably a human, intramuscularly five times during a RSV season, wherein said antibodies or an antibody fragment has an increased *in vivo* half-life. In another embodiment, approximately 5 mg/kg or less (preferably 1.5 mg/kg or less) of an antibody or

fragment thereof in the liquid formulation of the present invention which immunospecifically binds to a RSV antigen with a higher avidity and/or higher affinity than previously known antibodies such as, *e.g.*, SYNAGIS®, is administered five times, 3 times, or 1 to 2 times during a RSV season to a mammal, preferably a human. In another embodiment, 3 mg/kg of antibody or a fragment thereof in the liquid formulation of the present invention which immunospecifically binds to a RSV antigen with a higher avidity and/or a higher affinity known antibodies such as, *e.g.*, SYNAGIS® and which has an increased *in vivo* half-life is administered monthly three times during a RSV season to a mammal, preferably a human, intramuscularly. In another embodiment, 5 mg/kg of antibody or a fragment thereof in the liquid formulation of the present invention which immunospecifically binds to a RSV antigen with a higher avidity and/or a higher affinity than known antibodies such as, *e.g.*, SYNAGIS® and which has an increased *in vivo* half-life is administered to a mammal, preferably a human, intramuscularly twice times during a RSV season.

## 5.7 Biological Assays

### 5.7.1 Immunospecificity of the Antibodies of the Invention

Antibodies of the present invention or fragments thereof may be characterized in a variety of ways well-known to one of skill in the art. In particular, antibodies of the invention or fragments thereof may be assayed for the ability to immunospecifically bind to an epitope of a respiratory syncytial virus. Such an assay may be performed in solution (*e.g.*, Houghten, 1992, Bio/Techniques 13:412-421), on beads (Lam, 1991, Nature 354:82-84), on chips (Fodor, 1993, Nature 364:555-556), on bacteria (U.S. Patent No. 5,223,409), on spores (U.S. Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (Cull *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:1865-1869) or on phage (Scott and Smith, 1990, Science 249:386-390; Cwirla *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:6378-6382; and Felici, 1991, J. Mol. Biol. 222:301-310) (each of these references is incorporated herein in its entirety by reference). An antibody of the invention or a fragment thereof in a liquid formulation of the present invention can be assayed for its specificity and affinity.

An antibody or a fragment thereof of the present invention may be assayed for immunospecific binding to a RSV antigen and cross-reactivity with other antigens by any method known in the art. Immunoassays which can be used to analyze immunospecific

binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), “sandwich” immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well-known in the art (see, *e.g.*, Ausubel *et al.*, eds., 1994, *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety).

### **5.7.2 *In Vitro* and *In Vivo* Assays**

An antibody or a fragment thereof, the liquid formulations of the invention, or a combination therapy of the present invention can be tested *in vitro* and/or *in vivo* in various assays or suitable animal model systems for its activity.

A liquid formulation of the present invention for treating, managing, preventing, or ameliorating a RSV infection or one or more symptoms thereof can be tested for its ability to inhibit viral replication or reduce viral load in *in vitro* assays. For example, viral replication can be assayed by a plaque assay such as described, *e.g.*, by Johnson *et al.*, 1997, *Journal of Infectious Diseases* 176:1215-1224 176:1215-1224. A liquid formulation of the invention administered according to the methods of the invention can also be assayed for their ability to inhibit or down-regulate the expression of viral polypeptides. Techniques known to those of skill in the art, including, but not limited to, western blot analysis, northern blot analysis, and RT-PCR can be used to measure the expression of viral polypeptides and/or viral titers.

A liquid formulation of the invention can be tested in suitable animal model systems prior to use in humans. Such animal model systems include, but are not limited to, rats, mice, chicken, cows, monkeys, pigs, dogs, rabbits, etc. Any animal system well-known in the art may be used. Several aspects of the procedure may vary; said aspects include, but are not limited to, the temporal regime of administering the therapies (*e.g.*, prophylactic and/or therapeutic agents) whether such therapies are administered separately or as an admixture, and the frequency of administration of the therapies.

Animal models can be used to assess the efficacy of the methods of the invention for treating, managing, preventing, or ameliorating a RSV infection or one or more symptom

thereof. Animal models for RSV infection include, but are not limited to, those as described by, *e.g.*, Piedimonte *et al.*, Am J Physiol 1999, 277:L831-L840; McArthur-Vaughan *et al.*, J. Med. Primatol. 2002, 31(2):61-73; and Byrd *et al.*, Clin. Infect. Dis. 1997, 25(6):1363-8. In a specific embodiment, cotton rats are administered a liquid formulation comprising an antibody or a fragment thereof that immunospecifically binds to a RSV antigen according to the methods of the invention, challenged with  $10^5$  pfu of RSV, and four or more days later, the rats are sacrificed and RSV titer and anti-RSV antibody serum titer is determined. Accordingly, a dosage that results in a 2 log decrease or a 99% reduction in RSV titer in the cotton rat challenged with  $10^5$  pfu of RSV relative to the cotton rat challenged with  $10^5$  pfu of RSV but not administered the formulation is the dosage of the formulation that can be administered to a human for the treatment, prevention or amelioration of a RSV infection or one or more symptoms thereof. Further, this embodiment, the tissues (*e.g.*, the lung tissues) from the sacrificed rats can be examined for histological changes.

The administration of a liquid formulation of the invention according to the methods of the present invention can be tested for its ability to decrease the time course of a RSV infection by at least 25%, preferably at least 50%, at least 60%, at least 75%, at least 85%, at least 95%, or at least 99% relative to a negative control. A liquid formulation of the invention can also be tested for its ability to increase the survival period of humans suffering from a RSV infection by at least 25%, preferably at least 50%, at least 60%, at least 75%, at least 85%, at least 95%, or at least 99% relative to a negative control. Further, a liquid formulation of the invention can be tested for its ability to reduce the hospitalization period of a human suffering from RSV infection by at least 60%, preferably at least 75%, at least 85%, at least 95%, or at least 99% relative to a negative control. Techniques known to those of skill in the art can be used to analyze the function of a liquid formulation of the invention *in vivo*.

Further, any *in vitro* or *in vivo* assays known to those skilled in the art can be used to evaluate the prophylactic and/or therapeutic utility of a liquid formulation of the invention disclosed herein for a RSV infection or one or more symptoms thereof.

### 5.7.3 Toxicity Assays

The toxicity and/or efficacy of the prophylactic and/or therapeutic protocols of the instant invention can be determined by standard pharmaceutical procedures in cell cultures

or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Therapies that exhibit large therapeutic indices are preferred. While therapies that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage of the prophylactic and/or therapeutic agents for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any therapy used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

### 5.8 Kits

The invention provides a pharmaceutical pack or kit comprising one or more containers filled with a liquid formulation of the invention for the prevention, treatment, management or amelioration of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection. The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with a liquid formulation of the invention for the detection, diagnosis or monitoring of a RSV infection. In a specific embodiment, the liquid formulations of the invention comprise an antibody or a fragment thereof that immunospecifically binds to a RSV antigen recombinantly fused or chemically conjugated to another moiety, including but not limited to, a heterologous protein, a heterologous polypeptide, a heterologous peptide, a large molecule, a small molecule, a marker sequence, a diagnostic or detectable agent, a

therapeutic moiety, a drug moiety, a radioactive metal ion, a second antibody, and a solid support.

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises a liquid formulation of the invention, in one or more containers. In another embodiment, a kit comprises a liquid formulation of the invention, in one or more containers, and one or more other prophylactic or therapeutic agents useful for the prevention, management or treatment of a RSV infection, one or more symptoms thereof, or a respiratory condition associated with, potentiated by or potentiating a RSV infection in one or more other containers. Preferably, the kit further comprises instructions for preventing, treating, managing or ameliorating a RSV infection (e.g., using the liquid formulations of the invention alone or in combination with another prophylactic or therapeutic agent), as well as side effects and dosage information for method of administration. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

### **5.9 Use of Liquid Formulations in the Diagnosis of RSV Infection**

The liquid formulations of the invention comprising labeled antibodies or fragments, derivatives and analogues thereof that immunospecifically bind to a RSV antigen can be used for diagnostic purposes to detect, diagnose, or monitor a RSV infection. Such diagnostic techniques are known in the art, including but not limited to, those disclosed in International Publication No. WO 01/58483, U.S. Patent No. 6,248,326, Pecheur et al., The FASEB J. 16(10):1266-8 (2002), Almed et al., The Journal of Histochemistry & Cytochemistry 50:1371-1379 (2002), all of which are incorporated herein by reference. In a preferred embodiment, antibodies which immunospecifically bind to a RSV antigen are used for diagnostic purposes to detect, diagnosis, or monitor a RSV infection. The detection or diagnosis of a infection can be conducted utilizing an effective amount (i.e., an amount effective to be able to detect the expression of a RSV antigen) of a liquid formulation of the invention in an *in vitro* assay using techniques well-known to one of skilled in the art, including but not limited to, assaying a sample taken from a subject,

wherein such sample can be, but not limited to, secretions from a subject's respiratory tract (e.g., sputum and saliva) and blood.

The liquid formulations of the present invention can be used in any *in vitro* immunoassay known in the art, such as ELISA, to detect, diagnose or monitor a RSV infection. In specific embodiments, the invention provides methods of detecting, diagnosing or monitoring a RSV infection, said methods comprising: a) combining an effective amount of a liquid formulation of the invention comprising a labeled antibody or antibody fragment that immunospecifically binds to a RSV antigen with a sample from a subject; b) waiting for a time interval to permit the labeled antibody or antibody fragment to preferentially bind to a RSV antigen if present in the sample; c) removing the unbound antibodies from the sample, and d) detecting the labeled antibody or antibody fragment in the sample. In specific embodiments, an antibody or fragment of the liquid formulations of the invention is not labeled, and a second labeled antibody or antibody fragment that recognizes the antibody or fragment of the liquid formulation of the invention can be used.

In some embodiments, monitoring of an infection is carried out by repeating the method for diagnosing the infection, for example, one month after initial diagnosis, six month after initial diagnosis, and one year after initial diagnosis.

The liquid formulations of the invention can also be administered to a subject to detect, diagnose or monitor a RSV infection.

## 6. EXAMPLES

### Stability Study

An antibody formulation of the present invention comprising, in an aqueous carrier, 25 mM of histidine, 1.6 mM of glycine, and an anti-RSV antibody is prepared according to the following protocol:

For a 1 kg solution of buffer: In 800 g water, 3.875 g histidine (free base) and 0.12 g glycine are dissolved. The pH is adjusted with 6 N HCl to  $6.0 \pm 0.2$ . Water is added to bring the total mass up to 1.0 kg (qs).

For the difiltration: After the chromatography steps in the purification process, the antibody is concentrated to  $150 \pm 15$  g/L. The concentrated product is difiltered into formulation buffer. The formulated product is diluted to a target manufacturing concentration of  $103 \pm 3$  g/L.

For a stability study, two formulations are prepared: one contains 105 mg/ml of the antibody and the other contained 160 mg/ml of the antibody. The stability of each formulation is measured using HPSEC in terms of degrees of aggregate formation and fragmentation during the storage at 2-8°C for up to 15 months and at 38-42°C for up to 1 year. For the HPSEC analysis, typically, Tosohas G3000WXL column with a mobile phase containing 0.1 M sodium phosphate and 0.1 M sodium sulfate, pH 6.8, is used at a flow rate of 0.8 ml/min. A sample containing 250 mg of protein in an appropriate volume is injected into the column and protein peaks are detected by 280 nm UV and/or fluorescence (280 nm excitation and 340 nm emission).

#### Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

Citation or discussion of a reference herein shall not be construed as an admission that such is prior art to the present invention.

## CLAIMS

What is claimed is:

1. A liquid antibody formulation comprising: (a) at least 15 mg/ml of an antibody, or a fragment thereof that immunospecifically binds to a RSV antigen; and (b) histidine in an aqueous carrier, wherein said formulation is substantially free of surfactant, inorganic salts or other excipients, and wherein said antibody or antibody fragment is not SYNAGIS® or a fragment thereof.
2. A liquid antibody formulation comprising:
  - (a) at least 15 mg/ml of one or more antibodies or fragments thereof that immunospecifically binds to a RSV antigen; and
  - (b) histidine in an aqueous carrier, wherein the formulation is substantially free of surfactant, inorganic salts or other excipients, and wherein at least one of said antibodies or antibody fragments is SYNAGIS® or a fragment thereof.
3. The formulation of claim 1 or 2, wherein the aqueous carrier is distilled water.
4. The formulation of claim 1, wherein the formulation is sterile.
5. The formulation of claim 1, wherein the formulation is homogenous.
6. The formulation of claim 1 or 2, wherein the formulation has a pH in the range between about 5.5 to 6.5.
7. The formulation of claim 1, wherein the antibody or antibody fragment is at a concentration of at least 100 mg/ml.
8. The formulation of claim 1, wherein the antibody or antibody fragment is at a concentration of at least 110 mg/ml.
9. The formulation of claim 8, wherein the antibody or antibody fragment is at a concentration of at least 150 mg/ml.

10. The formulation of claim 9, wherein the antibody or antibody fragment is at a concentration of at least 160 mg/ml.

11. The formulation of claim 1 or 2, wherein histidine is at concentration of about 10 to about 30 mM.

12. The formulation of claim 1 or 2 further comprising glycine.

13. The formulation of claim 12, wherein glycine is at a concentration of less than 3.0 mM.

14. The formulation of claim 1, wherein the antibody or antibody fragment that immunospecifically binds to a RSV antigen is stable at 40°C for at least 100 days as determined by HPSEC.

15. The formulation of claim 1, wherein the antibody or antibody fragment that immunospecifically binds to a RSV antigen is stable at about ambient temperature for at least 1 year as determined by HPSEC.

16. The formulation of claim 1, wherein the antibody or antibody fragment that immunospecifically binds to a RSV antigen is stable at 4°C for at least 3 years as determined by HPSEC.

17. The formulation of claim 1, wherein the antibody or antibody fragment that immunospecifically binds to a RSV antigen is stable at 4°C for 3-5 years as determined by HPSEC.

18. The formulation of claim 1, wherein the antibody or antibody fragment that immunospecifically binds to a RSV antigen is stable at 4°C for at least 5 years as determined by HPSEC.

19. The formulation of claim 1, wherein less than 2% of the antibody or antibody fragment forms an aggregate as measured by HPSEC.

20. The formulation of claim 19, wherein less than 1% of the antibody or antibody fragment forms an aggregate as measured by HPSEC.

21. The formulation of claim 20, wherein less than 0.5% of the antibody or antibody fragment forms an aggregate as measured by HPSEC.
22. The formulation of claim 1 or 2 further comprising an excipient.
23. The formulation of claim 22, wherein the excipient is a saccharide.
24. The formulation of claim 23, wherein the saccharide is sucrose.
25. The formulation of claim 24, wherein sucrose is at a concentration of about 1% to about 20%.
26. The formulation of claim 22, wherein the excipient is a polyol other than mannitol.
27. The formulation of claim 26, wherein the polyol is polysorbate.
28. The formulation of claim 27, wherein Tween is at a concentration of about 0.001% to about 1%.
29. The formulation of claim 4, wherein the antibody is AFFF, p12f2, p12f4, p11d4, A1e109, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L215B10, A13A11, A1H5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S.
30. The formulation of claim 4, wherein the antibody or antibody fragment comprises a variable heavy (VH) domain having the amino acid sequence of SEQ ID NOs: 9, 17, 24, 28, 33, 36, 40, 44, 48, 51, 55, 67, 70, or 78.
31. The formulation of claim 4, wherein the antibody or antibody fragment comprises a variable light (VL) domain having the amino acid sequence of SEQ ID NOs: 11, 13, 21, 26, 30, 34, 38, 42, 46, 49, 52, 54, 56, 58, 60, 62, 64, 65, 68, 71, 74, or 76.
32. The formulation of claim 4, wherein the antibody is A4B4, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S.
33. The formulation of claim 4, wherein the antibody or antibody fragment comprises a variable heavy (VH) domain of SEQ ID NO:48.

34. The formulation of claim 4, wherein the antibody or antibody fragment comprises a variable light (VL) domain having the amino acid sequence of SEQ ID NO:11, 49, 74 or 76.

35. The formulation of claim 4, wherein the antibody or antibody fragment comprises a VH CDR or the antibody A4B4, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S.

36. The formulation of claim 4, wherein the antibody or antibody fragment comprises a VL CDR of the antibody A4B4, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S.

37. The formulation of claim 4, wherein the antibody or antibody fragment comprises a VHCDR1 having the amino acid sequence of SEQ ID NO:10, a VHCDR2 having the amino acid sequence of SEQ ID NO:19, and a VHCDR3 having the amino acid sequence of SEQ ID NO:20.

38. The formulation of claim 4, wherein the antibody or antibody fragment comprises a VLCDR1 having the amino acid sequence of SEQ ID NO:39, a VLCDR2 having the amino acid sequence of SEQ ID NO:5, and a VLCDR3 having the amino acid sequence of SEQ ID NO:6.

39. The formulation of claim 4, wherein the antibody or antibody fragment comprises a VLCDR1 having the amino acid sequence of SEQ ID NO:39, a VLCDR2 having the amino acid sequence of SEQ ID NO:77, and a VLCDR3 having the amino acid sequence of SEQ ID NO:6.

40. The formulation of claim 1 which has been prepared by a process in which, for each step of said process, said antibody or a fragment thereof is in an aqueous phase.

41. The formulation of claim 1 which has been prepared by a process that does not have a drying step.

42. The formulation of claim 1 which has been prepared by a process that does not have a lyophilization step.

43. A pharmaceutical unit dosage form suitable for parenteral administration to a human which comprises an antibody formulation of claim 1, 4, or 29 in a suitable container.

44. The pharmaceutical unit dosage form of claim 43, wherein said antibody or a fragment thereof has a concentration of from about 15 mg/ml to about 300 mg/ml in a volume of from 1 ml to 20 ml.

45. The pharmaceutical unit dosage form of claim 44, wherein said antibody or a fragment thereof has a concentration of 100 mg/ml in a volume of 1.2 ml.

46. The pharmaceutical unit dosage form of claim 43, wherein the antibody formulation is suitable for subcutaneous administration.

47. The pharmaceutical unit dosage form of claim 43, wherein the antibody formulation is suitable for intravenous administration.

48. The pharmaceutical unit dosage form of claim 43, wherein the antibody formulation is suitable for intramuscular administration.

49. A pharmaceutical unit dosage form suitable for aerosol administration to a human which comprises an antibody formulation of claim 1, 4, or 29 in a suitable container.

50. The pharmaceutical unit dosage form of claim 49, wherein said antibody or a fragment thereof has a concentration of from about 15 mg/ml to about 300 mg/ml in a volume of from 1.1 ml to 1.3 ml.

51. A sealed container comprising a formulation of claim 1, 3, 4, or 29.

52. A method of preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a subject, said method comprising administering a prophylactically or therapeutically effective amount of the formulation of claim 1, 4, 29, 32, 35 or 36.

53. The method of claim 52, wherein the formulation is administered parenterally.

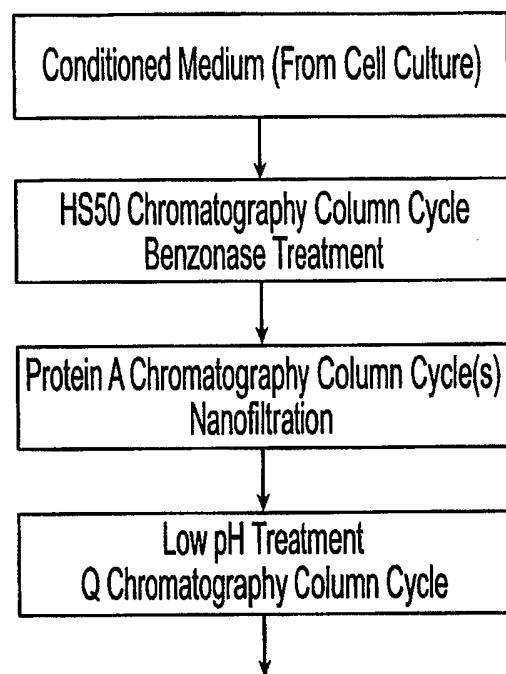
54. The method of claim 52, wherein the formulation is administered intramuscularly.

55. The method of claim 52, wherein the formulation is administered intravenously.

56. The method of claim 52, wherein the formulation is administered subcutaneously.

57. The method of claim 52, wherein the formulation is administered intranasally.

1/1



*Fig. 1*

## SEQUENCE LISTING

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<120> STABILIZED ANTI-RESPIRATORY SYNCYTIAL VIRUS (RSV) ANTIBODY FORMULATIONS

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Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
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Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> 27

<211> 7

<212> PRT

<213> Homo sapiens

<400> 27

Asp Thr Arg Gly Leu Pro Ser  
 1 5

<210> 28

<211> 120

<212> PRT

<213> Homo sapiens

<400> 28

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro  
 20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser  
 50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln  
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
 115 120

<210> 29

<211> 10

<212> PRT  
<213> Homo sapiens

<400> 29  
Asp Met Ile Phe Asn Trp Tyr Phe Asp Val  
1 5 10

<210> 30  
<211> 106  
<212> PRT  
<213> Homo sapiens

<400> 30  
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Pro Ser Ser Arg Val Gly Tyr Met  
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
35 40 45

Asp Thr Met Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
100 105

<210> 31  
<211> 10  
<212> PRT  
<213> Homo sapiens

<400> 31  
Ser Pro Ser Ser Arg Val Gly Tyr Met His  
1 5 10

<210> 32  
<211> 7  
<212> PRT  
<213> Homo sapiens

<400> 32  
Asp Thr Met Arg Leu Ala Ser  
1 5

<210> 33  
<211> 120  
<212> PRT  
<213> Homo sapiens

<400> 33  
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro  
 20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser  
 50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln  
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
 115 120

<210> 34

<211> 106

<212> PRT

<213> Homo sapiens

<400> 34

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Phe Lys Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> 35

<211> 7

<212> PRT

<213> Homo sapiens

<400> 35

Asp Thr Phe Lys Leu Ser Ser

1 5

<210> 36

<211> 120

<212> PRT

<213> Homo sapiens

<400> 36  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser  
 115 120

<210> 37  
 <211> 16  
 <212> PRT  
 <213> Homo sapiens

<400> 37  
 Asp Ile Trp Trp Asp Gly Lys Lys Asp Tyr Asn Pro Ser Leu Lys Asp  
 1 5 10 15

<210> 38  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 38  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Phe Lys Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> 39  
 <211> 10  
 <212> PRT

<213> Homo sapiens

<400> 39  
 Ser Ala Ser Ser Arg Val Gly Tyr Met His  
 1 5 10

<210> 40  
 <211> 120  
 <212> PRT

<213> Homo sapiens

<400> 40  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser  
 50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
 115 120

<210> 41  
 <211> 16  
 <212> PRT

<213> Homo sapiens

<400> 41  
 Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser Leu Lys Asp  
 1 5 10 15

<210> 42  
 <211> 106  
 <212> PRT

<213> Homo sapiens

<400> 42  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Met Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> 43

<211> 7

<212> PRT

<213> Homo sapiens

<400> 43

Asp Thr Met Tyr Gln Ser Ser  
 1 5

<210> 44

<211> 120

<212> PRT

<213> Homo sapiens

<400> 44

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser  
 50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
 115 120

<210> 45

<211> 16

<212> PRT

<213> Homo sapiens

<400> 45  
 Asp Ile Trp Trp Asp Asp Lys Lys Ser Tyr Asn Pro Ser Leu Lys Asp  
 1 5 10 15

<210> 46  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 46  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Leu Pro Ser Ser Arg Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Met Tyr Gln Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Phe Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 47  
 <211> 10  
 <212> PRT  
 <213> Homo sapiens

<400> 47  
 Leu Pro Ser Ser Arg Val Gly Tyr Met His  
 1 5 10

<210> 48  
 <211> 120  
 <212> PRT  
 <213> Homo sapiens

<400> 48  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser  
 50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
 115 120

<210> 49  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 49  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Phe Phe Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 50  
 <211> 7  
 <212> PRT  
 <213> Homo sapiens

<400> 50  
 Asp Thr Phe Phe Leu Asp Ser  
 1 5

<210> 51  
 <211> 120  
 <212> PRT  
 <213> Homo sapiens

<400> 51  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Ser Tyr Asn Pro Ser  
 50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln  
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
 115 120

<210> 52

<211> 106

<212> PRT

<213> Homo sapiens

<400> 52

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Arg Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 53

<211> 7

<212> PRT

<213> Homo sapiens

<400> 53

Asp Thr Arg Tyr Gln Ser Ser  
 1 5

<210> 54

<211> 106

<212> PRT

<213> Homo sapiens

<220>

<221> misc\_feature

<223> VL Domain

<400> 54

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 55

<211> 120

<212> PRT

<213> Homo sapiens

<400> 55  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Ala  
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
 115 120

<210> 56

<211> 106

<212> PRT

<213> Homo sapiens

<220>

<221> misc\_feature

<223> VL Domain

<400> 56  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 57

<211> 106

<212> PRT

<213> Homo sapiens

<220>

<221> misc\_feature

<223> VL Domain

<400> 57

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Tyr Lys Gln Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 58

<211> 7

<212> PRT

<213> Homo sapiens

<400> 58

Asp Thr Tyr Lys Gln Thr Ser  
 1 5

<210> 59  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> VL Domain

<400> 59  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Arg Tyr Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 60  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 60  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Phe Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 61  
 <211> 9

<212> PRT  
 <213> Homo sapiens

<400> 61  
 Phe Gln Gly Ser Phe Tyr Pro Phe Thr  
 1 5

<210> 62  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> VL Domain

<400> 62  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Phe Lys Leu Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 63  
 <211> 7  
 <212> PRT  
 <213> Homo sapiens

<400> 63  
 Asp Thr Phe Lys Leu Thr Ser  
 1 5

<210> 64  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> VL Domain

<400> 64  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met

20	25	30
His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr		
35	40	45
Asp Thr Phe Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser		
50	55	60
Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp		
65	70	75
Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr		
85	90	95
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys		
100	105	
<210> 65		
<211> 106		
<212> PRT		
<213> Homo sapiens		
<400> 65		
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly		
1	5	10
Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met		
20	25	30
His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr		
35	40	45
Asp Thr Phe Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser		
50	55	60
Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp		
65	70	75
Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr		
85	90	95
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys		
100	105	
<210> 66		
<211> 7		
<212> PRT		
<213> Homo sapiens		
<400> 66		
Asp Thr Phe Arg Leu Ala Ser		
1	5	
<210> 67		
<211> 120		
<212> PRT		
<213> Homo sapiens		
<400> 67		
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln		
1	5	10

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser  
 50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Ala  
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
 115 120

<210> 68

<211> 106

<212> PRT

<213> Homo sapiens

<400> 68  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Tyr Arg His Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 69

<211> 7

<212> PRT

<213> Homo sapiens

<400> 69

Asp Thr Tyr Arg His Ser Ser  
 1 5

<210> 70

<211> 106

<212> PRT

<213> Homo sapiens

<400> 70  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Tyr Lys Gln Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 71  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 71  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Phe His Arg Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 72  
 <211> 10  
 <212> PRT  
 <213> Homo sapiens

<400> 72  
 Ser Leu Ser Ser Ser Val Gly Tyr Met His  
 1 5 10

<210> 73

<211> 7  
 <212> PRT  
 <213> Homo sapiens

<400> 73  
 Asp Thr Phe Phe His Arg Ser  
 1 5

<210> 74  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 74  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Leu Leu Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 75  
 <211> 7  
 <212> PRT  
 <213> Homo sapiens

<400> 75  
 Asp Thr Leu Leu Leu Asp Ser  
 1 5

<210> 76  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 76  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

Asp Thr Ser Phe Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

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