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(54) Title: INTERFERON-ALPHA CONSTRUCTS FOR USE IN THE TREATMENT OF SARS

Sample	Compound ID	IC50	TC50	Units	SI	Start Conc (ng/ml)	Stock Conc (ug/ml)	Stock Units	Plate	Dilution Box Conc	ul of stock drug to add to COL 1	IC50 Sign	Relative IC50	TC50 Sign	Relative TC50
43	INF-alpha-n	1404.0 >	28000.0	U/ml >	18.5	1.3E+06	26000	U/ml	43	1E+05	12.0 =	5.40E-02 >	1.00E+00		
44	INF-alpha-o	3680.0 >	80000.0	U/ml >	21.7	4.0E+06	80000	U/ml	44	3E+05	12.0 =	4.80E-02 >	1.00E+00		
45	CM-1	240.0 >	320.0	U/ml >	1.3	16000	320	U/ml	45	1280	12.0 =	7.50E-01 >	1.00E+00		
46	CM-2	100.0 >	4000.0	U/ml >	40.0	200000	4000	U/ml	46	16000	12.0 =	2.50E-02 >	1.00E+00		
47	CM-3	298.0 >	1000.0	U/ml >	3.4	50000	1000	U/ml	47	4000	12.0 =	2.96E-01 >	1.00E+00		
48	CM-4	162.0 >	2000.0	U/ml >	12.3	100000	2000	U/ml	48	8000	12.0 =	8.10E-02 >	1.00E+00		
50	HY-1	184.0 >	800.0	U/ml >	4.3	40000	800	U/ml	50	3200	12.0 =	2.30E-01 >	1.00E+00		
51	HY-2	83.5 >	500.0	U/ml >	6.0	25000	500	U/ml	51	2000	12.0 =	1.67E-01 >	1.00E+00		
52	HY-4	448.0 =	740.0	U/ml =	1.7	100000	2000	U/ml	52	8000	12.0 =	2.24E-01 =	3.70E-01		
53	HY-5	15.8 >	70.0	U/ml >	4.4	3500	70	U/ml	53	280	12.0 =	2.25E-01 >	1.00E+00		
54	SDM-1	58.0 >	4000.0	U/ml >	71.4	200000	4000	U/ml	54	16000	12.0 =	1.40E-02 >	1.00E+00		
55	SDM-2	8.0 >	4000.0	U/ml >	600.0	200000	4000	U/ml	55	16000	12.0 =	2.00E-03 >	1.00E+00		
56	SDM-3	80.0 >	2000.0	U/ml >	25.0	100000	2000	U/ml	56	8000	12.0 =	4.00E-02 >	1.00E+00		
57	SDM-4	55.0 >	1000.0	U/ml >	18.2	50000	1000	U/ml	57	4000	12.0 =	5.50E-02 >	1.00E+00		

(57) Abstract: The invention relates to the production and use of novel interferon-alpha polypeptides for treating or preventing a SARS-associated coronaviral infection.

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INTERFERON-ALPHA CONSTRUCTS FOR USE
IN THE TREATMENT OF SARS

This application claims priority from U.S. Application Ser. No. 60/567,083 filed April 30, 2004, which is hereby incorporated by reference in its entirety.

Government Funding

The invention described herein was developed with support from the National Institutes of Health. The U.S. Government has certain rights in the invention.

Field of the Invention

The invention relates to production and characterization of novel interferon alpha polypeptides for treatment of respiratory diseases, for example, severe acute respiratory syndrome caused by the SARS coronavirus.

Background of the Invention

Severe acute respiratory syndrome (SARS) has rapidly spread from Southeast Asia to numerous countries, including Canada and the United States. A new coronavirus has been isolated and detected from many affected patients. The mortality rate worldwide is approximately 10.5%. From five cohorts, the intensive care unit admission rate ranged from 20% to 38%. Fifty-nine percent to 100% of the intensive care unit patients required mechanical ventilatory support. The mortality rate of SARS patients admitted to the intensive care unit ranged from 5% to 67%. The most common clinical symptoms and signs are fever, cough, dyspnea, myalgias, malaise, and inspiratory crackles. Common laboratory abnormalities included mild leukopenia, lymphopenia, and increased aspartate transaminase, alanine transaminase, lactic dehydrogenase, and creatine kinase. The chest radiograph pattern ranged from focal infiltrates to diffuse airspace disease. Management consisted of isolation, strict respiratory and contact precautions, ventilatory support as needed, empiric broad-spectrum antibiotics, ribavirin, and corticosteroids. Predictors of mortality included

advanced age, the presence of comorbidities, and a high lactic dehydrogenase or high neutrophil count at admission.

SARS is a highly contagious, infectious process that can advance to significant hypoxemic respiratory failure requiring intensive care unit
5 monitoring and support. Early recognition is critical for effective management and containment of this disease.

The mechanism of SARS pathogenesis may involve both direct viral cytotoxic effects on the target cells and immune-mediated mechanisms.

The life cycle of the SARS virus is largely unknown. Vaccines offer an
10 important preventive measure for possible future recurrences of SARS, but the prospect for their development is still unknown because of the uncertainty regarding the role of immune responses in SARS virus pathogenesis. Accordingly, new treatment methods are needed for SARS and related respiratory diseases.

15

Summary of the Invention

The invention describes the production and characterization of novel interferon alpha polypeptides and nucleic acids therefor, and provides a method for treating or preventing a SARS-associated coronaviral infection in a mammal.
20 The method involves administering to the mammal a therapeutically effective amount of an interferon-alpha, or a combination of interferon-alpha polypeptides. Interferon-beta and/or interferon-gamma can also be administered with the interferon-alpha polypeptides of the invention. Kits and compositions for treating SARS are also provided.

25 In one embodiment, the invention provides a method for treating or preventing a SARS-associated coronaviral infection in a mammal, which comprises administering to the mammal a therapeutically effective amount of a composition that includes interferon-alpha. In some embodiments, the interferon-alpha is any one of SEQ ID NO:1-14, or a combination thereof. In
30 other embodiments, the interferon-alpha is any one of SEQ ID NO:1-16, or a combination thereof. In further embodiments, the interferon-alpha is any one of SEQ ID NO:11-14, or a combination thereof. In still further embodiments, the interferon-alpha is any one of SEQ ID NO:7, 8, 9, 12, or a combination thereof. Moreover, according to the invention, inclusion of interferon-beta and/or

interferon-gamma in the interferon-alpha compositions administered to the mammal can be beneficial.

In another embodiment, the invention provides a method for treating or preventing a SARS-associated coronaviral infection in a mammal, which comprises administering to the mammal a therapeutically effective amount of a composition that includes an interferon-alpha having a therapeutic index of 5.0 or more. Examples of interferon-alpha polypeptides with a therapeutic index of 5.0 or more include those with SEQ ID NO:7, 8, 9, 12, or a combination thereof. Interferon-beta and/or interferon-gamma can be included in the interferon-alpha compositions administered to the mammal.

The invention further provides novel interferon-alpha polypeptides that have any one of SEQ ID NO:11-14.

Description of the Figure

FIG. 1A-B provides a chart illustrating the influence of interferon-alpha on SARS-associated coronaviral infection.

Detailed Description of the Invention

The invention provides methods for treating respiratory conditions and diseases such as severe acute respiratory syndrome by administering an effective amount of one or more interferon-alpha polypeptides. In particular, the invention provides a method for treating or preventing a SARS-associated coronaviral infection in a mammal, which comprises administering to the mammal a therapeutically effective amount of an interferon-alpha. Such an interferon-alpha preferably is active against infection of the SARS-associated coronavirus and gives rise to few adverse side effects. For example, the interferon-alpha can have a therapeutic index of 5.0 or more. Moreover, the invention provides novel interferon-alpha polypeptides, for example, interferon-alpha polypeptides having any one of SEQ ID NO:11-14. According to the invention, addition of interferon-beta and/or interferon-gamma to the interferon-alpha compositions administered to the mammal can be beneficial.

Definitions

An individual who is "at risk of being exposed" to a virus is an individual who may encounter the virus such that the virus infects the individual (i.e., virus enters cells and replicates). In the context of respiratory viruses that cause acute infection and resolution of infection and symptoms, the individual may or may not have previously been exposed to virus. Because respiratory viruses are ubiquitous, generally any individual is at risk for exposure to the virus. In some contexts, an individual is determined to be "at risk" because exposure to the virus has higher probability of leading to infection (such as with immunocompromised, elderly and/or very young children and infants), which can further result in serious symptoms, conditions, and/or complications. In some settings, including, but not limited to, institutions such as hospitals, schools, day care facilities, military facilities, nursing homes and convalescent homes, an individual is determined to be "at risk" because of time spent in close proximity to others who may be infected.

A "biological sample" encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom, and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as proteins or polynucleotides. The term "biological sample" encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples.

As used herein, "delaying" development of a viral infection or a symptom of viral infection means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease or symptom when compared to not using the method(s) of the invention. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease.

An "effective amount" or a "sufficient amount" of a substance is an amount sufficient to effect beneficial or desired results, including clinical results.

An effective amount can be administered in one or more administrations. A "therapeutically effective amount" is an amount to effect beneficial clinical results, including, but not limited to, alleviation of one or more symptoms associated with viral infection as well as prevention of disease (e.g., prevention
5 of one or more symptoms of infection).

"Exposure" to a virus denotes encounter with virus which allows infection, such as, for example, upon contact with an infected individual.

An "individual" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, humans, farm animals, sport
10 animals, rodents, primates and certain pets. Vertebrates also include, but are not limited to, birds (i.e., avian individuals) and reptiles (i.e., reptilian individuals).

The term "infected individual", as used herein, refers to an individual who has been infected by a respiratory virus, for example, SARS coronavirus. Symptoms of respiratory virus infection are well known in the art and have been
15 described herein.

"Palliating" a disease or one or more symptoms of a disease or infection means lessening the extent and/or time course of undesirable clinical manifestations of a disease state or infection in an individual or population of individuals treated with interferon-alpha in accordance with the invention.

20 "Preventing a symptom of infection" by a respiratory virus means that the symptom does not appear after exposure to the virus. Examples of symptoms have been described above.

"Reducing duration of viral infection" means the length of time of viral infection (usually indicated by symptoms) is reduced, or shortened, as compared
25 to not administering interferon-alpha.

"Reducing severity of a symptom" or "ameliorating a symptom" of viral infection means a lessening or improvement of one or more symptoms of viral infection as compared to not administering interferon-alpha. "Reducing severity" also includes shortening or reduction in duration of a symptom. For respiratory
30 viruses like SARS, these symptoms are well known in the art and include, but are not limited to, inflammation of respiratory mucosa, fever, body aches, coughing, wheezing, sneezing, nasal discharge and chest pain.

An individual is "PCR-negative" for a virus if transcripts specific for the virus cannot be detected in blood or serum samples from the individual using

nucleic acid amplification methods standard in the art, such as polymerase chain reaction (PCR). Conversely, an individual is "PCR-positive" for a virus if transcripts specific for the virus can be detected in blood or serum samples from the individual using nucleic acid amplification methods standard in the art (e.g.,
5 PCR).

An individual is "seronegative" for a virus if antibodies specific to the virus cannot be detected in blood or serum samples from the individual using methods standard in the art, such as ELISA. Conversely, an individual is "seropositive" for a virus if antibodies specific for the virus can be detected in
10 blood or serum samples from the individual using methods standard in the art, such as ELISA. An individual is said to "seroconvert" for a virus when antibodies to the virus can be detected in blood or serum from an individual who was previously seronegative.

"Suppressing" viral infection indicates any aspect of viral infection, such
15 as viral replication, time course of infection, amount (titer) of virus, lesions, and/or one or more symptoms is curtailed, inhibited, or reduced (in terms of severity and/or duration) in an individual or a population of individuals treated with interferon-alpha in accordance with the invention as compared to an aspect of viral infection in an individual or a population of individuals not treated in
20 accordance with the invention. Reduction in viral titer includes, but is not limited to, elimination of the virus from an infected site or individual. Viral infection can be assessed by any means known in the art, including, but not limited to, measurement of virus particles, viral nucleic acid or viral antigens, detection of symptoms and detection and/or measurement of anti-virus antibodies. Anti-virus
25 antibodies are widely used to detect and monitor viral infection and generally are commercially available.

"Viral titer" is a term well known in the art and indicates the amount of virus in a given biological sample. "Viremia" is a term well-known in the art as the presence of virus in the blood stream and/or viral titer in a blood or serum
30 sample. Amount of virus are indicated by various measurements, including, but not limited to, amount of viral nucleic acid; presence of viral particles; replicating units (RU); plaque forming units (PFU). Generally, for fluid samples such as blood and urine, amount of virus is determined per unit fluid, such as milliliters. For solid samples such as tissue samples, amount of virus is

determined per weight unit, such as grams. Methods for determining amount of virus are known in the art and described herein.

SARS Virus

5 The virus responsible for severe acute respiratory syndrome (SARS) is a novel coronavirus, which may have an origin in wild animals such as civet cats in southern China. The SARS-associated coronavirus (SARS-CoV) is a member of the coronavirus family not observed previously in humans. Because its sequence differs from that of known human coronaviruses, SARS-CoV is
10 suspected to have crossed the species barrier between an animal host and humans. The SARS outbreak began in China's Guangdong Province, where approximately 1,500 probable cases were identified during November 2002-June 2003. Detection of SARS-like coronavirus has been reported previously in masked palm civets (sometimes called civet cats) and a raccoon dog for sale in a
15 live animal market in Shenzhen municipality.

 Investigations conducted by public health authorities in Guangdong Province were conducted that compared the seroprevalence of SARS-CoV IgG antibody in animal traders (i.e., workers in live animal markets) with that of persons in control groups. The results indicated that while 13% of the animal
20 traders had IgG antibody to SARS-CoV, none of these animal traders had SARS. In contrast, only 1%-3% of persons in three control groups had IgG antibody to SARS-CoV. These results provide indirect support for the hypothesis of an animal origin for SARS.

 The genome structure, gene expression pattern and protein profiles of the
25 SARS-CoV are similar to those of other coronaviruses. A joint study by Shenzhen CDC and Hong Kong University determined that the sequence of coronavirus isolated from masked palm civets is 99% identical to human SARS-CoV. Guan et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science (2003) 302:276-78. These
30 studies support the hypothesis that an animal reservoir exists for SARS-CoV or an antigenically related virus. However, the findings are not sufficient to identify either the natural reservoir for SARS-CoV or the animal(s) responsible for crossover to humans. The potential mutability of the coronavirus genome may pose problems in the control of future SARS outbreaks. -

Primary modes of SARS transmission probably are direct contact or droplet spread from a patient symptomatic with SARS; however, other routes of transmission might exist. Approximately 63% of Guangdong Province patients with clinically defined SARS had no known history of exposure to other SARS patients. This trend of unknown exposure also was observed in other areas. Therefore, the possibility of unrecognized sources of infection or infection from asymptomatic carriers of the virus cannot be excluded, although some patients might also have pneumonia caused by etiologies other than SARS-CoV.

10 Interferon

According to the invention, administration of interferon-alpha can ameliorate the symptoms of SARS. According to the invention, any interferon-alpha can be used in the methods of the invention. In some embodiments, human interferon-alpha is preferred.

Human interferon-alpha (HuIFN- α) is encoded by a family of about eighteen genes, where each gene encodes a single subtype of the HuIFN- α . Amino acid sequence identity among IFN- α subtypes is generally 75-99%. Zoon, K.C., Bekisz, J.B., Miller, D. Human Interferon-alpha Family: Protein Structure and Function in: Interferon Principles and Medical Application, eds. Baron, S. et al., pp. 95-105 (1992) ; Horisberger and Di Marco (1995) "Interferon-alpha hybrids," *Pharmac. Ther.* **55**:507-534; Zoon, K.C., Miller, D., Bekisz, J.B., Zur Nedden, D., Enterline, J.C., Nguyen, N.Y., Hu, R. Purification and Characterization of Multiple Components of Human Lymphoblastoid Interferon-alpha. *J. Biol. Chem.* **267**:15210-15216 (1992). Individual IFN- α subtypes can have different biological activities. For example, IFN- α 1 and IFN- α 2 have distinct target-cell specificities. IFN- α 2 shows high specific activity on bovine and human cells (similar to most HuIFN- α s), whereas IFN- α 1 shows high activity only on bovine cells.

Many types of interferon-alpha exist and can be used in the practice of the invention. For example, suitable interferon-alpha polypeptides include, but are not limited to, recombinant interferon alfa-2b, such as Intron-A interferon available from Schering Corporation, Kenilworth, N.J., recombinant interferon alfa-2a, such as Roferon interferon available from Hoffmann-La Roche, Nutley,

N.J., recombinant interferon alpha-2c, such as Berofer alpha 2 interferon available from Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, Conn., interferon alpha-n1, a purified blend of natural alpha interferons, such as Sumiferon available from Sumitomo, Japan or as Wellferon interferon alpha-ni
5 (INS) available from the Glaxo-Wellcome Ltd., London, Great Britain, or a consensus alpha interferon, such as those described in U.S. Pat. Nos. 4,897,471 and 4,695,623 (see, Examples 7, 8 or 9 thereof) and the product available from Amgen, Inc., Newbury Park, Calif., or interferon alfa-n3 a mixture of natural alpha interferons made by Interferon Sciences and available from the Purdue
10 Frederick Co., Norwalk, Conn., under the Alferon Tradename. The manufacture of interferon alpha 2b is described in U.S. Pat. No. 4,530,901.

Examples of nucleic acid and amino acid sequences for different types and species of interferon-alpha can be found in the art, for example, in the NCBI database. See website at ncbi.nlm.nih.gov.

15 Also, according to the invention, hybrid interferons are useful for treating SARS and can be used in the compositions and methods of the invention. Thus, the hybrid interferons described in U.S. Patent 6,685,933, which is incorporated in its entirety by reference herein, can be used in the practice of the present invention. Six of the hybrids provided by U.S. Patent 6,685,933 are termed
20 HY-1, HY-2, HY-3, HY-4, HY-5, and HY-6, and are composed as follows:

HY-1: IFN- α 21a(1-75)/IFN- α 2c(76-166);

HY-2: IFN- α 21a(1-95)/IFN- α 2c(96-166);

HY-3: IFN- α 2c(1-95)/IFN- α 21a(96-166);

HY-4: IFN- α -21a(1-75)/IFN- α 2c(76-81)/IFN- α 21a(82-95)/IFN- α 2c(96-
25 166);

HY-5: IFN- α -21a(1-75)/IFN- α 21a(76-81)/IFN- α 2c(82-95)/IFN- α 2c(96-166);

HY-6: IFN- α 21a(1-75)/IFN- α 2c(76-95)/IFN- α 21a(96-166).

This nomenclature indicates that HY-1 is comprised of amino acids 1-75 of IFN-
30 α 21a fused to amino acids 76-166 of IFN- α 2c; HY-2 is comprised of amino acids 1-95 of IFN- α 21a fused to amino acids 96-166 of IFN- α 2c; HY-3 is comprised of amino acids 1-95 of IFN- α 2c fused to amino acids 96-166 of IFN- α 21a; and so forth for the remaining mutants. HY-3 is 165 amino acids long due

to alignment numbering. In addition to being available in U.S. Patent 6,685,933, the sequences for several of these hybrid interferons are available at the ncbi.nlm.nih.gov website, including HY-1 (accession number AF085803), HY-2 (accession number AF085804), HY-3 (accession number AF085805). The

5 sequences for these interferon-alpha polypeptides are provided below.

HY-1 (SEQ ID NO:1):

```

      1   Cys Asp Leu Pro Gln Thr His Ser Leu Gly
      11  Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln
10     21  Met Gly Arg Ile Ser Pro Phe Ser Cys Leu
      31  Lys Asp Arg His Asp Phe Gly Phe Pro Gln
      41  Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys
      51  Ala Gln Ala Ile Ser Val Leu His Glu Met
      61  Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr
15     71  Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
      81  Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr
      91  Gln Gln Leu Asn Asp Leu Glu Ala Cys Val
     101  Ile Gln Gly Val Gly Val Thr Glu Thr Pro
     111  Leu Met Lys Glu Asp Ser Ile Leu Ala Val
20     121 Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr
     131  Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala
     141  Trp Glu Val Val Arg Ala Glu Ile Met Arg
     151  Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
     161  Ser Leu Arg Ser Lys Glu
25

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HY-2 (SEQ ID NO:2):

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      1   Cys Asp Leu Pro Gln Thr His Ser Leu Gly
      11  Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln
30     21  Met Gly Arg Ile Ser Pro Phe Ser Cys Leu
      31  Lys Asp Arg His Asp Phe Gly Phe Pro Gln
      41  Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys
      51  Ala Gln Ala Ile Ser Val Leu His Glu Met
      61  Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr
35     71  Lys Asp Ser Ser Ala Thr Trp Glu Gln Ser
      81  Leu Leu Glu Lys Phe Ser Thr Glu Leu Asn
      91  Gln Gln Leu Asn Asp Leu Glu Ala Cys Val
     101  Ile Gln Gly Val Gly Val Thr Glu Thr Pro
     111  Leu Met Lys Glu Asp Ser Ile Leu Ala Val
40     121 Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr
     131  Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala
     141  Trp Glu Val Val Arg Ala Glu Ile Met Arg
     151  Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
     161  Ser Leu Arg Ser Lys Glu
45

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HY-3 (SEQ ID NO:3):

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      1   Cys Asp Leu Pro Gln Thr His Ser Leu Gly

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	11	Ser	Arg	Arg	Thr	Leu	Met	Leu	Leu	Ala	Gln
	21	Met	Arg	Arg	Ile	Ser	Leu	Phe	Ser	Cys	Leu
	31	Lys	Asp	Arg	Arg	Asp	Phe	Gly	Phe	Pro	Gln
	41	Glu	Glu	Phe	Gly	Asn	Gln	Phe	Gln	Lys	Ala
5	51	Glu	Thr	Ile	Pro	Val	Leu	His	Glu	Met	Ile
	61	Gln	Gln	Ile	Phe	Asn	Leu	Phe	Ser	Thr	Lys
	71	Asp	Ser	Ser	Ala	Ala	Trp	Asp	Glu	Thr	Leu
	81	Leu	Asp	Lys	Phe	Tyr	Thr	Glu	Leu	Tyr	Gln
	91	Gln	Leu	Asn	Asp	Leu	Glu	Ala	Cys	Val	Ile
10	101	Gln	Glu	Val	Gly	Val	Glu	Glu	Thr	Pro	Leu
	111	Met	Asn	Val	Asp	Ser	Ile	Leu	Ala	Val	Lys
	121	Lys	Tyr	Phe	Gln	Arg	Ile	Thr	Leu	Tyr	Leu
	131	Thr	Glu	Lys	Lys	Tyr	Ser	Pro	Cys	Ala	Trp
	141	Glu	Val	Val	Arg	Ala	Glu	Ile	Met	Arg	Ser
15	151	Phe	Ser	Leu	Ser	Lys	Ile	Phe	Gln	Glu	Arg
	161	Leu	Arg	Arg	Lys	Glu					

HY-4 (SEQ ID NO:4):

20	1	Cys	Asp	Leu	Pro	Gln	Thr	His	Ser	Leu	Gly
	11	Asn	Arg	Arg	Ala	Leu	Ile	Leu	Leu	Ala	Gln
	21	Met	Gly	Arg	Ile	Ser	Pro	Phe	Ser	Cys	Leu
	31	Lys	Asp	Arg	His	Asp	Phe	Gly	Phe	Pro	Gln
	41	Glu	Glu	Phe	Asp	Gly	Asn	Gln	Phe	Gln	Lys
25	51	Ala	Gln	Ala	Ile	Ser	Val	Leu	His	Glu	Met
	61	Ile	Gln	Gln	Thr	Phe	Asn	Leu	Phe	Ser	Thr
	71	Lys	Asp	Ser	Ser	Ala	Ala	Trp	Asp	Glu	Thr
	81	Leu	Leu	Glu	Lys	Phe	Ser	Thr	Glu	Leu	Asn
	91	Gln	Gln	Leu	Asn	Asp	Leu	Glu	Ala	Cys	Val
30	101	Ile	Gln	Gly	Val	Gly	Val	Thr	Glu	Thr	Pro
	111	Leu	Met	Lys	Glu	Asp	Ser	Ile	Leu	Ala	Val
	121	Arg	Lys	Tyr	Phe	Gln	Arg	Ile	Thr	Leu	Tyr
	131	Leu	Lys	Glu	Lys	Lys	Tyr	Ser	Pro	Cys	Ala
	141	Trp	Glu	Val	Val	Arg	Ala	Glu	Ile	Met	Arg
35	151	Ser	Phe	Ser	Leu	Ser	Thr	Asn	Leu	Gln	Glu
	161	Ser	Leu	Arg	Ser	Lys	Glu				

HY-5 (SEQ ID NO:5):

40	1	Cys	Asp	Leu	Pro	Gln	Thr	His	Ser	Leu	Gly
	11	Asn	Arg	Arg	Ala	Leu	Ile	Leu	Leu	Ala	Gln
	21	Met	Gly	Arg	Ile	Ser	Pro	Phe	Ser	Cys	Leu
	31	Lys	Asp	Arg	His	Asp	Phe	Gly	Phe	Pro	Gln
	41	Glu	Glu	Phe	Asp	Gly	Asn	Gln	Phe	Gln	Lys
45	51	Ala	Gln	Ala	Ile	Ser	Val	Leu	His	Glu	Met
	61	Ile	Gln	Gln	Thr	Phe	Asn	Leu	Phe	Ser	Thr
	71	Lys	Asp	Ser	Ser	Ala	Thr	Trp	Glu	Gln	Ser
	81	Leu	Leu	Asp	Lys	Phe	Tyr	Thr	Glu	Leu	Tyr
	91	Gln	Gln	Leu	Asn	Asp	Leu	Glu	Ala	Cys	Val
50	101	Ile	Gln	Gly	Val	Gly	Val	Thr	Glu	Thr	Pro
	111	Leu	Met	Lys	Glu	Asp	Ser	Ile	Leu	Ala	Val

	121	Arg	Lys	Tyr	Phe	Gln	Arg	Ile	Thr	Leu	Tyr
	131	Leu	Lys	Glu	Lys	Lys	Tyr	Ser	Pro	Cys	Ala
	141	Trp	Glu	Val	Val	Arg	Ala	Glu	Ile	Met	Arg
	151	Ser	Phe	Ser	Leu	Ser	Thr	Asn	Leu	Gln	Glu
5	161	Ser	Leu	Arg	Ser	Lys	Glu				

HY-6 (SEQ ID NO:6):

	1	Cys	Asp	Leu	Pro	Gln	Thr	His	Ser	Leu	Gly
10	11	Asn	Arg	Arg	Ala	Leu	Ile	Leu	Leu	Ala	Gln
	21	Met	Gly	Arg	Ile	Ser	Pro	Phe	Ser	Cys	Leu
	31	Lys	Asp	Arg	His	Asp	Phe	Gly	Phe	Pro	Gln
	41	Glu	Glu	Phe	Asp	Gly	Asn	Gln	Phe	Gln	Lys
	51	Ala	Gln	Ala	Ile	Ser	Val	Leu	His	Glu	Met
15	61	Ile	Gln	Gln	Thr	Phe	Asn	Leu	Phe	Ser	Thr
	71	Lys	Asp	Ser	Ser	Ala	Ala	Trp	Asp	Glu	Thr
	81	Leu	Leu	Asp	Lys	Phe	Tyr	Thr	Glu	Leu	Tyr
	91	Gln	Gln	Leu	Asn	Asp	Leu	Glu	Ala	Cys	Val
	101	Ile	Gln	Glu	Val	Gly	Val	Glu	Glu	Thr	Pro
20	111	Leu	Met	Asn	Val	Asp	Ser	Ile	Leu	Ala	Val
	121	Lys	Lys	Tyr	Phe	Gln	Arg	Ile	Thr	Leu	Tyr
	131	Leu	Thr	Glu	Lys	Lys	Tyr	Ser	Pro	Cys	Ala
	141	Trp	Glu	Val	Val	Arg	Ala	Glu	Ile	Met	Arg
	151	Ser	Phe	Ser	Leu	Ser	Lys	Ile	Phe	Gln	Glu
25	161	Arg	Leu	Arg	Arg	Lys	Glu				

SDM-1 (SEQ ID NO:7):

	1	Cys	Asp	Leu	Pro	Gln	Thr	His	Ser	Leu	Gly
30	11	Asn	Arg	Arg	Ala	Leu	Ile	Leu	Leu	Ala	Gln
	21	Met	Gly	Arg	Ile	Ser	Pro	Phe	Ser	Cys	Leu
	31	Lys	Asp	Arg	His	Asp	Phe	Gly	Phe	Pro	Gln
	41	Glu	Glu	Phe	Asp	Gly	Asn	Gln	Phe	Gln	Lys
	51	Ala	Gln	Ala	Ile	Ser	Val	Leu	His	Glu	Met
35	61	Ile	Gln	Gln	Thr	Phe	Asn	Leu	Phe	Ser	Thr
	71	Lys	Asp	Ser	Ser	Ala	Ala	Trp	Asp	Glu	Thr
	81	Leu	Leu	Glu	Lys	Phe	Tyr	Thr	Glu	Leu	Asn
	91	Gln	Gln	Leu	Asn	Asp	Leu	Glu	Ala	Cys	Val
	101	Ile	Gln	Gly	Val	Gly	Val	Thr	Glu	Thr	Pro
40	111	Leu	Met	Lys	Glu	Asp	Ser	Ile	Leu	Ala	Val
	121	Arg	Lys	Tyr	Phe	Gln	Arg	Ile	Thr	Leu	Tyr
	131	Leu	Lys	Glu	Lys	Lys	Tyr	Ser	Pro	Cys	Ala
	141	Trp	Glu	Val	Val	Arg	Ala	Glu	Ile	Met	Arg
	151	Ser	Phe	Ser	Leu	Ser	Thr	Asn	Leu	Gln	Glu
45	161	Ser	Leu	Arg	Ser	Lys	Glu				

SDM-2 (SEQ ID NO:8):

	1	Cys	Asp	Leu	Pro	Gln	Thr	His	Ser	Leu	Gly
50	11	Asn	Arg	Arg	Ala	Leu	Ile	Leu	Leu	Ala	Gln
	21	Met	Gly	Arg	Ile	Ser	Pro	Phe	Ser	Cys	Leu

31 Lys Asp Arg His Asp Phe Gly Phe Pro Gln
 41 Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys
 51 Ala Gln Ala Ile Ser Val Leu His Glu Met
 61 Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr
 5 71 Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
 81 Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr
 91 Gln Gln Leu Asn Asp Leu Glu Ala Cys Val
 101 Ile Gln Gly Val Gly Val Thr Glu Thr Pro
 111 Leu Met Lys Glu Asp Ser Ile Leu Ala Val
 10 121 Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr
 131 Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala
 141 Trp Glu Val Val Arg Ala Glu Ile Met Arg
 151 Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
 161 Ser Leu Arg Ser Lys Glu
 15

SDM-3 (SEQ ID NO:9):

1 Cys Asp Leu Pro Gln Thr His Ser Leu Gly
 11 Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln
 20 21 Met Gly Arg Ile Ser Pro Phe Ser Cys Leu
 31 Lys Asp Arg His Asp Phe Gly Phe Pro Gln
 41 Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys
 51 Ala Gln Ala Ile Ser Val Leu His Glu Met
 61 Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr
 25 71 Lys Asp Ser Ser Ala Thr Trp Glu Gln Ser
 81 Leu Leu Asp Lys Phe Ser Thr Glu Leu Tyr
 91 Gln Gln Leu Asn Asp Leu Glu Ala Cys Val
 101 Ile Gln Gly Val Gly Val Thr Glu Thr Pro
 111 Leu Met Lys Glu Asp Ser Ile Leu Ala Val
 30 121 Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr
 131 Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala
 141 Trp Glu Val Val Arg Ala Glu Ile Met Arg
 151 Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
 161 Ser Leu Arg Ser Lys Glu
 35

SDM-4 (SEQ ID NO:10):

1 Cys Asp Leu Pro Gln Thr His Ser Leu Gly
 11 Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln
 40 21 Met Gly Arg Ile Ser Pro Phe Ser Cys Leu
 31 Lys Asp Arg His Asp Phe Gly Phe Pro Gln
 41 Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys
 51 Ala Gln Ala Ile Ser Val Leu His Glu Met
 61 Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr
 45 71 Lys Asp Ser Ser Ala Thr Trp Glu Gln Ser
 81 Leu Leu Asp Lys Phe Tyr Thr Glu Leu Asn
 91 Gln Gln Leu Asn Asp Leu Glu Ala Cys Val
 101 Ile Gln Gly Val Gly Val Thr Glu Thr Pro
 111 Leu Met Lys Glu Asp Ser Ile Leu Ala Val
 50 121 Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr
 131 Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala

141 Trp Glu Val Val Arg Ala Glu Ile Met Arg
 151 Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
 161 Ser Leu Arg Ser Lys Glu

5

CM-1 (SEQ ID NO:11):

1 Cys Asp Leu Pro Gln Thr His Ser Leu Gly
 11 Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln
 21 Met Gly Arg Ile Ser Pro Phe Ser Cys Leu
 10 31 Lys Asp Arg His Asp Phe Gly Phe Pro Gln
 41 Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys
 51 Ala Gln Ala Ile Ser Val Leu His Glu Met
 61 Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr
 71 Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
 15 81 Leu Leu Glu Lys Phe Asp Thr Glu Leu Asn
 91 Gln Gln Leu Asn Asp Leu Glu Ala Cys Val
 101 Ile Gln Gly Val Gly Val Thr Glu Thr Pro
 111 Leu Met Lys Glu Asp Ser Ile Leu Ala Val
 121 Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr
 20 131 Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala
 141 Trp Glu Val Val Arg Ala Glu Ile Met Arg
 151 Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
 161 Ser Leu Arg Ser Lys Glu

25

CM-2 (SEQ ID NO:12):

1 Cys Asp Leu Pro Gln Thr His Ser Leu Gly
 11 Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln
 21 Met Gly Arg Ile Ser Pro Phe Ser Cys Leu
 30 31 Lys Asp Arg His Asp Phe Gly Phe Pro Gln
 41 Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys
 51 Ala Gln Ala Ile Ser Val Leu His Glu Met
 61 Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr
 71 Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
 35 81 Leu Leu Glu Lys Phe Ile Thr Glu Leu Asn
 91 Gln Gln Leu Asn Asp Leu Glu Ala Cys Val
 101 Ile Gln Gly Val Gly Val Thr Glu Thr Pro
 111 Leu Met Lys Glu Asp Ser Ile Leu Ala Val
 121 Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr
 40 131 Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala
 141 Trp Glu Val Val Arg Ala Glu Ile Met Arg
 151 Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
 161 Ser Leu Arg Ser Lys Glu

45

CM-3 (SEQ ID NO:13):

1 Cys Asp Leu Pro Gln Thr His Ser Leu Gly
 11 Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln
 21 Met Gly Arg Ile Ser Pro Phe Ser Cys Leu
 50 31 Lys Asp Arg His Asp Phe Gly Phe Pro Gln
 41 Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys

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      51  Ala Gln Ala Ile Ser Val Leu His Glu Met
      61  Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr
      71  Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
      81  Leu Leu Glu Lys Phe Lys Thr Glu Leu Asn
5      91  Gln Gln Leu Asn Asp Leu Glu Ala Cys Val
     101  Ile Gln Gly Val Gly Val Thr Glu Thr Pro
     111  Leu Met Lys Glu Asp Ser Ile Leu Ala Val
     121  Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr
     131  Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala
10    141  Trp Glu Val Val Arg Ala Glu Ile Met Arg
     151  Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
     161  Ser Leu Arg Ser Lys Glu

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15 CM-4 (SEQ ID NO:14):

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      1  Cys Asp Leu Pro Gln Thr His Ser Leu Gly
     11  Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln
     21  Met Gly Arg Ile Ser Pro Phe Ser Cys Leu
     31  Lys Asp Arg His Asp Phe Gly Phe Pro Gln
20    41  Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys
     51  Ala Gln Ala Ile Ser Val Leu His Glu Met
     61  Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr
     71  Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
     81  Leu Leu Glu Lys Phe Ala Thr Glu Leu Asn
25    91  Gln Gln Leu Asn Asp Leu Glu Ala Cys Val
    101  Ile Gln Gly Val Gly Val Thr Glu Thr Pro
    111  Leu Met Lys Glu Asp Ser Ile Leu Ala Val
    121  Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr
    131  Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala
30    141  Trp Glu Val Val Arg Ala Glu Ile Met Arg
     151  Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
     161  Ser Leu Arg Ser Lys Glu

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35 The mature form of human interferon alpha 21a was used to generate these hybrid interferons and has the following sequence (SEQ ID NO:15).

```

      1  CDLPQTHSLG NRRALILLAQ MGRISPFSCS KDRHDFGFPO
     41  EEFDGNQFQK AQAISVLHEM IQQTFNLFST KDSSATWEQS
     81  LLEKFSTELN QQLNDLEACV IQEVGVEETP LMNVDSILAV
40    121  KKYFQRITLY LTEKKYSPCA WEVVRAEIMR SFSLSKIFQE
     161  RLRRKE

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The precursor of human interferon alpha 21 (interferon alpha-F) has accession number P01568 (gi: 20178289). See website at ncbi.nlm.nih.gov.

45 This sequence for this human interferon alpha 21 precursor is provided below (SEQ ID NO:16).

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      1  MALSFSLMA VLVLSYKSIC SLGCDLPQTH SLGNRRALIL

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41 LAQMGRISPF SCLKDRHDFG FPQEEFDGNQ FQKAQAISVL
 81 HEMIQQTFNL FSTKDSSATW EQSLLEKFST ELNQQQLNDLE
 121 ACVIQEVGVE ETPLMNVDSI LAVKKYFQRI TLYLTEKKYS
 161 PCAWEVVRAE IMRSFSLSKI FQERLRRKE

5

Nucleic acid and amino acid sequences for other species of interferon-alpha can also be found in the NCBI database. See website at ncbi.nlm.nih.gov. Thus, many interferon alpha polypeptides are available and can be used in the invention.

The production of a number of hybrid interferons has also been described by Hu, R.C., Bekisz, J., Hayes, M., Audet, S., Beeler, J., Petrocoin, E., Zoon, K.C. Divergence of Binding, Signaling, and Biological Responses to Recombinant Human Hybrid IFN-1. *The Journal of Immunology* 163:854-860 (1999); Hu, R., Bekisz, J., Schmeisser, H., McPhie, P. and Zoon, K. Human IFN-alpha Protein Engineering: The Amino Acid Residues at Positions 86 and 90 are Important for Antiproliferative Activity. *Journal of Immunology* 167:1482-1489 (2001); Schmeisser, H., Hu, R., Bekisz, J. and Zoon, K.C. Amino Acid Substitutions in Loop BC and Helix C Affect Antigenic Properties of Helix D in Hybrid Interferon a21a/a2c Molecules. *Journal of Interferon and Cytokine Research*, 22:463-472 (2002); Horisberger and Di Marco (1995) "Interferon-alpha hybrids." *Pharmac. Ther.* 55:507-534. Hence, the procedures and interferons described in these articles can be used in the practice of the present invention.

Further examples of methods for construction of hybrid interferons can be found, for example, in Protein Engineering of Interferon-alphas, Renqiu Hu, Ke-jian Lei, Joseph Bekisz and Kathryn C. Zoon, in *Methods in Molecular Medicine, Interferon Methods and Protocols*, ed. D.J.J. Carr (Humana Press, Totowa, NJ); PCT Publication WO 00/06735 ("Interferon Alpha Hybrids"); U.S. Patent No. 6,685,933 ("Interferon Alpha Hybrids"); U.S. Patent No. 4,892,743 ("Novel hybrid interferon species"); U.S. Pat. No. 5,071,761 "Hybrid Interferons"; U.S. Pat. No. 4,758,428 ("Multiclass hybrid interferons"); and U.S. Pat. No. 4,716,217 ("Hybrid lymphoblastoid-leukocyte human interferons").

Generally, two procedures are used to create hybrid interferon-alphas. First, some researchers have taken advantage of the presence of naturally

occurring restriction endonuclease cleavage sites within interferon-encoding sequences to piece together homologous coding fragments. See, e.g., U.S. Pat. No. 5,071,761 "Hybrid Interferons." The second general procedure for construction of hybrid interferon-alphas uses PCR amplification to create
5 specific desired nucleic acid fragments, thereby gaining the potential to piece together new pieces of different interferons. Horton et al., (1989) "Engineering hybrid genes without the use of restriction enzymes." *Gene* 77:61-68. Further details on making hybrid interferons are provided in U.S. Patent 6,685,933 and U.S. Patent Publication No. 20040018172.

10 Recombinant interferons can be made by using genetic engineering techniques, for example, in large scale using *Escherichia coli* as a host. Goeddel et al., *Nature*, 287:411 (1980); Streuli et al., *Science*, 209:1343 (1980). For example, interferon- $\alpha 2$ is used in the products Intron™ A (interferon- $\alpha 2b$) by Schering Plough and Roferon™ A (interferon- $\alpha 2a$) by Hoffman-La Roche.
15 Further information on producing recombinant interferons, for example, the novel recombinant interferons of the invention (e.g., SEQ ID NO:11-14), is provided below.

However, recombinantly produced interferons are often composed of only a single species, and if such recombinant interferons are produced in
20 bacteria, they may not be post-translationally modified. Such recombinantly expressed interferons may therefore not have optimal activity. Therefore interferons derived from natural sources can also be used in the invention.

HuIFN- α polypeptides can be produced by a number of human cell lines and human leukocyte cells after exposure to viruses or double-stranded RNA, or
25 in transformed leukocyte cell lines (for example, lymphoblastoid lines). Examples of natural sources from which various types of interferon-alpha can be obtained include those from the human lymphoblastoid cell line, Namalwa (Mizrahi, *Meth. Enzymol.*, 78:54 (1981); Phillips et al., *Meth. Enzymol.*, 119:35 (1986)), and those from human peripheral blood leukocytes (Mogensen et al.,
30 *Pharmacol. Ther. Part C*, 1:369 (1977); Cantell et al., *Methods Enzymol.*, 78:29 (1981); Horowitz, *Methods Enzymol.*, 119:39 (1986)). See also, Hayes and Zoon (1993) "Priming of Human Monocytes for enhanced lipopolysaccharide responses: Expression of alpha interferon, interferon regulatory factors, and tumor necrosis factor." *Infect. Immun.* 61:3222-3227; Zoon, K.C., Miller, D.,

Bekisz, J.B., Zur Nedden, D., Enterline, J.C., Nguyen, N.Y., Hu, R. Purification and Characterization of Multiple Components of Human Lymphoblastoid Interferon-alpha. J. Biol. Chem. 267:15210-15216 (1992).

Such natural sources of interferon-alpha provide multiple species of
5 interferon-alpha, each with different structural and biological activity. These
"natural" interferons are considered by some researchers to provide potentially
better therapeutic efficacy than a single species of recombinant interferon. For
example, natural interferon-alpha can be used at a four times lower dosage to
treat *Condyloma* than the recombinant products. See, e.g., Physicians Desk
10 Reference, 47th edit., eds. Medical Economics Data, Montvale, N.J., p. 1879 and
2194 (1993). Another advantage of using natural leukocyte interferon as a
therapeutic agent is that such natural interferon generally is not particularly
immunogenic. Evidence exists that patients treated with the recombinant
interferons identified above, and the lymphoblastoid interferon, Wellferon™
15 (interferon alpha-n1, Burroughs Wellcome) can develop neutralizing antibodies
to interferon. Lok et al., Hepatology, 12:1266 (1990); Jacobs et al., J. Biol.
Resp. Mod., 7:447 (1988); Weck et al., J. Interferon Res., 1(Suppl):S37 (1989).
However, patients treated with leukocyte derived interferon (interferon alpha-n3
or Cantell's partially purified interferon preparation) generally do not generate
20 detectable serum antibody to interferon Von Wussow et al., Lancet, 2:635
(1987); Liao et al., J. Infect. Dis., 165:757-760 (1992). The presence of
neutralizing anti-interferon antibodies may potentially block the therapeutic
effect of the interferon and therefore may be a significant factor in the course of
clinical treatment.

25 The IFN α -n3a protein population contains proteins defined by their
amino acid sequences that can be identified by the interferon gene sequences
reported in the literature. Specifically, the IFN α -n3a composition comprises a
mixture of at least six human interferon-alpha protein species. The sequences of
these interferon species are available on a variety of commercial databases. See
30 also, K. Zoon, "Purification and Characterization of Human Interferon from
Lymphoblastoid (Namalva) Cultures", Meth. Enzymol., 78:457-465 (1981).

According to the invention, one or more of these interferon-alphas can be
administered to patients suffering from, or suspected of suffering from,
respiratory diseases such as SARS. In some embodiments, interferon-alpha

polypeptides with high therapeutic indices (SI values) are selected for treating SARS. The therapeutic index (SI) is the ratio of the therapeutic activity of a polypeptide over its toxicity. Hence, larger SI values indicate that the polypeptide is more potent for SARS treatment. Thus, for example, highly effective interferon-alpha polypeptides include those with SI values of about 5 or more. Examples of interferon-alpha polypeptides with SI values of about 5 or more include SDM-2 (SI = 500), SDM-1 (SI = 71.4), CM-2 (SI = 40), SDM-3 (SI = 25), Interferon-alpha-o (SI = 21.7), Interferon-alpha-n (SI = 18.5), SDM-4 (SI = 18.2), CM-4 (SI = 12.3), HY-2 (SI = 6.0), Interferon-alpha-d (SI = 5) polypeptides. The SDM-2, SDM-1, CM-2, SDM-3, SDM-4, CM-4 and HY-2 interferon-alpha polypeptides have SEQ ID Nos: 8, 7, 12, 9, 10, 14 and 2, respectively. Some of the interferon-alpha preparations employed, for example, the interferon alpha pre-a through o preparations listed in FIG. 1A-B, were purified from natural lymphoblastoid interferon using the methods described in Zoon et al., *Purification and Characterization of Multiple Components of Human Lymphoblastoid Interferon-alpha*. J. Biol. Chem. 267(21):15210-16 (1992). The letters pre-a through o are used to identify the order in which different interferon preparations eluted from an HPLC column.

There are many assays available to those skilled in the art that measure the degree of resistance of cells to viruses. See McNeill (1981) "Interferon assay." J. Immunological Methods 46:121-127. These assays generally can be categorized into three types: inhibition of cytopathic effect; virus plaque formation; and reduction of virus yield. Viral cytopathic effect assays measure the degree of protection induced in cell cultures pretreated with interferon and subsequently infected with viruses. Vesicular stomatitis virus, for example, has been tested in such an assay. This type of assay is convenient for screening numerous different interferons, as it can be performed in 96-well plates. See, Rubinstein et al., (1981) "Convenient assay for interferon." J. Virol. 37:755-758. Plaque-reduction assays measure the resistance of interferon-treated cell cultures to a plaque-forming virus. One benefit to this assay is that it allows precise measurement of a 50% reduction in plaque formation. Finally, virus yield assays measure the amount of virus released from cells during, for instance, a single growth cycle. Such assays are useful for testing the antiviral activity of interferons against viruses that do not cause cytopathic effects, or that do not

build plaques in target-cell cultures. The multiplicity of infection (moi) is an important factor to consider when using either plaque-reduction or virus-yield assays.

Other clinically important interferon characteristics are also easily assayed in the laboratory setting. One such characteristic is the ability of an interferon polypeptide to bind to specific cell-surface receptors. For instance, some interferon-alphas exhibit different cell-surface properties compared to interferon-alpha-2b, the interferon most widely used in clinical trials. While interferon-alpha-2b is an effective antiviral agent, it causes significant adverse side effects. Interferons that exhibit distinct binding properties from interferon-alpha-2b may not cause the same adverse effects. Therefore, interferons that compete poorly with interferon-alpha-2b for binding sites on cells are of clinical interest. Competitive interferon binding assays are well known in the art. See, Hu et al., (1993) "Evidence for multiple binding sites for several components of human lymphoblastoid interferon-alpha." J. Biol. Chem. 268:12591-12595; Di Marco et al., (1994) "Mutational analysis of the structure-function relationship in interferon-.alpha.." Biochem. Biophys. Res. Comm. 202:1445-1451. In general, such assays involve incubation of cell culture cells with a mixture of ¹²⁵I - labeled IFN-alpha-2b and an unlabeled interferon of interest. Unbound interferon is then removed, and the amount of bound label (and by extension, bound ¹²⁵I-labeled IFN-alpha-2b) is measured. By comparing the amount of label that binds to cells in the presence or absence of competing interferons, relative binding affinities can be calculated.

25 Nucleic acid constructs, expression cassettes, and recombinant expression

The present invention provides isolated nucleic acid segments that encode the interferon polypeptides of the invention. In some embodiments, the nucleic acid constructs and expression cassette encode interferon polypeptides CM-1 (SEQ ID NO:11), CM-2 (SEQ ID NO:12), CM-3 (SEQ ID NO:13) and/or CM-4 (SEQ ID NO:14). Nucleic acids encoding these interferons are provided below.

CM-1 (SEQ ID NO:17):

35 5' - TGT GAT CTG CCT CAG ACC CAC AGC CTG GGT AAT AGG
AGG GCC TTG ATA CTC CTG GCA CAA ATG GGA AGA ATC

TCT CCT TTC TCC TGC CTG AAG GAC AGA CAT GAC TTT
 GGA TTC CCC CAG GAG GAG TTT GAT GGC AAC CAG TTC
 CAG AAG GCT CAA GCC ATC TCT GTC CTC CAT GAG ATG
 ATC CAG CAG ACC TTC AAT CTC TTC AGC ACA AAG GAC
 5 TCA TCT GCT TGG GAT GAG ACC CTC CTA GAA AAA TTT
 GAT ACT GAA CTT AAC CAG CAG CTG AAT GAC CTC GAG
 GCC TGC GTG ATA CAG GGG GTG GGG GTG ACA GAG ACT
 CCC CTG ATG AAG GAG GAC TCC ATT CTG GCT GTG AGG
 AAA TAC TTC CAA AGA ATC ACT CTC TAT CTG AAA GAG
 10 AAG AAA TAC AGC CCT TGT GCC TGG GAA GTT GTC AGA
 GCA GAA ATC ATG AGA TCT TTT TCT TTG TCA ACA AAC
 TTG CAA GAA AGT TTA AGA AGT AAG GAA TG-3'

15 CM-2 (SEQ ID NO:18):

5' - TGT GAT CTG CCT CAG ACC CAC AGC CTG GGT AAT AGG
 AGG GCC TTG ATA CTC CTG GCA CAA ATG GGA AGA ATC
 TCT CCT TTC TCC TGC CTG AAG GAC AGA CAT GAC TTT
 20 GGA TTC CCC CAG GAG GAG TTT GAT GGC AAC CAG TTC
 CAG AAG GCT CAA GCC ATC TCT GTC CTC CAT GAG ATG
 ATC CAG CAG ACC TTC AAT CTC TTC AGC ACA AAG GAC
 TCA TCT GCT TGG GAT GAG ACC CTC CTA GAA AAA TTT
 ATA ACT GAA CTT AAC CAG CAG CTG AAT GAC CTC GAG
 25 GCC TGC GTG ATA CAG GGG GTG GGG GTG ACA GAG ACT
 CCC CTG ATG AAG GAG GAC TCC ATT CTG GCT GTG AGG
 AAA TAC TTC CAA AGA ATC ACT CTC TAT CTG AAA GAG
 AAG AAA TAC AGC CCT TGT GCC TGG GAA GTT GTC AGA
 GCA GAA ATC ATG AGA TCT TTT TCT TTG TCA ACA AAC
 30 TTG CAA GAA AGT TTA AGA AGT AAG GAA TG-3'

CM-3 (SEQ ID NO:19):

35 5' - TGT GAT CTG CCT CAG ACC CAC AGC CTG GGT AAT AGG
 AGG GCC TTG ATA CTC CTG GCA CAA ATG GGA AGA ATC
 TCT CCT TTC TCC TGC CTG AAG GAC AGA CAT GAC TTT
 GGA TTC CCC CAG GAG GAG TTT GAT GGC AAC CAG TTC
 CAG AAG GCT CAA GCC ATC TCT GTC CTC CAT GAG ATG
 40 ATC CAG CAG ACC TTC AAT CTC TTC AGC ACA AAG GAC
 TCA TCT GCT TGG GAT GAG ACC CTC CTA GAA AAA TTT
 AAA ACT GAA CTT AAC CAG CAG CTG AAT GAC CTC GAG
 GCC TGC GTG ATA CAG GGG GTG GGG GTG ACA GAG ACT
 CCC CTG ATG AAG GAG GAC TCC ATT CTG GCT GTG AGG
 45 AAA TAC TTC CAA AGA ATC ACT CTC TAT CTG AAA GAG
 AAG AAA TAC AGC CCT TGT GCC TGG GAA GTT GTC AGA
 GCA GAA ATC ATG AGA TCT TTT TCT TTG TCA ACA AAC
 TTG CAA GAA AGT TTA AGA AGT AAG GAA TG-3'

50

CM-4 (SEQ ID NO:20)

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5' - TGT GAT CTG CCT CAG ACC CAC AGC CTG GGT AAT AGG
AGG GCC TTG ATA CTC CTG GCA CAA ATG GGA AGA ATC
5 TCT CCT TTC TCC TGC CTG AAG GAC AGA CAT GAC TTT
GGA TTC CCC CAG GAG GAG TTT GAT GGC AAC CAG TTC
CAG AAG GCT CAA GCC ATC TCT GTC CTC CAT GAG ATG
ATC CAG CAG ACC TTC AAT CTC TTC AGC ACA AAG GAC
TCA TCT GCT TGG GAT GAG ACC CTC CTA GAA AAA TTT
10 GCT ACT GAA CTT AAC CAG CAG CTG AAT GAC CTC GAG
GCC TGC GTG ATA CAG GGG GTG GGG GTG ACA GAG ACT
CCC CTG ATG AAG GAG GAC TCC ATT CTG GCT GTG AGG
AAA TAC TTC CAA AGA ATC ACT CTC TAT CTG AAA GAG
AAG AAA TAC AGC CCT TGT GCC TGG GAA GTT GTC AGA
15 GCA GAA ATC ATG AGA TCT TTT TCT TTG TCA ACA AAC
TTG CAA GAA AGT TTA AGA AGT AAG GAA TG-3'

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The nucleic acid segments of the invention include segments that encode
 20 for the same amino acids as provided, for example, in SEQ ID NO:11-14, due to
 the degeneracy of the genetic code. Thus, for example, the amino acid threonine
 is encoded by ACU, ACC, ACA and ACG and is therefore degenerate. It is
 intended that the invention includes all variations of the polynucleotide segments
 that encode for the same amino acids. Such mutations are known in the art
 25 (Watson et al, Molecular Biology of the Gene, Benjamin Cummings 1987).
 Hence, variants of the SEQ ID NO:17-20 nucleic acids are contemplated by the
 invention where the variants encode the same or substantially the same amino
 acid sequences (SEQ ID NO:11-14) but where the variants have different
 nucleotide sequences than those of SEQ ID NO:17-20.

30 Conservative mutations in the amino acid sequences of the interferons
 used in the invention are also contemplated. Thus, interferon nucleic acid
 segments can encode polypeptides with conservative amino acid changes, for
 example, the substitution of leucine for isoleucine and so forth. Such mutations
 are also known in the art. Thus, the genes and nucleotide sequences of the
 35 invention include both the naturally occurring sequences as well as conservative
 mutant forms.

The nucleic acid segments of the invention may be contained within a
 vector. A vector may include, but is not limited to, any plasmid, phagemid, F-
 factor, virus, cosmid, or phage in double or single stranded linear or circular
 40 form which may or may not be self transmissible or mobilizable. The vector can

also transform a prokaryotic or eukaryotic host either by integration into the cellular genome or exist extra-chromosomally (e.g. autonomous replicating plasmid with an origin of replication).

Preferably the nucleic acid segment in the vector is under the control of,
5 and operably linked to, an appropriate promoter or other regulatory elements for transcription in vitro or in a host cell, such as a eukaryotic cell, or a microbe, e.g. bacteria. The vector may be a shuttle vector that functions in multiple hosts. The vector may also be a cloning vector that typically contains one or a small number of restriction endonuclease recognition sites at which foreign DNA sequences
10 can be inserted in a determinable fashion. Such insertion can occur without loss of essential biological function of the cloning vector. A cloning vector may also contain a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Examples of marker genes are tetracycline resistance or ampicillin resistance. Many cloning vectors are
15 commercially available (Stratagene, New England Biolabs, Clontech).

The nucleic acid segments of the invention may also be inserted into an expression vector. In some embodiments, such an expression vector contains prokaryotic DNA elements coding for a bacterial replication origin and an antibiotic resistance gene to provide for the amplification and selection of the
20 expression vector in a bacterial host; regulatory elements that control initiation of transcription such as a promoter; and DNA elements that control the processing of transcripts such as introns, or a transcription termination / polyadenylation sequence.

Methods to introduce nucleic acid segment into a vector are available in
25 the art (Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001)). Briefly, a vector into which a nucleic acid segment is to be inserted is treated with one or more restriction enzymes (restriction endonuclease) to produce a linearized vector having a blunt end, a "sticky" end with a 5' or a 3' overhang, or any combination
30 of the above. The vector may also be treated with a restriction enzyme and subsequently treated with another modifying enzyme, such as a polymerase, an exonuclease, a phosphatase or a kinase, to create a linearized vector that has characteristics useful for ligation of a nucleic acid segment into the vector. The nucleic acid segment that is to be inserted into the vector is treated with one or

more restriction enzymes to create a linearized segment having a blunt end, a "sticky" end with a 5' or a 3' overhang, or any combination of the above. The nucleic acid segment may also be treated with a restriction enzyme and subsequently treated with another DNA modifying enzyme. Such DNA
5 modifying enzymes include, but are not limited to, polymerase, exonuclease, phosphatase or a kinase, to create a nucleic acid segment that has characteristics useful for ligation of a nucleic acid segment into the vector.

The treated vector and nucleic acid segment are then ligated together to form a construct containing a nucleic acid segment according to methods
10 available in the art (Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001)). Briefly, the treated nucleic acid fragment and the treated vector are combined in the presence of a suitable buffer and ligase. The mixture is then incubated under appropriate conditions to allow the ligase to ligate the nucleic acid fragment into
15 the vector.

The invention also provides an expression cassette which contains a nucleic acid sequence capable of directing expression of a particular nucleic acid segment of the invention, such as any of SEQ ID NO:11-14, either in vitro or in a host cell. The expression cassette is an isolatable unit such that the expression
20 cassette may be in linear form and functional for in vitro transcription and translation assays. The materials and procedures to conduct these assays are commercially available, for example, from Promega Corp. (Madison, Wisconsin). For example, an in vitro transcript may be produced by placing a nucleic acid sequence under the control of a T7 promoter and then using T7
25 RNA polymerase to produce an in vitro transcript. This transcript may then be translated in vitro through use of a rabbit reticulocyte lysate. Alternatively, the expression cassette can be incorporated into a vector allowing for replication and amplification of the expression cassette within a host cell or also in vitro transcription and translation of a nucleic acid segment.

30 Such an expression cassette may contain one or a plurality of restriction sites allowing for placement of the nucleic acid segment under the regulation of a regulatory sequence. The expression cassette can also contain a termination signal operably linked to the nucleic acid segment as well as regulatory sequences required for proper translation of the nucleic acid segment. The

expression cassette containing the nucleic acid segment may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression cassette may also be one which is naturally occurring but has been obtained in a recombinant form useful
5 for heterologous expression. Expression of the nucleic acid segment in the expression cassette may be under the control of a constitutive promoter or an inducible promoter which initiates transcription only when the host cell is exposed to some particular external stimulus.

The expression cassette may include in the 5'-3' direction of transcription,
10 a transcriptional and translational initiation region, a nucleic acid segment and a transcriptional and translational termination region functional in vivo and /or in vitro. The termination region may be native with the transcriptional initiation region, may be native with the nucleic acid segment, or may be derived from another source.

15 The regulatory sequence can be a polynucleotide sequence located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influences the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences can include, but are not limited to, enhancers, promoters,
20 repressor binding sites, translation leader sequences, introns, and polyadenylation signal sequences. They may include natural and synthetic sequences as well as sequences which may be a combination of synthetic and natural sequences. While regulatory sequences are not limited to promoters, some useful regulatory sequences include constitutive promoters, inducible
25 promoters, regulated promoters, tissue-specific promoters, viral promoters and synthetic promoters.

A promoter is a nucleotide sequence which controls the expression of the coding sequence by providing the recognition for RNA polymerase and other factors required for proper transcription. A promoter includes a minimal
30 promoter, consisting only of all basal elements needed for transcription initiation, such as a TATA-box and/or initiator that is a short DNA sequence comprised of a TATA-box and other sequences that serve to specify the site of transcription initiation, to which regulatory elements are added for control of expression. A promoter may be derived entirely from a native gene, or be

composed of different elements derived from different promoters found in nature, or even be comprised of synthetic DNA segments. A promoter may contain DNA sequences that are involved in the binding of protein factors which control the effectiveness of transcription initiation in response to physiological or developmental conditions.

The invention also provides a construct containing a vector and an expression cassette. The vector may be selected from, but not limited to, any vector available to one of skill in the including some of those described herein. Into this vector may be inserted an expression cassette through methods known in the art and previously described (Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001)). In one embodiment, the regulatory sequences of the expression cassette may be derived from a source other than the vector into which the expression cassette is inserted. In another embodiment, a construct containing a vector and an expression cassette is formed upon insertion of a nucleic acid segment of the invention into a vector that itself contains regulatory sequences. Thus, an expression cassette is formed upon insertion of the nucleic acid segment into the vector. Vectors containing regulatory sequences are available commercially and methods for their use are known in the art (Clontech, Promega, Stratagene).

Administration

Administration of interferon-alpha (alone or in combination with interferon-beta or interferon-gamma) can result in prevention, palliation, and/or improvement in one or more symptoms of respiratory virus infection, such as SARS coronavirus infection. The exact form of prevention, palliation or improvement will depend on the particular respiratory virus. In some embodiments, administration of interferon-alpha results in a reduction in viral titer (a reduction of which indicates suppression of viral infection). In some embodiments, duration of respiratory viral infection is reduced. In other embodiments, viral infection is suppressed, which may be indicated by any one or more of a number of parameters, including, but not limited to, extent of one or more symptoms and viral titer.

Symptoms of infection may be assessed before and/or after

administration of interferon-alpha by the individual or the clinician. Rhinitis, nasal mucous production, severity of cough, myalgia, elevated body temperature, and other symptoms of respiratory virus infection may be easily measured using simple tests and/or scales as are known in the art.

- 5 Biological samples can be examined for evidence of virus and/or pathological effects of virus before, during and/or after administration of interferon-alpha. Biological samples for examination include, but are not limited to, lung tissue specimens, bronchoalveolar lavage specimens, sputum, and upper respiratory tract swab, aspirate and wash specimens. See Ksiazek et al. (N. Engl. J. Med., published at www.nejm.org on Apr. 10, 2003) for examples of
10 specimens in which SARS-associated coronavirus was identified.

- Viral titer may be assessed in biological samples using standard methods of the art. Levels of viral nucleic acid may be assessed by isolating nucleic acid from the sample and blot analysis using a viral polynucleotide sequence as a
15 probe, or by using PCR analysis. Another assay is to test for virus particles in the sample, for example, by observing whether plaque forming units (PFU) are present in the sample. The presence and or amount of viral particles may be measured from any infected area, such as infected tissue or mucosal discharge. When the sample is a liquid, viral titer is calculated in some indication of
20 number or amount of virus or virus particles (e.g., infectious particles, plaque forming units, infectious doses, or median tissue culture infectious doses (TCID₅₀)) per unit volume. In solid samples, such as a tissue sample, viral titer is calculated in virus particles per unit weight. Reduction is indicated by comparing an estimated titer (based, for example, on animal or clinical studies) that
25 represents untreated infection, and/or a titer measured at an earlier time point, with the measured viral titer after treatment.

Formulations

- An effective amount or a therapeutically effective amount of an
30 interferon-alpha formulation is typically used in formulations for treating SARS. An effective amount or a therapeutically effective amount of interferon-alpha is quantity of interferon- α sufficient to achieve a desired effect in a subject being treated. The interferon-alpha polypeptides of the invention, including their salts, are therefore administered so as to achieve a reduction in at least one symptom

associated with SARS. For instance, this can be the amount necessary to inhibit viral proliferation. See, U.S. Pat. Nos. 4,089,400 ("Polypeptides and process for the production thereof") and 5,503,828 ("Alpha interferon composition and method for its production from human peripheral blood leukocytes") for general disclosure as to the amounts of IFN- α that have proven efficacious in clinical settings. The same dose levels as are used in conventional (non-hybrid) interferon therapy may be used with hybrid interferons. In general, a dose of about 10^2 to about 10^8 IU, or a dose of about 10^4 to about 10^7 IU, will be appropriate and may be administered more than once, for example daily, during a course of treatment. However, the effective amount of interferon-alpha will be dependent on the subject being treated, the severity of the affliction, and the manner of administration of the interferon.

The interferon-alpha can be administered in combination with other interferons, for example, interferon-beta or interferon-gamma. For example, the formulations of the invention can include Betaseron[®] (Interferon beta-1b), AVONEX[®] (Interferon beta-1a, available from Biogen Idec), Rebif[®] (Interferon beta-1a), ACTIMMUNE[®] (Interferon gamma-1b) or other sources of interferon-beta and interferon-gamma.

Administration of the therapeutic agents in accordance with the present invention may be in a single dose, in multiple doses, in a continuous or intermittent manner, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the polypeptides of the invention may be essentially continuous over a pre-selected period of time or may be in a series of spaced doses. Both local and systemic administration is contemplated.

To prepare the composition, interferon polypeptides are synthesized or otherwise obtained, purified as necessary or desired and then lyophilized and stabilized. The polypeptide can then be adjusted to the appropriate concentration, and optionally combined with other agents. The absolute weight of a given polypeptide included in a unit dose can vary widely. For example, in some embodiments, about 1×10^6 to about 1×10^7 units, or about 3×10^6 to about 6×10^6 units, interferon alpha can be administered per dosage period, where the dosage period is once a day or once every other day.

The specific activity of interferon alpha formulations is typically about 1×10^8 to about 3×10^8 units/mg interferon alpha polypeptide. Thus, about 0.001 to about 0.1 mg, or about 0.01 to about 0.05 mg, or about 0.015 to about 0.03 mg of at least one polypeptide of the invention, or a plurality of interferon

5 polypeptides, can be administered as unit dosage.

Thus, one or more suitable unit dosage forms comprising the therapeutic polypeptides of the invention can be administered by a variety of routes including oral, parenteral (including subcutaneous, intravenous, intramuscular and intraperitoneal), rectal, dermal, transdermal, intrathoracic, intrapulmonary
10 and intranasal (respiratory) routes. The therapeutic polypeptides may also be formulated for sustained release (for example, using microencapsulation, see WO 94/ 07529, and U.S. Patent No.4,962,091). The formulations may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known to the pharmaceutical arts. Such
15 methods may include the step of mixing the therapeutic agent with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, introducing or shaping the product into the desired delivery system.

When the therapeutic polypeptides of the invention are prepared for oral
20 administration, they are generally combined with a pharmaceutically acceptable carrier, diluent or excipient to form a pharmaceutical formulation, or unit dosage form. For oral administration, the polypeptides may be present as a powder, a granular formulation, a solution, a suspension, an emulsion or in a natural or synthetic polymer or resin for ingestion of the active ingredients from a chewing
25 gum. The active polypeptides may also be presented as a bolus, electuary or paste. Orally administered therapeutic polypeptides of the invention can also be formulated for sustained release, e.g., the polypeptides can be coated, micro-encapsulated, or otherwise placed within a sustained delivery device. The total active ingredients in such formulations comprise from 0.1 to 99.9% by weight of
30 the formulation.

By "pharmaceutically acceptable" it is meant a carrier, diluent, excipient, and/or salt that is compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

Pharmaceutical formulations containing the therapeutic polypeptides of the invention can be prepared by procedures known in the art using well-known and readily available ingredients. For example, the polypeptide can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, solutions, suspensions, powders, aerosols and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include buffers, as well as fillers and extenders such as starch, cellulose, sugars, mannitol, and silicic derivatives. Binding agents can also be included such as carboxymethyl cellulose, hydroxymethylcellulose, hydroxypropyl methylcellulose and other cellulose derivatives, alginates, gelatin, and polyvinyl-pyrrolidone. Moisturizing agents can be included such as glycerol, disintegrating agents such as calcium carbonate and sodium bicarbonate. Agents for retarding dissolution can also be included such as paraffin. Resorption accelerators such as quaternary ammonium compounds can also be included. Surface active agents such as cetyl alcohol and glycerol monostearate can be included. Adsorptive carriers such as kaolin and bentonite can be added. Lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols can also be included. Preservatives may also be added. The compositions of the invention can also contain thickening agents such as cellulose and/or cellulose derivatives. They may also contain gums such as xanthan, guar or carbo gum or gum arabic, or alternatively polyethylene glycols, bentones and montmorillonites, and the like.

For example, tablets or caplets containing the polypeptides of the invention can include buffering agents such as calcium carbonate, magnesium oxide and magnesium carbonate. Caplets and tablets can also include inactive ingredients such as cellulose, pre-gelatinized starch, silicon dioxide, hydroxy propyl methyl cellulose, magnesium stearate, microcrystalline cellulose, starch, talc, titanium dioxide, benzoic acid, citric acid, corn starch, mineral oil, polypropylene glycol, sodium phosphate, zinc stearate, and the like. Hard or soft gelatin capsules containing at least one polypeptide of the invention can contain inactive ingredients such as gelatin, microcrystalline cellulose, sodium lauryl sulfate, starch, talc, and titanium dioxide, and the like, as well as liquid vehicles such as polyethylene glycols (PEGs) and vegetable oil. Moreover, enteric-coated caplets or tablets containing one or more polypeptides of the invention

are designed to resist disintegration in the stomach and dissolve in the more neutral to alkaline environment of the duodenum.

Pegylated interferon alpha formulations are available and are contemplated for use in the formulations and methods of the invention. See
5 Haagmans, et al Pegylated interferon-alpha protects type i pneumocytes against SARS coronavirus infection in macques. Nature Medicine 10: 290 (2004); U.S. Patent Nos. 5,762,923 and 6,685,931. The term "pegylated interferon-alpha" as used herein means polyethylene glycol modified conjugates of interferon-alpha. One type of polyethylene-glycol-interferon alfa -2b conjugate is PEG₁₂₀₀₀-
10 interferon alfa 2b, which is available from Schering-Plough Research Institute, Kenilworth, N.J. The phrases "12,000 molecular weight polyethylene glycol conjugated interferon alpha", "PEG₁₂₀₀₀ -IFN alfa-2b conjugate", and "PEG₁₂₀₀₀-IFN alfa" as used herein mean conjugates such as are prepared according to the methods of International Application No. WO 95/13090 and containing urethane
15 linkages between the interferon-alfa-2a or -2b amino groups and polyethylene glycol having an average molecular weight of 12000.

The therapeutic polypeptides of the invention can also be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous,
20 intraperitoneal or intravenous routes. The pharmaceutical formulations of the therapeutic polypeptides of the invention can also take the form of an aqueous or anhydrous solution or dispersion, or alternatively the form of an emulsion or suspension or salve.

Thus, the therapeutic polypeptides may be formulated for parenteral
25 administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion containers or in multi-dose containers. As noted above, preservatives can be added to help maintain the shelf life of the dosage form. The active polypeptides and other ingredients may form suspensions,
30 solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active polypeptides and other ingredients may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

These formulations can contain pharmaceutically acceptable carriers, vehicles and adjuvants that are well known in the art. It is possible, for example, to prepare solutions using one or more organic solvent(s) that is/are acceptable from the physiological standpoint, chosen, in addition to water, from solvents
5 such as acetone, ethanol, isopropyl alcohol, glycol ethers such as the products sold under the name "Dowanol," polyglycols and polyethylene glycols, C₁-C₄ alkyl esters of short-chain acids, ethyl or isopropyl lactate, fatty acid triglycerides such as the products marketed under the name "Miglyol," isopropyl myristate, animal, mineral and vegetable oils and polysiloxanes.

10 It is possible to add, if necessary, an adjuvant chosen from antioxidants, surfactants, other preservatives, film-forming, keratolytic or comedolytic agents, perfumes, flavorings and colorings. Antioxidants such as t-butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene and α -tocopherol and its derivatives can be added.

15 Also contemplated are combination products that include one or more polypeptides of the present invention and one or more anti-microbial or anti-viral agents. For example, a variety of antibiotics can be included in the pharmaceutical compositions of the invention, such as aminoglycosides (e.g., streptomycin, gentamicin, sisomicin, tobramycin and amikacin), ansamycins
20 (e.g. rifamycin), antimycotics (e.g. polyenes and benzofuran derivatives), β -lactams (e.g. penicillins and cephalosporins), chloramphenicol (including thiamphenol and azidamphenicol), linosamides (lincomycin, clindamycin), macrolides (erythromycin, oleandomycin, spiramycin), polymyxins, bacitracins, tyrothycin, capreomycin, vancomycin, tetracyclines (including oxytetracycline,
25 minocycline, doxycycline), phosphomycin and fusidic acid. Examples of anti-viral agents that can be used in the formulations of the invention include ribavirin, acyclovir, gancyclovir, vidarabidine, foscarnet, cidofovir, amantidine, trifluorothymidine, zidovudine, didanosine or zalcitabine.

Additionally, the polypeptides are well suited to formulation as sustained
30 release dosage forms and the like. The formulations can be so constituted that they release the active polypeptide, for example, in a particular part of the intestinal or respiratory tract, possibly over a period of time. Coatings, envelopes, and protective matrices may be made, for example, from polymeric substances, such as polylactide-glycolates, liposomes, microemulsions,

microparticles, nanoparticles, or waxes. These coatings, envelopes, and protective matrices are useful to coat indwelling devices, e.g., stents, catheters, peritoneal dialysis tubing, draining devices and the like.

For topical administration, the therapeutic agents may be formulated as is known in the art for direct application to a target area. Forms chiefly conditioned for topical application take the form, for example, of creams, milks, gels, dispersion or microemulsions, lotions thickened to a greater or lesser extent, impregnated pads, ointments or sticks, aerosol formulations (e.g., sprays or foams), soaps, detergents, lotions or cakes of soap. Other conventional forms for this purpose include wound dressings, coated bandages or other polymer coverings, ointments, creams, lotions, pastes, jellies, sprays, and aerosols. Thus, the therapeutic polypeptides of the invention can be delivered via patches or bandages for dermal administration. Alternatively, the polypeptide can be formulated to be part of an adhesive polymer, such as polyacrylate or acrylate/vinyl acetate copolymer. For long-term applications it might be desirable to use microporous and/or breathable backing laminates, so hydration or maceration of the skin can be minimized. The backing layer can be any appropriate thickness that will provide the desired protective and support functions. A suitable thickness will generally be from about 10 to about 200 microns.

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents. The active polypeptides can also be delivered via iontophoresis, e.g., as disclosed in U.S. Patent Nos. 4,140,122; 4,383,529; or 4,051,842. The percent by weight of a therapeutic agent of the invention present in a topical formulation will depend on various factors, but generally will be from 0.01% to 95% of the total weight of the formulation, and typically 0.1-85% by weight.

Drops, such as eye drops or nose drops, may be formulated with one or more of the therapeutic polypeptides in an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents. Liquid sprays are conveniently delivered from pressurized packs. Drops

can be delivered via a simple eye dropper-capped bottle, or via a plastic bottle adapted to deliver liquid contents dropwise, via a specially shaped closure.

The therapeutic polypeptide may further be formulated for topical administration in the mouth or throat. For example, the active ingredients may
5 be formulated as a lozenge further comprising a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the composition in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the composition of the present invention in a suitable liquid carrier.

The pharmaceutical formulations of the present invention may include, as
10 optional ingredients, pharmaceutically acceptable carriers, diluents, solubilizing or emulsifying agents, and salts of the type that are available in the art. Examples of such substances include normal saline solutions such as physiologically buffered saline solutions and water. Specific non-limiting examples of the carriers and/or diluents that are useful in the pharmaceutical
15 formulations of the present invention include water and physiologically acceptable buffered saline solutions such as phosphate buffered saline solutions pH 7.0-8.0.

The polypeptides of the invention can also be administered to the respiratory tract. Thus, the present invention also provides aerosol
20 pharmaceutical formulations and dosage forms for use in the methods of the invention. In general, such dosage forms comprise an amount of at least one of the agents of the invention effective to treat or prevent the clinical symptoms of a specific infection, indication or disease (e.g. SARS). Any statistically significant attenuation of one or more symptoms of an infection, indication or
25 disease that has been treated pursuant to the method of the present invention is considered to be a treatment of such infection, indication or disease within the scope of the invention.

Alternatively, for administration by inhalation or insufflation, the composition may take the form of a dry powder, for example, a powder mix of
30 the therapeutic agent and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges, or, e.g., gelatin or blister packs from which the powder may be administered with the aid of an inhalator, insufflator, or a metered-dose inhaler (see, for example, the pressurized metered dose inhaler (MDI) and the

dry powder inhaler disclosed in Newman, S. P. in Aerosols and the Lung, Clarke, S. W. and Davia, D. eds., pp. 197-224, Butterworths, London, England, 1984).

Therapeutic polypeptides of the present invention can also be
5 administered in an aqueous solution when administered in an aerosol or inhaled form. Thus, other aerosol pharmaceutical formulations may comprise, for example, a physiologically acceptable buffered saline solution containing between about 0.1 µg/ml and about 100 µg/ml of one or more of the polypeptides of the present invention specific for the indication or disease to be
10 treated. Dry aerosol in the form of finely divided solid polypeptide particles that are not dissolved or suspended in a liquid are also useful in the practice of the present invention. Polypeptides of the present invention may be formulated as dusting powders and comprise finely divided particles having an average particle size of between about 1 and 5 µm, alternatively between 2 and 3 µm. Finely
15 divided particles may be prepared by pulverization and screen filtration using techniques well known in the art. The particles may be administered by inhaling a predetermined quantity of the finely divided material, which can be in the form of a powder. It will be appreciated that the unit content of active ingredient or ingredients contained in an individual aerosol dose of each dosage form need not
20 in itself constitute an effective amount for treating the particular infection, indication or disease since the necessary effective amount can be reached by administration of a plurality of dosage units. Moreover, the effective amount may be achieved using less than the dose in the dosage form, either individually, or in a series of administrations.

25 For administration to the upper (nasal) or lower respiratory tract by inhalation, the therapeutic polypeptides of the invention are conveniently delivered from a nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane,
30 dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Nebulizers include, but are not limited to, those described in U.S. Patent Nos. 4,624,251; 3,703,173; 3,561,444; and 4,635,627. Aerosol delivery systems of the type disclosed herein are available from

numerous commercial sources including Fisons Corporation (Bedford, Mass.), Schering Corp. (Kenilworth, NJ) and American Pharmoseal Co., (Valencia, CA). For intra-nasal administration, the therapeutic agent may also be administered via nose drops, a liquid spray, such as via a plastic bottle atomizer or metered-dose inhaler. Typical of atomizers are the Mistometer (Wintrop) and the Medihaler (Riker).

Furthermore, the active ingredients may also be used in combination with other therapeutic agents, for example, pain relievers, anti-inflammatory agents, antihistamines, bronchodilators, anti-microbial agents, anti-viral agents and the like, whether for the conditions described or some other condition.

The present invention further pertains to a packaged pharmaceutical composition for controlling viral infections such as a kit or other container. The kit or container holds a therapeutically effective amount of a pharmaceutical composition for controlling viral infections and instructions for using the pharmaceutical composition for control of the viral infection. The pharmaceutical composition includes at least one interferon-alpha polypeptide, in a therapeutically effective amount such that a viral infection is controlled.

The invention will be further described by reference to the following detailed example, which is given for illustration of the invention, and are not intended to be limiting thereof.

EXAMPLE 1: Effect of Interferon-alpha on SARS Coronavirus Infection

This Example illustrates that interferon-alpha treatment ameliorates infection by SARS coronavirus.

Twenty-four hour old cultures of Vero E6 or Vero 76 cells were grown in six well plates for 24 hours. Confluent monolayers were propagated in Earles minimal essential medium (MEM) supplemented with 5% bovine calf serum, L-glutamine, antibiotics, and sodium bicarbonate. Cells were incubated at 37 °C in a 5% CO₂ humidified atmosphere. Prior to treatment, the monolayers were rinsed twice with MEM without serum.

Some of the interferon-alpha preparations employed, for example, the interferon alpha pre-a through o preparations listed in FIG. 1A-B, were purified from natural lymphoblastoid cell interferon using the methods described

in Zoon et al., *Purification and Characterization of Multiple Components of Human Lymphoblastoid Interferon-alpha*. J. Biol. Chem. 267(21):15210-16 (1992).

Various concentrations of different interferon-alpha preparations were prepared in MEM without serum. The selected interferon-alpha-MEM media mixture (0.5 ml) was added to the cultures. After 1 minute at room temperature the cells were gently scraped off the plates with a rubber policeman. The cells were returned to the CO₂ incubator for 10 minutes, then diluted into 8 ml of MEM with calf serum and dispersed in 0.1 ml per well of a 96-well plate containing log dilutions of SARS corona, urbani strain (8 wells per dilution). The plates were incubated for 72 hours at 37 °C in the CO₂ incubator, after which time they were examined under a light microscope for the presence of cytopathic effect. Viral titers (TCID₅₀) were determined by the method of Spearman-Karber.

The results of these experiments are shown in FIG. 1A-B. Note that the therapeutic index (SI) is the ratio of the therapeutic activity of a polypeptide over its toxicity. Hence, larger SI values indicate that the polypeptide is more potent for SARS treatment. Thus, highly effective interferon-alpha polypeptides include the SDM-2 (SI = 500), SDM-1 (SI = 71.4), CM-2 (SI = 40), SDM-3 (SI = 25), Interferon-alpha-o (SI = 21.7), Interferon-alpha-n (SI = 18.5), SDM-4 (SI = 18.2), CM-4 (SI = 12.3), HY-2 (SI = 6.0), Interferon-alpha-d (SI = 5) polypeptides.

EXAMPLE 2: Interferon Are Highly Effective Inhibitors of SARS CoV Replication

This Example illustrates the antiviral activity of certain interferons when used either alone or in combination with other interferons.

Materials and Methods

Cells and viruses. African green monkey kidney cells (Vero 76) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were routinely grown in minimal essential medium (MEM) supplemented with 5% heat-inactivated fetal bovine serum (FBS; Hyclone

Laboratories; Logan UT, USA). For antiviral assays, the serum was reduced to 2% and gentamicin was added to medium at a final concentration of 50 µg/ml.

Severe acute respiratory syndrome coronavirus (SARS CoV), strain Urbani

5 (200300592), was obtained from the CDC and routinely passaged in Vero 76 cells.

Cytopathic Effect (CPE) Inhibition Assay. Compounds were tested at varying concentrations (four one log₁₀ or seven half log₁₀ dilutions) one to two times with this assay and the activity was then verified spectrophotometrically
10 by neutral red (NR) uptake on the same plate (see below). An equal volume of each compound, followed by virus (multiplicity of infection [MOI] = 0.001) within 10 min of compound addition to cells, was added to 80-90% confluent cell monolayers in 96-well tissue culture plates. Alternatively, each compound was added 24 h prior to virus exposure (24 h pretreatment). The MOI used was
15 such that 100% of the cells in the virus alone controls exhibited cytopathic effects (CPE) within 3-5 days. The plates were incubated at 37°C until the cells in the virus only control wells showed complete viral CPE as observed by light microscopy. Each concentration of drug was assayed for inhibition of viral CPE in triplicate and for cytotoxicity in duplicate. Six wells per microplate were set
20 aside as uninfected, untreated cell controls and six wells received virus in medium only per microplate and represented controls for virus replication.

When doing a combination study, one compound was evaluated at one concentration, while varying the concentration of the second compound. Those studies used the pretreatment procedure or the near simultaneous addition of
25 virus procedure. For all CPE-based assays, the 50% effective concentrations (EC₅₀) were calculated by linear regression analysis of the means of the CPE ratings expressed as percentages of untreated, uninfected controls for each concentration.

Morphological changes resulting from cytotoxicity of a compound were
30 graded on a scale of 0-5; where 5 was defined as complete cytotoxicity. The 50% cytotoxic dosage (IC₅₀) was calculated by regression analysis and a selectivity index (SI) was calculated using the formula: $SI = IC_{50} / EC_{50}$.

Neutral Red (NR) Uptake Assay of CPE Inhibition and Compound Cytotoxicity.

This assay was done on the same CPE inhibition test plates described above to verify the inhibitory activity and the cytotoxicity observed by visual observation. The usual correlation between visual and NR assays in our hands has been greater than 95%.

5 Medium was removed from each well of a plate, 0.034% NR was added to each well of the plate and the plate was incubated for 2 hr at 37°C in the dark. The NR solution was removed from the wells, rinsed and the remaining dye extracted using ethanol buffered with Sorenson's citrate buffer. Absorbances at 540 nm/450 nm were read with a microplate reader (Bio- Tek EL 1309; Bio- Tek
10 Instruments, Inc., Winooski, VT). Absorbance values were expressed as percentages of untreated controls, and EC₅₀, IC₅₀ and SI values were calculated as described above.

Virus Yield Reduction Assay. All compounds were evaluated in a virus yield reduction assay to confirm the results of the CPE inhibition/NR uptake
15 assays. Infectious virus yields from each well from a second CPE inhibition assay were determined using currently available procedures. After CPE was scored as described earlier, each plate was frozen at -80°C and thawed. Sample wells at each compound concentration tested were pooled and titered in Vero cells for infectious virus by CPE assay as described above. A 90% reduction in
20 virus yield was then calculated by linear regression analysis. This represented a one-log 10 inhibition in titer when compared to untreated virus controls.

Results and Discussion

CM-2 (SEQ ID NO:2), SDM-2 (SEQ ID NO:8) and SDM-4 (SEQ ID
25 NO:10) constructs were evaluated against SARS CoV replication in Vero 76 cells using procedures described above. All of these compounds were very potent inhibitors of SARS CoV replication, with EC₅₀ values ranging from 0.3 to 1.3 ng/ml (Table 1) by visual assay. Similar results were obtained by neutral red assay. The most potent compound was beta interferon, these findings agreeing
30 with a number of published findings reporting that beta interferons are the most potent inhibitors of Saco replication.

In the sensitive virus yield reduction assay (Table 2), all compounds were still potent inhibitors of infectious virus production with the exception of SDM-2 and SDM-4, which were less inhibitory of virus production than of viral CPE.

This coincides with the microscopic observation that viral cytopathic effects were detected at most every concentration tested for those two compounds, whereas for the other interferons, virus cytopathic effects were often not detected microscopically at the higher concentrations evaluated.

5 In two preliminary synergy studies, varying concentrations of CM-2 were evaluated in combination with one concentration of beta IFN 1a (0.1 ng/ml) or with gamma IFN (1 ng/ml), using a 24 h pretreatment regimen with both compounds (Table 3) or added 10 to 15 min prior to virus exposure (Table 4). The data suggest that when used as a pretreatment that there might be a slight
10 synergistic effect with the combination of beta IFN (added at 0.1 ng/ml) and CM-2 (for CM, EC90 = 0.3 ng/ml; for beta IFN, EC90 = 0.5 ng/ml; together, EC90 = 0.2 ng/ml). However, when used in combination with gamma interferon, there was an apparent enhancement of antiviral activity when compared to either compound alone (for CM-2, EC90 = 0.3 ng/ml; for IFN
15 gamma, EC90 = 2 ng/ml; together, EC90 = 0.01 ng/ml). As expected, there was an apparent loss of activity when the combination of interferons was added near simultaneously with virus (Table 4).

The CM-2 compound was the most potent of the new interferons tested. Moreover, when used in combination with IFN gamma, the CM-2 compound
20 was much more effective in reducing virus yields than either compound used alone.

Table 1. Effects of interferon treatment on SARS CoV replication in African green monkey kidney cells (Vero 76) when added 24 h prior to virus exposure.

25

Interferon	Visual assay EC ₅₀ (ng/ml)	Neutral red uptake assay EC ₅₀ (ng/ml)
CM	1.3	3
SDM-2	3	4
SDM-4	3	2
21a	0.3	1
Alpha 2a	0.5	2
IFN gamma	1	0.4

IFN beta 1a	0.01	<0.01
Alferon	0.32	<0.32

Table 2. Effects of interferon treatment on SARS CoV virus yields in African green monkey kidney cells (Vero 76) when added 24 h prior to virus exposure.

Interferon	Virus yield reduction assay EC ₉₀ (ng/ml)
CM-2	0.3
SDM-2	>10
SDM-4	>10
Alpha 2a	0.08
Alpha 2la	2
IFN gamma	2
IFN beta 1a	0.5
Alferon	0.4

Table 3. Effects of combinations of interferons on SARS CoV virus yields in African green monkey kidney cells (Vero 76) when added 24 h prior to virus exposure.

Interferon	Visual Assay EC ₅₀ (ng/ml)	Virus Yield Reduction Assay EC ₉₀ (ng/ml)
CM-2 + Interferon beta (0.1 ng/ml)	< 0.0027	0.2
CM-2 + Interferon beta (1 ng/ml)	< 0.0027	0.01

Table 4. Effects of combinations of interferons on SARS CoV replication in African green monkey kidney cells (Vero 76) when added within 10 min of virus exposure.

Interferon	Visual Assay EC₅₀ (ng/ml)	Virus Yield Reduction Assay EC₉₀ (ng/ml)
CM-2 + Interferon beta (0.1 ng/ml)	2	2
CM-2 + Interferon beta (1 ng/ml)	2	0.9

5

All patents and publications referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced patent or publication is hereby incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents or publications.

The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims. As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality (for example, a culture or population) of such host cells, and so forth.

Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein.

Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent
5 and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of
10 such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the
15 concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic
20 disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where
25 features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

WHAT IS CLAIMED:

1. An isolated interferon-alpha polypeptide having any one of SEQ ID NO:11-14, or a combination thereof.
2. An isolated nucleic acid encoding an interferon-alpha polypeptide having any one of SEQ ID NO:11-14.
3. The isolated nucleic acid of claim 2, wherein the nucleic acid comprises any one of SEQ ID NO:17-20.
4. A pharmaceutical composition for treating or preventing a SARS-associated coronaviral infection comprising a therapeutically effective amount of an interferon-alpha polypeptide having any one of SEQ ID NO:1-14, or a combination thereof, and a pharmaceutically acceptable carrier.
5. The composition of claim 4, wherein the therapeutically effective amount of the interferon-alpha polypeptide is about 100 IU to about 50,000,000 IU interferon-alpha.
6. The composition of claim 4, wherein the interferon-alpha polypeptide has a therapeutic index of 5.0 or more.
7. The composition of claim 4, wherein the interferon-alpha polypeptide is a polypeptide having any one of SEQ ID NO:11-14, or a combination thereof.
8. The composition of claim 4, wherein the interferon-alpha polypeptide is a polypeptide having any one of SEQ ID NO:2, 7, 8, 9, 10, 12, 14 or a combination thereof.
9. The composition of claim 4, wherein the composition further comprises an effective amount of interferon-beta, interferon-gamma or a combination thereof.
10. A kit which comprises packaging material, a composition comprising at least one interferon-alpha polypeptide selected from the group consisting of SEQ ID NO:1-14, or a combination thereof, and instructions for use in treating a SARS-associated coronaviral infection.
11. The kit of claim 10, wherein the interferon-alpha polypeptide has a therapeutic index of 5.0 or more.

12. The kit of claim 10, wherein the interferon-alpha polypeptide is a polypeptide having any one of SEQ ID NO:11-14, or a combination thereof.
- 5 13. The kit of claim 10, wherein the interferon-alpha polypeptide is a polypeptide having any one of SEQ ID NO:2, 7, 8, 9, 10, 12, 14, or a combination thereof.
14. The kit of claim 10, wherein the composition further comprises an effective amount of interferon-beta, interferon-gamma or a combination thereof.
- 10 15. A method for treating or preventing a SARS-associated coronaviral infection in a mammal, which comprises administering to the mammal a therapeutically effective amount of a composition comprising an interferon-alpha having any one of SEQ ID NO:1-14, or a combination thereof.
- 15 16. The method of claim 15, wherein the therapeutically effective amount of the interferon-alpha polypeptide is about 100 IU to about 50,000,000 IU interferon-alpha.
17. The method of claim 15, wherein the interferon-alpha polypeptide has a therapeutic index of 5.0 or more.
- 20 18. The method of claim 15, wherein the interferon-alpha polypeptide is a polypeptide having any one of SEQ ID NO:1-14, or a combination thereof.
19. The method of claim 15, wherein the interferon-alpha polypeptide is a polypeptide having any one of SEQ ID NO:11-14, or a combination thereof.
- 25 20. The method of claim 15, wherein the interferon-alpha polypeptide is a polypeptide having any one of SEQ ID NO:2, 7, 8, 9, 10, 12, 14, or a combination thereof.
21. The method of claim 15, wherein the composition further comprises an effective amount of interferon-beta, interferon-gamma or a combination thereof.
- 30

Sample	Compound ID	IC50	TC50	Units	SI	Start Conc (mg/ml)	Start Conc (ug/ml)	Stock Units	Plate	Dilution Box Conc	ul of stock drug to add to COL 1	IC50 Sign	Relative IC50	TC50 Sign	Relative TC50
27	INF-alpha-pre-a	80000.0	80000.0	U/ml	1.0	4.0E+06	80000	U/ml	27	3E+05	12.0	>	1.00E+00	>	1.00E+00
28	INF-alpha-a	160000.0	160000.0	U/ml	1.0	8.0E+06	160000	U/ml	28	6E+05	12.0	=	1.00E+00	>	1.00E+00
29	INF-alpha-b	560000.0	1600000.0	U/ml	2.9	8.0E+07	1600000	U/ml	29	6E+06	12.0	=	3.50E-01	>	1.00E+00
30	INF-alpha-b'	22000.0	100000.0	U/ml	4.5	5.0E+06	100000	U/ml	30	4E+05	12.0	=	2.20E-01	>	1.00E+00
31	INF-alpha-pre-c	50000.0	125000.0	U/ml	2.5	6.3E+06	125000	U/ml	31	5E+05	12.0	=	4.00E-01	>	1.00E+00
32	INF-alpha-c	48000.0	80000.0	U/ml	1.7	4.0E+06	80000	U/ml	32	3E+05	12.0	=	6.00E-01	>	1.00E+00
33	INF-alpha-d	25000.0	125000.0	U/ml	5.0	6.3E+06	125000	U/ml	33	5E+05	12.0	=	2.00E-01	>	1.00E+00
34	INF-alpha-e	47520.0	160000.0	U/ml	3.4	8.0E+06	160000	U/ml	34	6E+05	12.0	=	2.97E-01	>	1.00E+00
35	INF-alpha-f	5000.0	5000.0	U/ml	1.0	2.5E+05	5000	U/ml	35	20000	12.0	=	1.00E+00	>	1.00E+00
36	INF-alpha-pre-g	12500.0	12500.0	U/ml	1.0	6.3E+05	12500	U/ml	36	50000	12.0	=	1.00E+00	>	1.00E+00
37	INF-alpha-g	224000.0	320000.0	U/ml	1.4	1.6E+07	320000	U/ml	37	1E+06	12.0	=	7.00E-01	>	1.00E+00
38	INF-alpha-h	48000.0	80000.0	U/ml	1.7	4.0E+06	80000	U/ml	38	3E+05	12.0	=	6.00E-01	>	1.00E+00
39	INF-alpha-i	6400.0	6400.0	U/ml	1.0	3.2E+05	6400	U/ml	39	25600	12.0	>	1.00E+00	>	1.00E+00
40	INF-alpha-j	125000.0	125000.0	U/ml	1.0	6.3E+06	125000	U/ml	40	5E+05	12.0	>	1.00E+00	>	1.00E+00
41	INF-alpha-k	12500.0	12500.0	U/ml	1.0	6.3E+05	12500	U/ml	41	50000	12.0	=	1.00E+00	>	1.00E+00
42	INF-alpha-m	16120.0	26000.0	U/ml	1.6	1.3E+06	26000	U/ml	42	1E+05	12.0	=	6.20E-01	>	1.00E+00

1/2

FIG. 1A

Sample	Compound ID	IC50	TC50	Units	SI	Start Conc (mg/ml)	Start Conc (ug/ml)	Stock Units	Plate	Dilution Box Conc	ul of stock drug to add to COL 1	IC50 Sign	Relative IC50	TC50 Sign	Relative TC50
43	INF-alpha-n	1404.0	>		18.5	1.3E+06	26000	U/ml	43	1E+05	12.0	=	5.40E-02	>	1.00E+00
44	INF-alpha-o	3680.0	>	>	21.7	4.0E+06	80000	U/ml	44	3E+05	12.0	=	4.60E-02	>	1.00E+00
45	CM-1	240.0	>	>	1.3	16000	320	U/ml	45	1280	12.0	=	7.50E-01	>	1.00E+00
46	CM-2	100.0	>	>	40.0	200000	4000	U/ml	46	16000	12.0	=	2.50E-02	>	1.00E+00
47	CM-3	296.0	>	>	3.4	50000	1000	U/ml	47	4000	12.0	=	2.96E-01	>	1.00E+00
48	CM-4	162.0	>	>	12.3	100000	2000	U/ml	48	8000	12.0	=	8.10E-02	>	1.00E+00
50	HY-1	184.0	>	>	4.3	40000	800	U/ml	50	3200	12.0	=	2.30E-01	>	1.00E+00
51	HY-2	83.5	>	>	6.0	25000	500	U/ml	51	2000	12.0	=	1.67E-01	>	1.00E+00
52	HY-4	448.0	=	=	1.7	100000	2000	U/ml	52	8000	12.0	=	2.24E-01	=	3.70E-01
53	HY-5	15.8	>	>	4.4	3500	70	U/ml	53	280	12.0	=	2.25E-01	>	1.00E+00
54	SDM-1	56.0	>	>	71.4	200000	4000	U/ml	54	16000	12.0	=	1.40E-02	>	1.00E+00
55	SDM-2	8.0	>	>	500.0	200000	4000	U/ml	55	16000	12.0	=	2.00E-03	>	1.00E+00
56	SDM-3	80.0	>	>	25.0	100000	2000	U/ml	56	8000	12.0	=	4.00E-02	>	1.00E+00
57	SDM-4	55.0	>	>	18.2	50000	1000	U/ml	57	4000	12.0	=	5.50E-02	>	1.00E+00

2/2

FIG. 1B

SEQUENCE LISTING

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5 Zoon, Kathryn
Hu, Renqiu
Bekisz, Joseph

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Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
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25Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
      50          55          60
Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Glu Gln Ser
65          70          75          80
Leu Leu Glu Lys Phe Ser Thr Glu Leu Asn Gln Gln Leu Asn Asp Leu
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 Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp
 20 25 30
 Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
 30 35 40 45
 Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
 50 55 60
 Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
 65 70 75 80
 35Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
 85 90 95
 Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met
 100 105 110
 Asn Val Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile Thr
 40 115 120 125
 Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
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Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Lys Ile Phe Gln Glu
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 Arg Leu Arg Arg Lys Glu
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 <213> Artificial Sequence

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 Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
 20 35 40 45
 Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
 50 55 60
 Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
 65 70 75 80
 25Leu Leu Glu Lys Phe Tyr Thr Glu Leu Asn Gln Gln Leu Asn Asp Leu
 85 90 95
 Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
 100 105 110
 Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
 30 115 120 125
 Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
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 35Ser Leu Arg Ser Lys Glu
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40<212> PRT

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

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 Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Glu Gln Ser
 65 70 75 80
 5Leu Leu Asp Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
 85 90 95
 Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
 100 105 110
 Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
 10 115 120 125
 Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
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 Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
 35 40 45
 Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
 50 55 60
 35Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Glu Gln Ser
 65 70 75 80
 Leu Leu Asp Lys Phe Tyr Thr Glu Leu Asn Gln Gln Leu Asn Asp Leu
 85 90 95
 Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
 40 100 105 110

9

Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
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 Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
 145 150 155 160
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 Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
 35 40 45
 Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
 50 55 60
 25Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
 65 70 75 80
 Leu Leu Glu Lys Phe Asp Thr Glu Leu Asn Gln Gln Leu Asn Asp Leu
 85 90 95
 Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
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 115 120 125
 Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
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 Ser Leu Arg Ser Lys Glu
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           20             25             30
   Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
15       35             40             45
   Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
       50             55             60
   Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
   65             70             75             80
20Leu Leu Glu Lys Phe Ile Thr Glu Leu Asn Gln Gln Leu Asn Asp Leu
           85             90             95
   Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
           100            105            110
   Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
25       115            120            125
   Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
       130            135            140
   Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
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 Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
 35 40 45
 Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
 50 55 60
 10 Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
 65 70 75 80
 Leu Leu Glu Lys Phe Lys Thr Glu Leu Asn Gln Gln Leu Asn Asp Leu
 85 90 95
 Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
 15 100 105 110
 Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
 115 120 125
 Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
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 Ser Leu Arg Ser Lys Glu
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 Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
 35 40 45
 40 Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
 50 55 60

12

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
 65 70 75 80
 Leu Leu Glu Lys Phe Ala Thr Glu Leu Asn Gln Gln Leu Asn Asp Leu
 85 90 95
 5Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
 100 105 110
 Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
 115 120 125
 Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
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 Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
 35 40 45
 Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
 50 55 60
 30Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Glu Gln Ser
 65 70 75 80
 Leu Leu Glu Lys Phe Ser Thr Glu Leu Asn Gln Gln Leu Asn Asp Leu
 85 90 95
 Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met
 35 100 105 110
 Asn Val Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile Thr
 115 120 125
 Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
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Arg Leu Arg Arg Lys Glu
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Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser
15 35 40 45
Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
50 55 60
Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
65 70 75 80
20His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser
85 90 95
Ser Ala Thr Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu
100 105 110
Asn Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly
25 115 120 125
Val Glu Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala Val Lys
130 135 140
Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
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caggggggtg	gggtgacaga	gactcccctg	atgaaggagg	actccattct	ggctgtgagg	360
aaatacttcc	aaagaatcac	tctctatctg	aaagagaaga	aatacagccc	ttgtgcctgg	420
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caggggggtg	gggtgacaga	gactcccctg	atgaaggagg	actccattct	ggctgtgagg	360
aaatacttcc	aaagaatcac	tctctatctg	aaagagaaga	aatacagccc	ttgtgcctgg	420
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15

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ctagaaaaat ttaaaactga acttaaccag cagctgaatg acctcgaggc ctgcgtgata 300
cagggggtgg gggtgacaga gactcccctg atgaaggagg actccattct ggctgtgagg 360
aaatacttcc aaagaatcac tctctatctg aaagagaaga aatacagccc ttgtgcctgg 420
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gaggagtttg atggcaacca gttccagaag gctcaagcca tctctgtcct ccatgagatg 180
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