Hybrid with Interferon-α and an Immunoglobulin Fc Linked through a Non-Immunogenic Peptide

Background of the invention

Interferon-α ("IFNα") was among the first of the cytokines to be produced by recombinant DNA technology and has been shown to have therapeutic value in conditions such as inflammatory, viral, and malignant diseases. Several IFNα preparations, including those purified from the natural sources and those generated by recombinant DNA technology, have been used or are being tested in a variety of malignant and viral diseases. IFNα can cause regression of some established tumors and induce positive responses in some viral infections. So far, IFNα has been approved or tested in many countries for indications such as: Kaposi's sarcoma; hairy cell leukemia; malignant melanoma; basal cell carcinoma; multiple myeloma; renal cell carcinoma, hepatitis B; hepatitis C; venereal warts, Herpes I/II, varicella/herpes zoster; and mycosis fungoides.

Most cytokines, including IFNα, have relatively short circulation half-lives since they are produced *in vivo* to act locally and transiently. The serum half-life of IFNα is only about two to eight hours (Roche Labs. Referon A, Schering Intron A, *Physicians' Desk Reference*, 47th edition, 1993, pp. 2006-2008, 2194-2201). To use IFNα as an effective systemic therapeutic, one needs relatively large doses and frequent administrations. For example, one of the recommended therapeutic strategies for the AIDS-related Kaposi's sarcoma starts with an induction dose of 36 million IU daily for 10 to 12 weeks, administered as an intramuscular or subcutaneous injection, followed by a maintenance dose of 36 million IU, three times a week. (Roche Labs. Referon A, *Physicians' Desk Reference*, 47th edition, 1993, pp. 2006-2008). Such frequent parenteral administrations are inconvenient and painful. Further, toxic effects, which are probably caused by the high dosage, are a problem for certain

patients. Skin, neurologic, endocrine, and immune toxicity have been reported. To overcome these disadvantages, one can modify the molecule to increase its circulation half-life or change the drug's formulation to extend its release time. The dosage and administration frequency can then be reduced while increasing the efficacy. It was reported that doses of less than nine million units had been well tolerated, while doses more than 36 million units can induce severe toxicity and significantly alter patient status. (Quesada, J.R. et al., J. Clin. Oncol., 4:234-43, 1986). It is possible to decrease substantially the toxic effects by producing a new form IFNα which is more stable in the circulation and requires smaller doses. Efforts have been made to create a recombinant IFNα-gelatin conjugate with an extended retention time (Tabata, Y. et al., *Cancer Res.* 51:5532-8, 1991). A lipid-based encapsulated IFNα formulation has also been tested in animals and achieved an extended release of the protein in the peritoneum (Bonetti, A. and Kim, S. *Cancer Chemother Pharmacol.* 33:258-261, 1993).

Immunoglobulins of IgG and IgM class are among the most abundant proteins in the human blood. They circulate with half-lives ranging from several days to 21 days. IgG has been found to increase the half-lives of several ligand binding proteins (receptors) when used to form recombinant hybrids, including the soluble CD4 molecule, LHR, and IFN-γ receptor (Mordenti J. *et al., Nature,* 337:525-31, 1989; Capon, D.J. and Lasky, L.A., U.S. Patent number 5,116,964; Kurschner, C. *et al., J. Immunol.* 149:4096-4100, 1992). However, such hybrids can present problems in that the peptide at the C-terminal of the active moeity and the peptide at the N-terminal of the Fc portion at the fusion point creates a new peptide sequence, which is a neoantigen, and which can be immunogenic. The invention relates to a IFNα-Fc hybrid which is designed to overcome this problem and extend the half-life of the IFNα.

Summary of the invention

The present invention relates to a hybrid recombinant protein which consists of two subunits. Each subunit includes a human interferon, preferably IFNα, joined by a peptide linker which is primarily composed of a T cell inert sequence, linked to a human immunoglobulin Fc fragment, preferably the γ4 chain. The γ4 chain is preferred over the γ1 chain because the former has little or no complement activating ability.

The C-terminal end of the IFNα is linked to the N-terminal end of the Fc fragment. An additional IFNα (or other cytokine) can attach to the N-terminal end of any other unbound Fc chains in the Fc fragment, resulting in a homodimer for the γ4 chain. If the Fc fragment selected is another chain, such as the μ chain, then, because the Fc fragments form pentamers with ten possible binding sites, this results in a molecule with interferon or other cytokine linked at each of ten binding sites.

The two moieties of the hybrid are linked through a T cell immunologically inert peptide (*e.g.*, Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser (SEQ ID NO:1)). This peptide itself is immunologically inactive. The insertion of this peptide at the fusion point eliminates the neoantigenicity created by the joining of the two peptide moeities. The linker peptide also increases the flexibility of these moieties and allows retention of the biological activity. This relatively long linker peptide helps overcome the possible steric hindrance from the Fc portion of the hybrid, which could interfere with the activity of the hybrid.

The hybrid has a much longer half-life than the native IFNα. Due to the linker, it is also designed to reduce the possibility of generating a new immunogenic epitope (a neoantigen) at what would otherwise be the fusion point of the IFNα and the immunoglobulin Fc segment.

Cytokines are generally small proteins with relatively short half-lives which dissipate rapidly

among various tissues, including at undesired sites. It is believed that small quantities of some cytokines can cross the blood-brain barrier and enter the central nervous system, thereby causing severe neurological toxicity. The IFNα linked to Fcγ of the present invention would be especially suitable for treating hepatitis B or C, because these products will have a long retention time in the vasculature (upon intravenous adminstration) and will not penetrate undesired sites.

The specific hybrid described can also serve as a model for the design and construction of other cytokine-Fc hybrids. The same or a similar linker could be used in order to reduce the possibility of generating a new immunogenic epitope while allowing retention of the biological activity. Cytokine-Fc hybrids in which interleukin-2 is the cytokine, or hybrids including other cytokines, could be made using the same techniques.

Detailed Description of Making and Using the Invention

The hybrid molecule of the invention includes an interferon moiety linked through a unique linker to an immunoglobulin Fc moiety. Preferably, the C-terminal ends of two interferon moieties are separately attached to each of the two N-terminal ends of a heavy chain γ4 Fc fragment, resulting in a homodimer structure. A unique linker peptide, Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser (SEQ ID NO:1), was created to link the two moieties. The complete nucleotide sequence of the preferred γ4 hybrid (including the linker and the Fc moiety) appears in SEQ ID NO: 7. The linker is located at amino acid residue numbers 189 to 204.

The advantage of the hybrid over the native cytokine is that the half-life *in vivo* is much longer. The hybrid including interferon and the γ4 chain Fc homodimer is larger than the native interferon. Because the pores in the blood vessels of the liver are large, this larger molecule is more suitable for use in treating hepatitis, where the virus responsible primarily affects the liver.

The linker peptide is designed to increase the flexibility of the two moieties and thus maintain their biological activity. Although the interferon and the immunoglobulin are both of human origin, there is always a possibility of generating a new immunogenic epitope at the fusion point of the two molecules. Therefore, the other advantage of the linker of the invention, which consists mainly of a T cell inert sequence, is to reduce immunogenicity at the fusion point. Referring to SEQ ID NO:7, it can be seen that if the linker (residue numbers 189-204) was not present, a new sequence consisting of the residues immediately before number 189 and immediately after 204 would be created. This new sequence would be a neoantigen for the human body.

Human IFNα is derived from a family of several different genes. More than 24 species have been identified so far, from gene and protein sequence data. They differ from each other by anywhere from a few to a maximum of 35 amino acids. Most of the species have a signal peptide sequence of 23 amino acid residues and a mature amino acid sequence of 166 amino acid residues (Goeddel, D.V. et al., *Nature*, 290:20-26, 1981; Weissmann, C. and Weber, H., *Prog. Nuc. Acid Res. Mol. Biol.* 33:251-300, 1986; Zoon, K.C., *Interferon*, 9:1-12, 1987).

IFNα2 (also called IFNαA) is one of the most intensively studied interferon species. The recombinant version of IFNα2 has been used as a therapeutic for several years. Two IFNα2 recombinant products, IFNα2a and IFNα2b, are now commercially available. They differ only in one amino acid at position 23, and there is no significant difference in biological activity between them (von Gabain, A., *et al., Eur. J. Biochem.* 190:257-61, 1990).

IFNα2a was selected as the fusion partner for the interferon hybrid of the invention, although the IFNα2b or any other interferon species (including IFNβ) can be used as well. It is also possible to make similar constructs with other cytokines, such as interleukin-1 or interleukin-2. The same linker

could be used, or another one which is not immunogenic and which maintains the biological activity of the contruct could be substituted.

The advantages of the $\gamma 4$ chain as the Fc moiety in the hybrid is that it is stable in the human circulation. The $\gamma 4$ chain (unlike the $\gamma 1$ chain) also avoids the wide spectrum of secondary biological properties, such as complement fixation and antibody-dependant cell-mediated cytotoxicity (ADCC), which may be undesirable properties.

The cDNA of the IFNα2a can be obtained by reverse transcription and PCR, using RNA extracted from leukocytes which express IFNα. One such cell line, KG-1, can be obtained from the American Type Culture Collection (ATCC) in Rockville, Maryland, where it is held under number CCL 246. In the procedure used in making the hybrid of the invention, before the RNA extraction, the cells were challenged by Sendai virus to increase their transcription of interferons (Cantell, K. et al., *Methods in Enzymology*, 78A:29-38, Adacemic Press, 1981).

As mentioned above, IFNα is a collection of IFN species and each cell expresses several different IFNα subspecies at the same time. The DNA sequence homology among these species is so high that RT-PCR would probably amplify a group of them instead a specific one. To obtain specifically the IFNα2a cDNA, the PCR primers were designed so that the last nucleotides of the two primers ended at positions where the amino acids coded are unique for IFNα2a. These are position S22 and 161, respectively (See Zoon, K.C. *Interferon*, 9:1-12, 1987).

By using an overlapping PCR technique (Daugherty, B.L. *et al., Nucleic Acids Res.* 19:2471-6, 1991), one can easily ligate two gene segments at any site as desired. However, one drawback of PCR amplification is the relatively high mutation rate (Saiki, R.K. *et al., Science*, 239:487, 1988). Thus, DNA sequencing was also done to check every DNA segment obtained through PCR for lack of

mutation. Sequencing can be tedious and time consuming when the size of the segment is over 1kb, as is the full length IFNα-Fc cDNA. However, a restriction endonuclease site, BamH I, can be incorporated into the linker nucleotide sequence without changing its amino acid sequence. This site is located between the nucleotide numbers 15 and 16 in SEQ ID NO:1.

The two gene segments from PCR can be separately cloned into cloning vectors. This makes the DNA sequencing easier and quicker since both segments are only a few hundred base pairs in length. Once the clones with the correct DNA sequences are identified, the two gene segments can be linked together through the BamH I site. No second round overlapping PCR and subsequent DNA sequencing of the full length segment are required.

There are several ways to express the recombinant protein *in vitro*, including in *E. coli*, baculovirus, yeast, mammalian cells or other expression systems. The prokaryotic system, *E. coli*, is not able to do post-translational modification, such as glycosylation. But this is probably not a serious problem for the IFNα-Fc hybrid since the native IFNα and immunoglobulin γ4 molecule are not heavily glycosylated. Further, it has been reported that recombinant IFNα without any glycosylation retained its biological activity (Baron, E. and Narula, S., *Bio/technology*, 10:179-190, 1990). However, the purification of recombinant protein from the *E. coli* lysate can be difficult. The foreign proteins expressed by *E. coli* often aggregate and form insoluble inclusion bodies. Thus, solubilization and subsequent refolding of the inclusion bodies is usually required (Schein, C.H. and Noteborn, H.M., *Bio/technology*, 6:291-294, 1988; Wilkinson, D.L. and Harrison, R.G., *Bio/technology*, 9:443-448, 1991).

The yeast expression system *Pichia Pastoris* (Invitrogen, San Diego, CA) overcomes some of the problems encountered when using the bacterial system. It usually gives a high yield and has the ability to do various post-translational modifications. The expressed foreign protein can be secreted

into the culture supernatant where not many other proteins reside, making protein purification and process scale-up much easier. This system was tried first to express either the IFNα-Fc hybrid or the wild type IFNα2a. Unfortunately the IFNα-Fc secreted was found to be partially degraded on SDS-PAGE, whereas the IFNα2a alone was not. The degradation was believed to be caused by the protease activities present in the yeast expression system, as reported by Scorer, C.A. et al., *Gene*, 136:111-9, 1993. The relatively weak spot in the hinge region is the possible target for the proteases.

A mammalian cell expression system for the IFNα-Fc hybrid was also tried. The mammalian expression vector, pCDNA3 (Invitrogen, San Diego, CA) which contains a CMV promoter and a NEO resistance gene, was employed. The host cells, NSO cells, were transfected by the pCDNA3/IFNα-Fc expression vector using the electroporation method. The cells were selected by G418 at a concentration of 0.8 mg/ml. The IFNα-Fc expressing clones were identified by ELISA. The hybrid was successfully expressed in this system and there was no degradation.

There are several advantages to this mammalian expression system. First, the recombinant protein is secreted into the culture supernatant and there is no aggregation, thereby simplifying purification. One chromatography step using a protein A column yields a purified IFNα-Fc protein. Also, the protein produced in this system has a glycosylation pattern very similar to the natural molecules since it is expressed by mammalian cells. Further, a native IFNα2a signal peptide sequence is included in the expression vector. Therefore the protein secreted from the cells has an authentic N-terminal, whereas in the *E. coli* or yeast expression systems there either is no signal peptide or a non-IFNα signal peptide is used. Either way, it will bring in additional artificial amino acid residue(s) at the N-terminal end of the recombinant IFNα-Fc.

As mentioned above, the purification of the IFNα-Fc recombinant protein from the culture

supernatant is relatively straightforward. The protein with a purity of more than 90%, as judged by SDS-PAGE, can be easily obtained by one step of affinity chromatography with a protein A column.

There are several assay methods available for the measuring of the IFNα bioactivity. Using an antiviral assay, it was demonstrated that the hybrid of SEQ ID NO:7 had a specific activity about 5 to 10 fold higher than a related IFNα-Fc hybrid, in which the linker molecule had the sequence Gly Gly Ser Gly Gly Ser (SEQ ID NO:2), and the Fc portion of the hybrid was derived from human IgG1 rather than IgG4. Nevertheless, although the biologicial activity of the hybrid shown in SEQ ID NO:7 was improved substantially over the similar hybrid, it was still lower than that of the native IFNα. However, it is expected that this hybrid will have a longer half-life *in vivo*, than the native IFNα. This expectation is based on results demonstrating that the related IFNα hybrid with the linker sequence shown in SEQ ID NO:2 and an IgG1 Fc portion showed a much longer half-life, in a pharmacokinetic study in a mouse model, than did the native IFNα.

Because the hybrid of SEQ ID NO:7 is expected to have a longer half-life *in vivo* than native IFNα, even though its specific activity is lower, this novel hybrid is expected to be preferred to the native IFNα for clinical use. This is because, as a result of the longer half-life, the Cxt (the area under the concentration vs. time curve) would be up to several hundred times greater than for the native IFNα. This means that at the equivalent molar dosage of the native IFNα and the hybrid, the latter would provide a several hundred fold increased exposure to IFNα, resulting in vastly increased efficacy at the same dosage, and less frequent administration.

In measuring specific activity, molar dosage is preferred instead of expressing activity as units per mass of protein. This is because interferons function through the binding to their specific receptors, which is directly related to the number of molecules present. Also, the molecular weight

of the IFNα-Fcγ4, 110 Kd, is more than five-fold larger than that of the wild type IFNα2a, which is 20kd. Taking this into consideration, measuring activity in units/μmol instead of the units/mg provides a better comparison of activity specifity.

Example I: Cloning human IFNα cDNA and constructing the IFNα-Fc expression vector

$6x10^6$ KG-1 cells (ATCC 246) were incubated with 200 units of Sendai virus at 37°C overnight. The cells were harvested and washed with PBS throughly. The total RNA was extracted by using the RNA-ZOL RNA isolation kit (BIOTEX, Houston, TX) following the procedure provided by the manufacturer. The first-strand cDNA was synthesized by reverse transcription using AMV reverse transcriptase with oligo(dT) as 3' primer in 50mM Tris-HCl (pH 8.3), 60mMKCl, and 6mM $MgCl_2$, incubated at 42°C for 1 hour. The reaction mixture was used directly as the template for PCR to amplify IFNα cDNA. The 5' primer for PCR contained a Hind III site and the coding sequence for the first 21 amino acids from the IFNα2a leader peptide (SEQ ID NO:3). The 3' primer contained the sequence coding for part of the linker (SEQ ID NO:1) and the last five amino acids of the IFNα2a, and a BamH I site integrated in the linker sequence (SEQ ID NO:4). The PCR buffer contained 50mM KCl, 10mMTris-Hcl (pH8.3), 1.5mM $MgCl_2$, 0.01% gelatin, 0.1 mmol each of dNTP, 0.5 μmol of each primers, 5 μl RT reaction mixture, and 1 unit of Taq DNA polymerase in a total of 50 μl volume. The PCR condition was 94°C (1 min), 55°C (2 min), and 72°C (2 min) for 40 cycles on a GeneAmp PCR System 9600 (Perkin Elmer, Norwalk, CT).

The cDNA of the human immunoglobulin γ4 Fc was obtained by reverse transcription and PCR performed the same way as described above. The RNA was extracted from the human tonsil B cells. The 5' primer had the sequence shown in SEQ ID NO:5. The 3' primer had the sequence shown in SEQ ID NO:6.

The two PCR amplified DNA segments were cloned into pUC18 vectors at sites Hind III/BamH I or sites BamH I/EcoR I respectively. After their DNA sequences were confirmed by DNA sequencing using the kit from USB (Cleveland, Ohio), the two segments were ligated together through the BamH I site by a second round cloning. The full length IFNα-Fc cDNA was then inserted into a mammalian expression vector pCDNA3 (Invitrogen, San Diego, CA) through the Hind III and EcoR I sites.

Example 2: Expressing IFNα-Fc in mammalian cells

10^7 NSO cells were mixed with 10μg linearized pCDNA3/IFNα-Fc plasmid in 0.8 ml PBS and kept on ice for 5 min. Electroporation was performed at 200v, 960μF using Gene Pulser (BioRad, Hircules, CA). The cells were then put back on ice for 20 minutes and transferred to a 100mm tissue culture plate in 10ml DMEM supplied with 2% FCS. After incubation at 37°C for two days, the cells were washed and resuspended in the same medium. 0.6 mg/ml G418 was added to start the selection. The cells were plated out in eight 96-well micro plates and incubated at 37°C. Colonies appeared in one week and they were ready for screening in two weeks. The supernatants from each well with a single colony growing were collected. The IFNα-Fc in the supernatant was quantitatively determined by an ELISA assay employing goat anti-human IgG and anti-human Fc conjugated with horseradish peroxidase. The clones with higher ELISA readings and smaller colony size were selected for subcloning. These colonies were transferred to a 24-well plate and supplied with a medium containing G418. The clone with the highest secretion level was expanded and adapted to grow in a spinner. For large scale preparation, the culture supernatant was collected and passed through a protein A agarose column equilibrized by PBS. The protein bound to the protein A was eluted by 50 mM citric acid (pH 3.0) and concentrated by lyophilization.

Example 3: Characterization of the IFNα-Fc hybrid.

The purity of the recombinant protein isolated from NSO culture medium was examined by SDS-PAGE and Western blot. Only one protein band was visible on the blotted membrane stained by ponceau s for total proteins, showing a homogeneity of the protein preparation. The apparent molecular weight of this protein is about 55kd under reducing conditions and 110kd under non-reducing conditions, which is exactly the predicted size for the IFNα-Fc hybrid. The doubling of its apparent molecular weight under non-reducing conditions suggests that the hybrid is in a dimeric form. The recombinant protein can be recognized by both anti-Fc and anti-IFNα antibodies, confirming that it consists of two moieties, the IFNα and the Fc fragment.

The bioactivity assay for the IFNα-Fc was an antiviral assay. Specifically, the assay method used was a modification of the protocol described by Robert M. Friedman et al (Measurement of antiviral activity induced by interferons α, β, and γ, *Current Protocols in Immunology*, 1994, pp. 6.9.1-6.9.8). Briefly, human lung carcinoma cells (A549, ATCC#CCL 185) were seeded in 96-well plates at a density of 40,000 cells/well and incubated at 37°C for 24 hours. 1:2 serially diluted IFNα-Fc hybrid or native IFNα (NIH# GxaO1-901-535) were added and incubated at 37°C for 24 hours. Every sample was done in triplicate. The culture medium was replaced with a fresh one containing encephalomyocarditis virus (ATCC #VR 1298) at a concentration of about 0.1 MOI/cell and incubated at 37°C for a further 48 hours. The dead cells were washed away by pipetting up and down vigorously with PBS. The attached cells were fixed by 2% formaldehyde and stained by giemsa stain. The plates were rinsed with tap water and allowed to dry. The stained cells were dissolved by methanol and the samples were read spectrophotometrically at 595nm. The antiviral activity of IFNα-Fc hybrid was calculated by comparing it with the IFNα standard, and was found to be about 30 to 60% of the activity of the IFNα standard.

It should be understood that the terms and expressions used herein are exemplary only and not limiting, and that the scope of the invention is defined only in the claims which follow, and includes all equivalents of the subject matter of those claims.

SEQUENCE LISTING

(1) General Information:
(i) Applicant: Yu, Liming; Chang, Tse Wen
(ii) Title of Invention: Hybrid with Interferon-α and an Immunoglobulin Fc Linked through a Non-Immunogenic Peptide
(iii) Number of Sequences: 7
(iv) Correspondence Address:
(A) Addressee: Tanox Biosystems, Inc.
(B) Street: 10301 Stella Link Rd.
(C) City: Houston
(D) State: Texas
(E) Country: USA
(F) Zip: 77025
(v) Computer Readable Form:
(A) Medium Type: Diskette, 3.5 inch
(B) Computer: Addonics C142 SVGA
(C) Operating System: DOS 3.30
(D) Software: Wordperfect 5.1
(vi) Current application data:
(A) Application Number:
(B) Filing Date:
(C) Classification:
(vii) Prior Application Data:
(A) Application Number: 08/579,211
(B) Filing Date: 12/28/95
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(C) Reference/Docket Number: 95-2-PCT
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(2) Information for SEQ ID NO:1:
(i) Sequence Characteristics:
(A) Length: 48 nucleic acids
(B) Type: nucleotide
(C) Strandedness: double stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:1:

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GGT GGA GGA TCA 48
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(2) Information for SEQ ID NO:2:
(i) Sequence Characteristics:
(A) Length: 6 amino acids
(B) Type: amino acid
(D) Topology: unknown
(xi) Sequence Description: SEQ ID NO:2:

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(2) Information for SEQ ID NO:3:
(i) Sequence Characteristics:

(A) Length: 81 nucleic acids
(B) Type: nucleic acid
(C) Strandedness: single stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:3:

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GGTGGCCCTC CTGGTGCTCA GCTGCAAGTC AAGCTGCTCT G 81

(2) Information for SEQ ID NO:4:
(i) Sequence Characteristics:
(A) Length: 40 nucleic acids
(B) Type: nucleic acid
(C) Strandedness: single stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:4:

CTCTGCGGAT CCACCTGAGC CACCTTCCTT ACTTCTTAAA 40

(2) Information for SEQ ID NO:5:
(i) Sequence Characteristics:
(A) Length: 58 nucleic acids
(B) Type: nucleic acid
(C) Strandedness: single stranded
(D) Topology: linear
(xi) Sequence Description: SEQ ID NO:5:

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AGTCCAAATA TGGTCCCC 58

(2) Information for SEQ ID NO:6:
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(A) Length: 42 nucleic acids
(B) Type: nucleic acid
(C) Strandedness: double stranded
(D) Topology: linear
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(2) Information for SEQ ID NO:7:
(i) Sequence Characteristics:
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(B) Type: nucleic acid
(C) Strandedness: double stranded
(D) Topology: linear
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1 5 10

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15 20 25

CCT CAA ACC CAC AGC CTG GGT AGC AGG AGG ACC TTG ATG 117
Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
30 35

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             95                 100

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105                 110                 115

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        250                 255                 260

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                265                 270

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    275                 280                 285

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            290                 295

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300                 305                 310

GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC 975
Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala
        315                 320                 325

AAA GGG CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG CCC 1014
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
                330                 335

CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG 1053
Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu
    340                 345                 350

ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC 1092
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
            355                 360

GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC 1131
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
365                 370                 375

AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC 1170
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
        380                 385                 390

TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG 1209
Phe Lys Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp
                395                 400

CAG GAG GGG AAT GTC TTC TCA TGC TCC GTG ATG CAT GAG 1248
Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu
    405                 410                 415

GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC TCC CTG 1287
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
            420                 425
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TCT CTG GGT AAA TAG 1302
Ser Leu Gly Lys
430



What Is Claimed Is:

1. A hybrid molecule comprising an interferon molecule joined at its C-terminal end through a peptide linker to the N-terminal end of the immunoglobulin Fc fragment, the peptide linker comprising the sequence Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser (SEQ ID NO:1).

2. The hybrid molecule of claim 1 in which another interferon molecule is joined at its C-terminal end through the peptide linker to the N-terminal end of a chain of the immunoglobulin Fc fragment, thereby forming a homodimer.

3. The hybrid molecule of claim 2 in which the interferon molecule is IFNα2a or IFNα2b.

4. The hybrid molecule of claim 2 in which the Fc fragment is a γ4 chain Fc fragment.

5. A method of treating hepatitis, hairy cell leukemia, multiple myeloma, or other cancers or viral diseases, comprising administering the hybrid molecule of any of claims 1 to 4.

THIS SHEET HAS BEEN REPLACED

