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(54) Titre : PROTEINES HYBRIDES D’ALBUMINE

(54) Title: ALBUMIN FUSION PROTEINS

(57) Abrégé/Abstract:
The present invention encompasses albumin fusion proteins. Nucleic acid molecules encoding the albumin fusion proteins of the invention are also encompassed by the invention, as are vectors containing these nucleic acids, host cells transformed with these nucleic acids vectors, and methods of making the albumin fusion proteins of the invention and using these nucleic acids, vectors, and/or host cells. Additionally the present invention encompasses pharmaceutical compositions comprising albumin fusion proteins and methods of treating, preventing, or ameliorating diseases, disorders or conditions using albumin fusion proteins of the invention.
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ABSTRACT

The present invention encompasses albumin fusion proteins. Nucleic acid molecules encoding the albumin fusion proteins of the invention are also encompassed by the invention, as are vectors containing these nucleic acids, host cells transformed with these nucleic acids vectors, and methods of making the albumin fusion proteins of the invention and using these nucleic acids, vectors, and/or host cells. Additionally the present invention encompasses pharmaceutical compositions comprising albumin fusion proteins and methods of treating, preventing, or ameliorating diseases, disorders or conditions using albumin fusion proteins of the invention.
THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A nucleic acid molecule comprising a polynucleotide encoding a HSA (human serum albumin)/kex2 leader sequence, a polynucleotide encoding mature human serum albumin, a polynucleotide encoding a mature granulocyte-colony stimulating factor (G-CSF), and a polynucleotide sequence comprising a promoter sequence, a selectable marker sequence, and a region for termination of transcription, wherein said HSA (human serum albumin)/kex2 leader sequence is fused to the N-terminus of mature human serum albumin, wherein said mature human serum albumin is fused to the N-terminus of mature G-CSF, and wherein said albumin fusion protein has G-CSF activity, optionally wherein the nucleic acid molecule is part of an expression cassette.

2. A nucleic acid molecule comprising the cDNA contained in ATCC Deposit No. PTA-3766.

3. A vector comprising the nucleic acid molecule of claim 1 or 2.

4. A vector according to Claim 3 comprising a promoter and termination region operably associated to a nucleic acid molecule encoding a leader sequence and an albumin fusion protein, the nucleic acid comprising the construct contained in ATCC Deposit No. PTA-3766, wherein said leader sequence is a hybrid HSA/kex2 leader sequence comprising the amino acids of SEQ ID NO:1111.

5. A vector according to Claim 3 comprising a promoter and termination region operably associated to a nucleic acid molecule encoding a leader sequence and an albumin fusion protein comprising amino acids 1 to 783 of SEQ ID NO:226, wherein said leader sequence is a hybrid HSA/kex2 leader sequence comprising the amino acids of SEQ ID NO:1111.

6. A vector according to any of Claims 3 to 5, wherein said promoter is a PRB1 promoter.

7. A vector according to any of Claims 3 to 6, wherein said vector is a pSAC35 expression vector.

8. A host cell comprising a vector according to any of Claims 3 to 7.

9. The host cell of claim 8, wherein said host cell is a yeast cell, optionally wherein the yeast cell is a *Saccharomyces cerevisiae* and/or wherein the
yeast cell is glycosylation deficient and/or wherein the yeast cell is protease deficient.

10. A method of producing an albumin fusion protein comprising:

(a) culturing a host cell according to claim 8 or 9 in suitable conditions for expression of the albumin fusion protein; and

(b) isolating the albumin fusion protein.

11. The albumin-G-CSF fusion protein encoded by the nucleic acid molecule according to any one of claims 1 to 7 for use in medicine.

12. The albumin-G-CSF fusion protein encoded by the nucleic acid molecule according to any one of claims 1 to 7 for use in the treatment of inflammatory disorders, cancer, leukaemia, such as myelocytic leukaemia, acute myelogeneous leukaemia and acute lymphoblastic leukaemia, neutropenia, such as primary neutropenia (e.g. Kostmann syndrome), secondary neutropenia, neutropenia in HIV-infected patients and neutropenia associated with chemotherapy, infections associated with neutropenia, myelodysplasia, autoimmune diseases and disorders, psoriasis, wound healing, lymphoma, such as non-Hodgkin's lymphoma, Hodgkin's disease or glycogen storage disease;

or for use in the prevention of inflammatory disorders, neutropenia, such as neutropenia in HIV-infected patients or neutropenia associated with chemotherapy;

or for use in the diagnosis of inflammatory diseases.
Figure 1B
Figure 1C
Figure 2
Figure 3

**Figure 4**

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Comparison of the effect of recombinant human Epo and Epo albumin fusion proteins on the % change in hematocrit from day 0 to day 7: dose-response analysis

Figure 5
Dose schedule: Comparison of the effect of Epo albumin fusion proteins on % change in hematocrit

Figure 6A
Dose schedule: Comparison of the effect of Epo albumin Fusion proteins on % change in hematocrit

% change in hematocrit: day 0 to day 14

Figure 6B
Comparison of the effect of Epo albumin fusion proteins and recombinant human Epo on the proliferation of TF-1 cells.

Figure 7
Comparison of the effect of recombinant human Epo and Epo albumin fusion protein encoded by CID 1997 on the % change in hematocrit from day 0 to day 8: dose-response analysis

Figure 8
Activity of IL-2 albumin fusion protein encoded by CID 1812 in the CTLL-2 proliferation assay

Figure 9
Effect of IL2 albumin fusion protein encoded by CID 1812 on RENCA tumor growth in BALB/c mice: Day 21

Figure 10

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Response of NFS-60 cells to GCSF albumin fusion proteins (supernatants)

GCSF albumin fusion protein supernatants (dilution)

Figure 11
Effects of GCSF albumin fusion protein (GCSF fusion) on total white blood cell count (WBC)

Figure 12

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Dose response of IFNb albumin fusion proteins

Figure 13
Expression of insulin mRNA in INS-1 (832/13) cells

Figure 14
Inhibition of proliferation of HS294T melanoma cells by IFNa albumin fusion protein

Figure 15
SEAP activation with IFNa albumin fusion proteins

Figure 16

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Figure 17