overall survival between placebo and Bevacizumab treated patients were reported in the pivotal AVOREN study presented at the ASCO meeting 2009 (Escudier *et al.,* 2010). Evasion of treatment could arise via different mechanisms including angiogenic redundancy or inefficacy due to the presence of anti-angiogenic forms of VEGF called VEGFxxxb, resulting from an alternative splicing of VEGF pre mRNA (Harper, 2008).

WO 2008/130969 discloses monoclonal antibodies obtained by the injection into a mouse of a mixture of said five antigens, said antibodies being able to bind to five antigens: human IL-8 (CXCL8), Gro-alpha (CXCL1), Gro-beta (CXCL2), Gro-gamma (CXCL3) and ENA-78 (CXCL5), and discloses the use of said antibodies in an inhaled LPS acute lung inflammatory model in non-human primates. However, WO 2008/130969 does not disclose a monoclonal antibody binding to CXCL1, CXCL7 and CXCL8.

WO 2011/100271 discloses monoclonal antibodies able to bind to human IL-8 (CXCL8), Gro-alpha (CXCL1), Gro-beta (CXCL2), Gro-gamma (CXCL3), GCP2, NAP2 (CXCL7) and ENA-78 (CXCL5). However, WO 2011/100271 does not disclose the affinity of said monoclonal antibodies for said antigens.

There remains therefore a significant need in the art for new and improved anti-angiogenic compounds that increase patients' lifespan and avoid relapse and disease progression to death. The present inventors have made a significant step forward with the invention disclosed herein.

SUMMARY OF INVENTION

In one aspect, the present invention provides an isolated antibody or a fragment thereof, which is directed to the human chemokines CXCL1, CXCL7 and CXCL8, said antibody or fragment being capable of binding to the human chemokine CXCL1 with an equilibrium dissociation constant (KD) of at most 16 nM, to the human chemokine CXCL7 with an equilibrium dissociation constant (KD) of at most 5 nM and to the human chemokine CXCL8 with an equilibrium dissociation constant (KD) of at most 45 nM, as determined by surface plasmon resonance wherein the light chain variable region (V_L) comprises:

- a light chain CDR1 (LC-CDR1) of sequence SEQ ID NO: 1 or of sequence having at least 90% of identity with SEQ ID NO: 1 over the entire length of SEQ ID NO: 1; and

- a light chain CDR2 (LC-CDR2) of sequence SEQ ID NO: 2 or of sequence having at least 90% of identity with SEQ ID NO: 2 over the entire length of SEQ ID NO: 2; and

- a light chain CDR3 (LC-CDR3) of sequence SEQ ID NO: 3 or of sequence having at least 90% of identity with SEQ ID NO: 3 over the entire length of SEQ ID NO: 3; and

wherein the heavy chain variable region (VH) comprises:

- a heavy chain CDR1 (HC-CDR1) of sequence SEQ ID NO: 4 or of sequence having at least 90% of identity with SEQ ID NO: 4 over the entire length of SEQ ID NO: 4; and

- a heavy chain CDR2 (HC-CDR2) of sequence SEQ ID NO: 5 or of sequence having at least 90% of identity with SEQ ID NO: 5 over the entire length of SEQ ID NO: 5; and

- a heavy chain CDR3 (HC-CDR3) of sequence SEQ ID NO: 6 or of sequence having at least 90% of identity with SEQ ID NO: 6 over the entire length of SEQ ID NO: 6;

or

wherein the light chain variable region (V_L) comprises:

- a light chain CDR1 (LC-CDR1) of sequence SEQ ID NO: 7 or of sequence having at least 90% of identity with SEQ ID NO: 7 over the entire length of SEQ ID NO: 7; and

- a light chain CDR2 (LC-CDR2) of sequence SEQ ID NO: 8 or of sequence having at least 90% of identity with SEQ ID NO: 8 over the entire length of SEQ ID NO: 8; and

- a light chain CDR3 (LC-CDR3) of sequence SEQ ID NO: 9 or of sequence having at least 90% of identity with SEQ ID NO: 9 over the entire length of SEQ ID NO: 9; and

wherein the heavy chain variable region (VH) comprises:

- a heavy chain CDR1 (HC-CDR1) of sequence SEQ ID NO: 10 or of sequence having at least 90% of identity with SEQ ID NO: 10 over the entire length of SEQ ID NO: 10; and

- a heavy chain CDR2 (HC-CDR2) of sequence SEQ ID NO: 11 or of sequence having at least 90% of identity with SEQ ID NO: 11 over the entire length of SEQ ID NO: 11; and

- a heavy chain CDR3 (HC-CDR3) of sequence SEQ ID NO: 12 or of sequence having at least 90% of identity with SEQ ID NO: 12 over the entire length of SEQ ID NO: 12.

In another aspect, the present invention provides an isolated nucleic acid molecule encoding an antibody or a fragment thereof as disclosed herein. In yet another aspect, the present invention provides a vector comprising a nucleic acid molecule as disclosed herein. In

yet another aspect, the present invention provides a host cell comprising a nucleic acid molecule as disclosed herein or a vector as disclosed herein.

In one aspect, the present invention also provides a pharmaceutical composition comprising at least one compound selected from the group consisting of an antibody and a fragment thereof as disclosed herein, an isolated nucleic acid molecule as disclosed herein and a vector as disclosed herein. In another aspect, the present invention provides an antibody or a fragment thereof as disclosed herein or an isolated nucleic acid molecule as disclosed herein or a vector as disclosed herein for use as a medicament.

In another aspect, the present invention provides an antibody or a fragment thereof as disclosed herein or an isolated nucleic acid molecule as disclosed herein or a vector as disclosed herein for use in the treatment and/or prevention of a pathological angiogenesis disease or a disease characterized by undesirable excessive neovascularization, in particular chosen in the group consisting of cancers with abnormal angiogenesis, ophthalmological diseases with abnormal angiogenesis, rheumatoid arthritis, psoriasis, angioma, endometriosis and kaposi sarcoma.

In another aspect, the present invention provides an *in vitro* diagnostic and/or prognostic method of a pathological angiogenesis disease or a disease characterized by undesirable excessive neovascularization in a subject, said method comprising: i) determining the expression and/or the level of expression of at least one human chemokine chosen in the group consisting of CXCL1, CXCL7 and CXCL8 in a biological sample of said subject using at least one antibody and/or fragment thereof as disclosed herein.

In yet another aspect, the present invention also provides a kit for carrying out the method as disclosed herein, comprising at least one antibody and/or fragment thereof as disclosed herein.

DESCRIPTION

The purpose of the invention is to fulfill this need by providing new compounds, which make it possible to solve in whole or part the problems mentioned-above.

Unexpectedly, the inventors have demonstrated that their newly developed antibodies directed to the chemokines CXCL1, CXCL7 and CXCL8 are able to inhibit efficiently tumor growth, in particular in a mouse model of RCC (Clear cell renal cell carcinoma). RCC is one

of the most relevant models for the study of anti-angiogenic therapies, since RCC are highly vascularized tumors (that arise due to mutations in the von Hippel Lindau gene).

These results are unexpected, since several recent papers describe that suppression of chemokines, including CXCL8, does not inhibit but on the contrary promotes tumor growth. In this way, Yao et al. (2007) have demonstrated that suppression of CXCL-8 promoted tumor growth in nude mice.

Surprisingly, these newly developed antibodies are able to inhibit tumor growth more efficiently than commercial anti-CXCL1, anti-CXCL7 and anti-CXCL8 antibodies used alone

CLAIMS

1. An isolated antibody or a fragment thereof, which is directed to the human chemokines CXCL1, CXCL7 and CXCL8, said antibody or fragment being capable of binding to the human chemokine CXCL1 with an equilibrium dissociation constant (K_D) of at most 16 nM, to the human chemokine CXCL7 with an equilibrium dissociation constant (K_D) of at most 5 nM and to the human chemokine CXCL8 with an equilibrium dissociation constant (K_D) of at most 45 nM, as determined by surface plasmon resonance, wherein the light chain variable region (V_L) comprises:

- a light chain CDR1 (LC-CDR1) of sequence SEQ ID NO: 1 or of sequence having at least 90% of identity with SEQ ID NO: 1 over the entire length of SEQ ID NO: 1; and

- a light chain CDR2 (LC-CDR2) of sequence SEQ ID NO: 2 or of sequence having at least 90% of identity with SEQ ID NO: 2 over the entire length of SEQ ID NO: 2; and

- a light chain CDR3 (LC-CDR3) of sequence SEQ ID NO: 3 or of sequence having at least 90% of identity with SEQ ID NO: 3 over the entire length of SEQ ID NO: 3; and

wherein the heavy chain variable region (VH) comprises:

- a heavy chain CDR1 (HC-CDR1) of sequence SEQ ID NO: 4 or of sequence having at least 90% of identity with SEQ ID NO: 4 over the entire length of SEQ ID NO: 4; and

- a heavy chain CDR2 (HC-CDR2) of sequence SEQ ID NO: 5 or of sequence having at least 90% of identity with SEQ ID NO: 5 over the entire length of SEQ ID NO: 5; and

- a heavy chain CDR3 (HC-CDR3) of sequence SEQ ID NO: 6 or of sequence having at least 90% of identity with SEQ ID NO: 6 over the entire length of SEQ ID NO: 6;

or

wherein the light chain variable region (V_L) comprises:

- a light chain CDR1 (LC-CDR1) of sequence SEQ ID NO: 7 or of sequence having at least 90% of identity with SEQ ID NO: 7 over the entire length of SEQ ID NO: 7; and

- a light chain CDR2 (LC-CDR2) of sequence SEQ ID NO: 8 or of sequence having at least 90% of identity with SEQ ID NO: 8 over the entire length of SEQ ID NO: 8; and

- a light chain CDR3 (LC-CDR3) of sequence SEQ ID NO: 9 or of sequence having at least 90% of identity with SEQ ID NO: 9 over the entire length of SEQ ID NO: 9; and

wherein the heavy chain variable region (VH) comprises:

- a heavy chain CDR1 (HC-CDR1) of sequence SEQ ID NO: 10 or of sequence having at least 90% of identity with SEQ ID NO: 10 over the entire length of SEQ ID NO: 10; and

- a heavy chain CDR2 (HC-CDR2) of sequence SEQ ID NO: 11 or of sequence having at least 90% of identity with SEQ ID NO: 11 over the entire length of SEQ ID NO: 11; and

- a heavy chain CDR3 (HC-CDR3) of sequence SEQ ID NO: 12 or of sequence having at least 90% of identity with SEQ ID NO: 12 over the entire length of SEQ ID NO: 12.

2. The antibody according to claim 1, wherein the light chain variable region (V_L) comprises:

- a light chain CDR1 (LC-CDR1) of sequence SEQ ID NO: 1 or of sequence having at least 95% of identity with SEQ ID NO: 1 over the entire length of SEQ ID NO: 1; and

- a light chain CDR2 (LC-CDR2) of sequence SEQ ID NO: 2 or of sequence having at least 95% of identity with SEQ ID NO: 2 over the entire length of SEQ ID NO: 2; and

- a light chain CDR3 (LC-CDR3) of sequence SEQ ID NO: 3 or of sequence having at least 95% of identity with SEQ ID NO: 3 over the entire length of SEQ ID NO: 3; and

wherein the heavy chain variable region (VH) comprises:

- a heavy chain CDR1 (HC-CDR1) of sequence SEQ ID NO: 4 or of sequence having at least 95% of identity with SEQ ID NO: 4 over the entire length of SEQ ID NO: 4; and

- a heavy chain CDR2 (HC-CDR2) of sequence SEQ ID NO: 5 or of sequence having at least 95% of identity with SEQ ID NO: 5 over the entire length of SEQ ID NO: 5; and

- a heavy chain CDR3 (HC-CDR3) of sequence SEQ ID NO: 6 or of sequence having at least 95% of identity with SEQ ID NO: 6 over the entire length of SEQ ID NO: 6;

or

wherein the light chain variable region (V_L) comprises:

- a light chain CDR1 (LC-CDR1) of sequence SEQ ID NO: 7 or of sequence having at least 95% of identity with SEQ ID NO: 7 over the entire length of SEQ ID NO: 7; and

- a light chain CDR2 (LC-CDR2) of sequence SEQ ID NO: 8 or of sequence having at least 95% of identity with SEQ ID NO: 8 over the entire length of SEQ ID NO: 8; and

- a light chain CDR3 (LC-CDR3) of sequence SEQ ID NO: 9 or of sequence having at least 95% of identity with SEQ ID NO: 9 over the entire length of SEQ ID NO: 9; and

wherein the heavy chain variable region (VH) comprises:

- a heavy chain CDR1 (HC-CDR1) of sequence SEQ ID NO: 10 or of sequence having at least 95% of identity with SEQ ID NO: 10 over the entire length of SEQ ID NO: 10; and

- a heavy chain CDR2 (HC-CDR2) of sequence SEQ ID NO: 11 or of sequence having at least 95% of identity with SEQ ID NO: 11 over the entire length of SEQ ID NO: 11; and

- a heavy chain CDR3 (HC-CDR3) of sequence SEQ ID NO: 12 or of sequence having at least 95% of identity with SEQ ID NO: 12 over the entire length of SEQ ID NO: 12.

3. The antibody according to claim 1, wherein the light chain variable region (V_L) comprises:

- a light chain CDR1 (LC-CDR1) of sequence SEQ ID NO: 1 or of sequence having at least 96% of identity with SEQ ID NO: 1 over the entire length of SEQ ID NO: 1; and

- a light chain CDR2 (LC-CDR2) of sequence SEQ ID NO: 2 or of sequence having at least 96% of identity with SEQ ID NO: 2 over the entire length of SEQ ID NO: 2; and

- a light chain CDR3 (LC-CDR3) of sequence SEQ ID NO: 3 or of sequence having at least 96% of identity with SEQ ID NO: 3 over the entire length of SEQ ID NO: 3; and

wherein the heavy chain variable region (VH) comprises:

- a heavy chain CDR1 (HC-CDR1) of sequence SEQ ID NO: 4 or of sequence having at least 96% of identity with SEQ ID NO: 4 over the entire length of SEQ ID NO: 4; and

- a heavy chain CDR2 (HC-CDR2) of sequence SEQ ID NO: 5 or of sequence having at least 96% of identity with SEQ ID NO: 5 over the entire length of SEQ ID NO: 5; and

- a heavy chain CDR3 (HC-CDR3) of sequence SEQ ID NO: 6 or of sequence having at least 96% of identity with SEQ ID NO: 6 over the entire length of SEQ ID NO: 6;

or

wherein the light chain variable region (V_L) comprises:

- a light chain CDR1 (LC-CDR1) of sequence SEQ ID NO: 7 or of sequence having at least 96% of identity with SEQ ID NO: 7 over the entire length of SEQ ID NO: 7; and

- a light chain CDR2 (LC-CDR2) of sequence SEQ ID NO: 8 or of sequence having at least 96% of identity with SEQ ID NO: 8 over the entire length of SEQ ID NO: 8; and

- a light chain CDR3 (LC-CDR3) of sequence SEQ ID NO: 9 or of sequence having at least 96% of identity with SEQ ID NO: 9 over the entire length of SEQ ID NO: 9; and

wherein the heavy chain variable region (VH) comprises:

- a heavy chain CDR1 (HC-CDR1) of sequence SEQ ID NO: 10 or of sequence having at least 96% of identity with SEQ ID NO: 10 over the entire length of SEQ ID NO: 10; and

- a heavy chain CDR2 (HC-CDR2) of sequence SEQ ID NO: 11 or of sequence having at least 96% of identity with SEQ ID NO: 11 over the entire length of SEQ ID NO: 11; and

- a heavy chain CDR3 (HC-CDR3) of sequence SEQ ID NO: 12 or of sequence having at least 96% of identity with SEQ ID NO: 12 over the entire length of SEQ ID NO: 12.

4. The antibody according to any one of claims 1 to 3, wherein the light chain variable region (V_L) comprises:

- a light chain CDR1 (LC-CDR1) of sequence SEQ ID NO: 1; and

- a light chain CDR2 (LC-CDR2) of sequence SEQ ID NO: 2; and

- a light chain CDR3 (LC-CDR3) of sequence SEQ ID NO: 3; and

wherein the heavy chain variable region (VH) comprises:

- a heavy chain CDR1 (HC-CDR1) of sequence SEQ ID NO: 4; and

- a heavy chain CDR2 (HC-CDR2) of sequence SEQ ID NO: 5; and

- a heavy chain CDR3 (HC-CDR3) of sequence SEQ ID NO: 6;

or

wherein the light chain variable region (V_L) comprises:

- a light chain CDR1 (LC-CDR1) of sequence SEQ ID NO: 7; and

- a light chain CDR2 (LC-CDR2) of sequence SEQ ID NO: 8; and

- a light chain CDR3 (LC-CDR3) of sequence SEQ ID NO: 9; and

wherein the heavy chain variable region (VH) comprises:

- a heavy chain CDR1 (HC-CDR1) of sequence SEQ ID NO: 10; and

- a heavy chain CDR2 (HC-CDR2) of sequence SEQ ID NO: 11; and

- a heavy chain CDR3 (HC-CDR3) of sequence SEQ ID NO: 12.

5. The antibody according to any one of claims 1 to 4, wherein the sequence of the light chain variable region (V_L) comprises or consists of the sequence SEQ ID NO: 13 or of a sequence having at least 90 % of identity with SEQ ID NO: 13 over the entire length of SEQ ID NO: 13; and wherein the sequence of the heavy chain variable region (V_H) comprises or consists of the sequence SEQ ID NO: 14 or of a sequence having at least 90 % of identity with SEQ ID NO: 14 over the entire length of SEQ ID NO: 14.

6. The antibody according to claim 5, wherein the sequence of the light chain variable region (V_L) comprises or consists of the sequence SEQ ID NO: 13 or of a sequence having at least 95 % of identity with SEQ ID NO: 13 over the entire length of SEQ ID NO: 13; and wherein the sequence of the heavy chain variable region (V_H) comprises or consists of the sequence SEQ ID NO: 14 or of a sequence having at least 95 % of identity with SEQ ID NO: 14 over the entire length of SEQ ID NO: 14.

7. The antibody according to claim 5, wherein the sequence of the light chain variable region (V_L) comprises or consists of the sequence SEQ ID NO: 13 or of a sequence having at least 96 % of identity with SEQ ID NO: 13 over the entire length of SEQ ID NO: 13; and wherein the sequence of the heavy chain variable region (V_H) comprises or consists of the sequence SEQ ID NO: 14 or of a sequence having at least 96 % of identity with SEQ ID NO: 14 over the entire length of SEQ ID NO: 14.

8. The antibody according to claim 5, wherein the sequence of the light chain variable region (V_L) comprises or consists of the sequence SEQ ID NO: 13; and wherein the sequence of the heavy chain variable region (V_H) comprises or consists of the sequence SEQ ID NO: 14.

9. The antibody according to any one of claims 1 to 4, wherein the sequence of the light chain variable region (V_L) comprises or consists of the sequence SEQ ID NO: 15 or of a sequence having at least 90 % of identity with SEQ ID NO: 15 over the entire length of SEQ ID NO: 15; and wherein the sequence of the heavy chain variable region (V_H) comprises or consists of the sequence SEQ ID NO: 16 or of a sequence having at least 90 % of identity with SEQ ID NO: 16 over the entire length of SEQ ID NO: 16.

10. The antibody according to claim 9, wherein the sequence of the light chain variable region (V_L) comprises or consists of the sequence SEQ ID NO: 15 or of a sequence having at least 95 % of identity with SEQ ID NO: 15 over the entire length of SEQ ID NO: 15; and wherein the sequence of the heavy chain variable region (V_H) comprises or consists of the sequence SEQ ID NO: 16 or of a sequence having at least 95 % of identity with SEQ ID NO: 16 over the entire length of SEQ ID NO: 16.

11. The antibody according to claim 9, wherein the sequence of the light chain variable region (V_L) comprises or consists of the sequence SEQ ID NO: 15 or of a sequence having at least 96 % of identity with SEQ ID NO: 15 over the entire length of SEQ ID NO: 15; and wherein the sequence of the heavy chain variable region (V_H) comprises or consists of the sequence SEQ ID NO: 16 or of a sequence having at least 96 % of identity with SEQ ID NO: 16 over the entire length of SEQ ID NO: 16.

12. The antibody according to claim 9, wherein the sequence of the light chain variable region (V_L) comprises or consists of the sequence SEQ ID NO: 15; and wherein the sequence of the heavy chain variable region (V_H) comprises or consists of the sequence SEQ ID NO: 16.

13. The antibody according to any one of claims 1 to 12, wherein said antibody is a monoclonal antibody.

14. The antibody according to any one of claims 5 to 8, said antibody being produced by the hybridoma deposited on 11.09.2012 at the Collection Nationale de Cultures de Microorganismes (CNCM) under the number CNCM I-4618.

15. The antibody according to any one of claims 9 to 12, said antibody being produced by the hybridoma deposited on 11.09.2012 at the Collection Nationale de Cultures de Microorganismes (CNCM) under the number CNCM I-4617.

16. An isolated nucleic acid molecule encoding an antibody or a fragment thereof as defined in any one of claims 1 to 15.

17. A vector comprising a nucleic acid molecule as defined in claim 16.

18. A host cell comprising a nucleic acid molecule as defined in claim 16 or a vector as defined in claim 17.

19. A pharmaceutical composition comprising at least one compound selected from the group consisting of an antibody and a fragment thereof as defined in any one of claims 1 to 15, an isolated nucleic acid molecule as defined in claim 16 and a vector as defined in claim 17.

20. An antibody or a fragment thereof as defined in any one of claims 1 to 15 or an isolated nucleic acid molecule as defined in claim 16 or a vector as defined in claim 17 for use as a medicament.

21. An antibody or a fragment thereof as defined in any one of claims 1 to 15 or an isolated nucleic acid molecule as defined in claim 16 or a vector as defined in claim 17 for use in the treatment and/or prevention of a pathological angiogenesis disease or a disease characterized by undesirable excessive neovascularization, in particular chosen in the group

consisting of cancers with abnormal angiogenesis, ophthalmological diseases with abnormal angiogenesis, rheumatoid arthritis, psoriasis, angioma, endometriosis and kaposi sarcoma.

22. An *in vitro* diagnostic and/or prognostic method of a pathological angiogenesis disease or a disease characterized by undesirable excessive neovascularization in a subject, said method comprising:

i) determining the expression and/or the level of expression of at least one human chemokine chosen in the group consisting of CXCL1, CXCL7 and CXCL8 in a biological sample of said subject using at least one antibody and/or fragment thereof according to any one of claim 1 to 15.

23. A kit for carrying out the method according to claim 22, comprising at least one antibody and/or fragment thereof according to any one of claim 1 to 15.