MODULATION OF MONOCYTES, OR PRECURSORS THEREOF, DIFFERENTIATION

~~SUMMARY OF THE INVENTION~~

The invention is generally directed to promoting immune responses driven by dendritic cells (DCs), and optionally M1-type (macrophage M1 polarization), by administering a compound that modulates monocyte, or precursors thereof, differentiation. The invention is directed to the use of an antibody able to bind to CSF-1R for modulating monocyte, or precursors thereof, differentiation, more particularly for inducing monocyte, or precursors thereof, differentiation towards dendritic cells and/or M1-polarized macrophages instead of M2-polarized macrophages. The invention is also directed to methods for evaluating the dose efficacy of an antibody able to bind to CSF-1R in a patient by assessing *in vivo* or *in vitro* monocyte, or precursors thereof, differentiation into macrophages (M1- or M2-type) and/or dendritic cells. The invention is further directed to post-treatment companion test and assays to assess the effect of an antibody able to bind to CSF-1R on a subject being treated.

During inflammation, circulating monocytes are recruited to the site of inflammation where they adopt a macrophage phenotype dictated by the presence of specific cytokines and growth factors. Mature macrophages are divided into two populations, M1-polarized or "classically activated" and M2-polarized or "alternatively activated." Macrophages are important tumor-infiltrating cells and play pivotal roles in tumor growth and metastasis. In most solid tumors, the

TAMs exhibit a M2 profile characterized not only by a IL-12^{low}IL-10^{high} phenotype but also high FcR-mediated phagocytic capacity associated with regulatory functions (Schmieder et al. 2012, *Semin Cancer Biol.*, 22, 289-297). The haemoglobin scavenger receptor (CD163) has been identified as a marker of M2-polarized macrophages which is expressed by TAMs (Ambarus et al. 2012, 375,196-206). TAMs can represent the most abundant immunosuppressive cell population in the tumor microenvironment, recruited by CSF-1 and CCL-2 (MCP-1) (Sica et al. 2006, *Eur J Cancer.*, 42, 717-727).

Similarly, the alternatively activated M2 macrophages have been implicated in several pathologies, the most prominent of which are allergy and asthma (Duffield, 2003, Clin. Sci. 104, 27; Gordon, 2003, Nat. Rev. Immunol., 3, 23; Dagupta and Keegan, J. Innate Immun., 2012, 4, 478).

The Inventors have now shown that certain monoclonal antibodies are able to promote DCs, and optionally M1-type (macrophage M1 polarization), immune response by administering a compound that modulates monocyte, or precursors thereof, differentiation. They have shown that the said monoclonal antibodies are able to down regulate surface FcγRI (CD64) and FcγRIII (CD16) to down regulate MCP-1 (Macrophage Chemotactic Protein 1, also called CCL-2), IL-6, MMP9 and/or IL-10 production, and to promote IL-12, IL-1□, TNF-α production. They have further shown that the said monoclonal antibodies inhibit the differentiation of $CD163^{+}$ M2-type macrophages from human monocytes and instead promote monocyte differentiation into DCs, and optionally macrophage M1 polarization.

Summary

In one aspect, there is provided use of an effective dose of an antibody able to bind to CSF-1R in the manufacture of a medicament for the generation of dendritic cells, wherein said medicament is to be exposed to suitable precursor cells.

In another aspect, there is provided a method for the generation ex vivo of dendritic cells by exposing suitable precursor cells to an effective dose of an antibody able to bind to CSF-1R.

Disclosure of Invention

The invention is broadly directed to methods for immunomodulation by modulating monocyte differentiation.

The invention is more specifically directed to methods for the generation *in vivo* or *ex vivo* of dendritic cells by

CLAIMS

1. A method for the generation *in vivo* or *ex vivo* of dendritic cells by exposing suitable precursor cells to an effective dose of an antibody able to bind to CSF-1R.

2. Method of claim 1 wherein said method is an *in vivo* method for increasing the dendritic cells pool in a patient suffering from conditions associated with undesirable macrophage differentiation and M2 polarization, wherein said method comprises the step of administering to the said patient an effective amount of an antibody able to bind to CSF-1R.

3. The method of claim 2, wherein patient is further suffering from conditions associated with CSF-1R activity.

4. The method of any of the preceding claims, wherein said method decreases the macrophage M2 pool.

5. The method of any of the preceding claims, wherein said method further increases M1-type macrophage pool.

6. The method of any of the preceding claims, wherein said method inhibits macrophage MCP-1, IL10 and IL-6 production.

7. The method of any of the preceding claims, wherein said method down regulates surface FcγRI (CD64) and FcγRIII (CD16) expression on macrophages.

8. The method of any of the preceding claims, wherein said method up-regulates IL-12/IL10 ratio in patient samples, e.g blood samples.

9. The method of any of the preceding claims, wherein said method reduces at least one of the followings:
(i) TAM recruitment into tumor;
(ii) at least one macrophage pro-tumoral functions;
(iii) tumor angiogenesis;
(iv) tumor invasion and metastasis;
(v) tumor growth;
(vi) tumor cell proliferation

10. The method of any of the preceding claims, wherein said conditions associated with undesirable M2 polarization is selected in the group consisting of cancer, asthma, allergy and progressive fibrosis diseases.

11. The method of any of the preceding claims, wherein said antibody able to bind to CSF-1R is an antibody that binds to at least one epitope located between position amino acids 20 to 41 of SEQ ID N0:23.

12. The method of any of the preceding claims, wherein said antibody able to bind to CSF-1R is an antibody that binds to one epitope located between position amino acids 20 to 39 of SEQ ID N0:23, to amino acids Asn72, Ser94-Ala95-Ala96, Lys102, Asp131-Pro132-Val133 and Trp159 of SEQ ID N0:23.

13. The method of any of the preceding claims, wherein said antibody able to bind to human CSF-1R is an antibody that

does not compete with IL-34 ligand for binding to the CSF-1R receptor.

14. The method of any of the preceding claims, wherein said antibody able to bind to human CSF-1R is an antibody that competes partially with CSF-1 ligand for binding to the CSF-1R receptor.

15. The method of any of the preceding claims, wherein said antibody able to bind to human CSF-1R is an antibody that binds specifically to human CSF-1R, comprising:

(a) a first variable region being defined by the following formula

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

wherein:

FR1, FR2, FR3 and FR4 are each framework regions;
CDR1, CDR2 and CDR3 are each complementarity determining regions;

wherein:

CDR1 has at least five consecutive amino acids and is defined in the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:1;
CDR2 has at least five consecutive amino acids and is defined in the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:1; and
CDR3 has at least five consecutive amino acids and is defined in the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:1;

and

(b a second variable region being defined by the following formula

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

wherein:

FR1, FR2, FR3 and FR4 are each framework regions;
CDR1, CDR2 and CDR3 are each complementarity determining regions;

wherein:

CDR1 has at least five consecutive amino acids and is defined in the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:2;
CDR2 has at least five consecutive amino acids and is defined in the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:2; and
CDR3 has at least five consecutive amino acids and is defined in the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:2.

16. The method of any of the preceding claims, wherein said antibody able to bind to CSF-1R is comprising (a) an heavy chain consisting in SEQ ID N0:24, and (b) a light chain consisting in SEQ ID N0:28.

CLAIMS

1. Use of an effective dose of an antibody able to bind to CSF-1R in the manufacture of a medicament for the generation of dendritic cells, wherein said medicament is to be exposed to suitable precursor cells.

2. The use of claim 1 wherein said medicament is for increasing the dendritic cells pool in a patient suffering from conditions associated with undesirable macrophage differentiation and M2 polarization.

3. The use of claim 2, wherein patient is further suffering from conditions associated with CSF-1R activity.

4. The use of any of the preceding claims, wherein said medicament decreases the macrophage M2 pool.

5. The use of any of the preceding claims, wherein said medicament further increases M1-type macrophage pool.

6. The use of any of the preceding claims, wherein said medicament inhibits macrophage MCP-1, IL10 and IL-6 production.

7. The use of any of the preceding claims, wherein said medicament down regulates surface FcγRI (CD64) and FcγRIII (CD16) expression on macrophages.

8. The use of any of the preceding claims, wherein said medicament up-regulates IL-12/IL10 ratio in patient samples, e.g blood samples.

9. The use of any of the preceding claims, wherein said medicament reduces at least one of the followings:

(i) TAM recruitment into tumor;
(ii) at least one macrophage pro-tumoral functions;
(iii) tumor angiogenesis;
(iv) tumor invasion and metastasis;
(v) tumor growth;
(vi) tumor cell proliferation

10. The use of any of the preceding claims, wherein said conditions associated with undesirable M2 polarization is selected in the group consisting of cancer, asthma, allergy and progressive fibrosis diseases.

11. The use of any of the preceding claims, wherein said antibody able to bind to CSF-1R is an antibody that binds to at least one epitope located between position amino acids 20 to 41 of SEQ ID N0:23.

12. The use of any of the preceding claims, wherein said antibody able to bind to CSF-1R is an antibody that binds to one epitope located between position amino acids 20 to 39 of SEQ ID N0:23, to amino acids Asn72, Ser94-Ala95-Ala96, Lys102, Asp131-Pro132-Val133 and Trp159 of SEQ ID N0:23.

13. The use of any of the preceding claims, wherein said antibody able to bind to human CSF-1R is an antibody that does not compete with IL-34 ligand for binding to the CSF-1R receptor.

14. The use of any of the preceding claims, wherein said antibody able to bind to human CSF-1R is an antibody that competes partially with CSF-1 ligand for binding to the CSF-1R receptor.

15. The use of any of the preceding claims, wherein said antibody able to bind to human CSF-1R is an antibody that binds specifically to human CSF-1R, comprising:

(a) a first variable region being defined by the following formula

FR1 - CDR1 – FR2 – CDR2 – FR3 – CDR3 – FR4

wherein:

FR1, FR2, FR3 and FR4 are each framework regions;

CDR1, CDR2 and CDR3 are each complementarity determining regions;

wherein:

CDR1 has at least five consecutive amino acids and is defined in the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:1;

CDR2 has at least five consecutive amino acids and is defined in the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:1; and

CDR3 has at least five consecutive amino acids and is defined in the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:1;

and

(b a second variable region being defined by the following formula

FR1 - CDR1 – FR2 – CDR2 – FR3 – CDR3 – FR4

wherein:

FR1, FR2, FR3 and FR4 are each framework regions;

CDR1, CDR2 and CDR3 are each complementarity determining regions;

wherein:

CDR1 has at least five consecutive amino acids and is defined in the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:2;

CDR2 has at least five consecutive amino acids and is defined in the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:2; and

CDR3 has at least five consecutive amino acids and is defined in the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:2.

16. The use of any of the preceding claims, wherein said antibody able to bind to CSF-1R is comprising (a) an heavy chain consisting in SEQ ID N0:24, and (b) a light chain consisting in SEQ ID N0:28.

17. A method for the generation ex vivo of dendritic cells by exposing suitable precursor cells to an effective dose of an antibody able to bind to CSF-1R.

18. The method of claim 17, wherein said method decreases the macrophage M2 pool.

19. The method of claim 17 or 18, wherein said method further increases M1-type macrophage pool.

20. The method of any one of the claims 17 to 19, wherein said method inhibits macrophage MCP-1, IL10 and IL-6 production.

21. The method of any one of the claims 17 to 20, wherein said method down regulates surface FcγRI (CD64) and FcγRIII (CD16) expression on macrophages.

22. The method of any one of the claims 17 to 21, wherein said method up-regulates IL-12/IL10 ratio in patient samples, e.g blood samples.

23. The method of any one of the claims 17 to 22, wherein said method reduces at least one of the followings:

(i) TAM recruitment into tumor;

(ii) at least one macrophage pro-tumoral functions;
(iii) tumor angiogenesis;
(iv) tumor invasion and metastasis;
(v) tumor growth;
(vi) tumor cell proliferation

24. The method of any one of the claims 17 to 23, wherein said conditions associated with undesirable M2 polarization is selected in the group consisting of cancer, asthma, allergy and progressive fibrosis diseases.

25. The method of any one of the claims 17 to 24, wherein said antibody able to bind to CSF-1R is an antibody that binds to at least one epitope located between position amino acids 20 to 41 of SEQ ID N0:23.

26. The method of any one of the claims 17 to 25, wherein said antibody able to bind to CSF-1R is an antibody that binds to one epitope located between position amino acids 20 to 39 of SEQ ID N0:23, to amino acids Asn72, Ser94-Ala95-Ala96, Lys102, Asp131-Pro132-Val133 and Trp159 of SEQ ID N0:23.

27. The method of any one of the claims 17 to 26, wherein said antibody able to bind to human CSF-1R is an antibody that does not compete with IL-34 ligand for binding to the CSF-1R receptor.

28. The method of any one of the claims 17 to 27, wherein said antibody able to bind to human CSF-1R is an antibody that competes partially with CSF-1 ligand for binding to the CSF-1R receptor.

29. The method of any one of the claims 17 to 28, wherein said antibody able to bind to human CSF-1R is an antibody that binds specifically to human CSF-1R, comprising:
(a) a first variable region being defined by the following formula

FR1 - CDR1 – FR2 – CDR2 – FR3 – CDR3 – FR4

wherein:
FR1, FR2, FR3 and FR4 are each framework regions;
CDR1, CDR2 and CDR3 are each complementarity determining regions;
wherein:
CDR1 has at least five consecutive amino acids and is defined in the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:1;

CDR2 has at least five consecutive amino acids and is defined in the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:1; and
CDR3 has at least five consecutive amino acids and is defined in the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:1;
and
(b a second variable region being defined by the following formula

FR1 - CDR1 – FR2 – CDR2 – FR3 – CDR3 – FR4

wherein:
FR1, FR2, FR3 and FR4 are each framework regions;
CDR1, CDR2 and CDR3 are each complementarity determining regions;
wherein:
CDR1 has at least five consecutive amino acids and is defined in the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:2;
CDR2 has at least five consecutive amino acids and is defined in the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:2; and
CDR3 has at least five consecutive amino acids and is defined in the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:2.

30. The method of any one of the claims 17 to 29, wherein said antibody able to bind to CSF-1R is comprising (a) an heavy chain consisting in SEQ ID N0:24, and (b) a light chain consisting in SEQ ID N0:28.