MODULATION OF MONOCYTES, OR PRECURSORS THEREOF, DIFFERENTIATION

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

existence of macrophages is advantageous for tumor growth and metastasis. Recent studies indicate that tumor-associated macrophages (TAMs) show a M2 phenotype. These tumor-associated macrophages (TAM) produce interleukin IL-10 and transforming growth factor (TGF) β to suppress general antitumor immune responses. Meanwhile, TAMs promote tumor neo-angiogenesis by the secretion of pro-angiogenic factors and define the invasive microenvironment to facilitate tumor metastasis and dissemination. For these reasons, selective depletion of M2 TAMs has been considered as a novel approach to anti-cancer therapy (Sica et al., 2006, European Journal of Cancer, 42,717-727).

Macrophages participate in immune responses to tumors in a polarized manner. The M1 differentiation is triggered by GM-CSF and further stimulated by interferon-γ (IFN-γ), bacterial lipopolysaccharide (LPS), or tumor necrosis factor α (TNFα), and is mediated by several signal transduction pathways involving signal transducer and activator of transcription (STAT), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), and mitogen-activated protein kinases (MAPK). These events enhance the production of agents such as the reactive oxygen species and nitric oxide (NO) and promote the subsequent inflammatory immune responses by increasing antigen presentation capacity and inducing the Th1 immunity through the production of cytokines such as IL12. In contrast, M2 macrophage polarization is used to describe macrophages activated in ways other than the M1 polarization including IL4/IL13-stimulated macrophages, IL10-induced macrophages and immune complex-triggered macrophages. Among many molecular differences between M1 versus M2 polarization, the ratio of IL12 and IL10 production is critical to distinguish M1 and M2 macrophages. Noticeably, TAMs share many properties of M2 macrophages.

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

Also disclosed is the 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 one 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, 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 NO: 23, and wherein said antibody comprises (a) heavy variable region consisting in SEQ ID NO: 29, and (b) a light variable region consisting in SEQ ID NO: 33.

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 i*n vivo* or *ex vivo* of dendritic cells by

exposing suitable precursor cells, usually monocytes or precursors thereof, to an effective dose of an antibody able to bind to CSF-1R. The dendritic cells thus generated may be characterized by expression of dendritic cell markers, such as CD1a, CD14, CD86, CD1 b, etc.

In the methods of the invention, dendritic cells are generated from precursor cells, usually monocytes, by contacting the precursor cells with an effective dose of an antibody able to bind to CSF-1R. The precursor cells may be isolated from a suitable biological sample by methods known in the art, and may be isolated, or provided in a complex cell population.

As used throughout the entire application, the terms "a" and "an" are used in the sense that they mean "at least one", "at least a first", "one or more" or "a plurality" of the referenced components or steps, unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

The term "about" or "approximately" as used herein means within 20%, preferably within 10%, and more preferably within 5% of a given value or range. The term "about x" further includes x value.

As used herein, "comprising" and "comprise" are intended to indicate that the kits of parts, products, compositions and methods include the referenced components or steps, but not excluding others. For example, "a composition comprising x and y" encompasses any composition that contains x and y, no matter what other components may be present in the composition. Likewise, "a method comprising the step of x"

encompasses any method in which x is carried out, whether x is the only step in the method or it is only one of the steps, no matter how many other steps there may be and no matter how simple or complex x is in comparison to them.

"Consisting essentially of" when used to define products, compositions and methods, shall mean excluding other components or steps of any essential significance. Thus, a composition consisting essentially of the recited components would not exclude trace contaminants and pharmaceutically acceptable carriers. "Consisting of" shall mean excluding more than trace elements of other components or steps.

According to a first embodiment, the present invention concerns an immunomodulation method by modulating monocyte or precursors thereof differentiation in a patient suffering from conditions associated with undesirable M2 macrophage 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. The invention is more specifically directed to such a method for immunomodulation wherein said patient is further suffering from conditions associated with CSF-1R activity.

According to one special embodiment, the present invention concerns the use of an antibody able to bind to human CSF-1R for modulating monocyte, or precursors thereof, differentiation, especially in a patient suffering from conditions associated with undesirable M2 macrophage polarization. According to one special embodiment, said patient is further suffering from conditions associated with CSF-1R activity.

The present application refers to "modulating monocyte, or precursors thereof, differentiation". This term means that the modulatory antibodies of the Invention cause a decrease in the M2 macrophage polarization pool and/or an increase in

dendritic cells (DCs) pool, and optionally M1-type (macrophage M1 polarization) pool, preferably a decrease in the M2 macrophage polarization pool and an increase in dendritic cells (DCs) pool. This can be indicated, as disclosed herein, by changes in the levels of factors that are associated with M2 macrophages. Examples of such factors are membrane markers such as CD64 or CD163, cytokines such as IL6, IL10 or IL12, MCP-1, MMP9, etc… This "modulating monocyte, or precursors thereof, differentiation" in patients can be appreciated for example by measuring increase of the IL12/IL10 ratio, MCP-1 or IL-6 level following administration to a patient of an antibody able to bind to CSF-1R of the Invention.

As used herein, dendritic cells (DCs) refers to any member of a diverse population of morphologically similar cell types found in lymphoid or non-lymphoid tissues. Dendritic cells (DC) are specialized antigen-presenting cells (APC) for the initiation of T cell-mediated immune responses. The efficiency of DC in inducing immunity is evident in tissue culture and whole animal models. This is especially apparent when T cells are quiescent or the precursor frequency for T cells reactive with a specific antigen (Ag) is low. DC are richly endowed with class I and II major histocompatibility complex (MHC) molecules, and their co-stimulatory and adhesive molecules provide them with accessory properties that are more developed and more potent than those of other APC.

DCs express high levels of class I and class II MHC products. These molecules are used to present peptide fragments from proteins to T lymphocytes. Antigen presentation by itself does not generate immunity. Additional "second signals" or accessory molecules are required. Many of these accessory molecules are membrane glycoproteins that interact with specific receptors on the T cell. These accessory molecules are expressed at high levels on DC and include: LFA-3 (CD58), B7-1 (CD80) and B7-2 (CD86), ICAM-1 (CD54) and ICAM-

3 (CD50), and CD40. Other APC can express these accessory molecules, but the levels on DC are particularly high.

DCs have much lower phagocytic capacities than their myeloid relatives and accordingly, a paucity of receptors for immune complexes (FcγR) or C3 and its fragments. Low levels of the CD32 FcγR and the CD11b C3biR can be found, particularly when the DC are not fully mature. Terminally differentiated DC express the marker CD1a and lack CD14, a receptor that is more abundant on macrophages and mediates the response of phagocytes to lipopolysaccharide (LPS) and LPS binding protein. In essence, the pathway of DC differentiation sacrifices many of the scavenging and antimicrobial properties of phagocytes for the very high levels of MHC products and accessory molecules that underlie efficient presentation of antigens to quiescent T cells.

Analyses of the cell surface in order to characterize /or identify/ or follow DCs occurrence according to the invention can be performed according to one embodiment using monoclonal antibodies using a flow cytometer, magnetic sorting, and the like. Briefly, the cells are either combined with monoclonal antibodies directly conjugated to fluorochromes, or with unconjugated primary antibody and subsequently with commercially available secondary antibodies conjugated to fluorochromes. The stained cells are analyzed using, for example, a flow cytometer (for example as available from Becton Dickinson, Mountain View, Calif.). Additional phenotypic characteristics of DCs can further be characterized by gene expression profiling e.g. by reverse-transcriptase polymerase chain reaction (RT-PCR).

According to another embodiment, the present invention concerns a method for increasing dendritic cells (DCs) pool, and optionally M1-type macrophage pool, in a patient especially a patient suffering from conditions associated with

undesirable 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. The invention is more specifically directed to such a method for immunomodulation wherein said patient is further suffering from conditions associated with CSF-1R activity.

According to special embodiment, said method for increasing dendritic cells (DCs) pool, and optionally M1-type macrophage pool, in a patient suffering from conditions associated with undesirable M2 polarization, further decreases the macrophage M2 pool.

"Patient" means a vertebrate, such as a mammal, such as a human. Mammals include, but are not limited to humans, dogs, cats, horses, cows, and pigs. According to the present invention, the "patient" is suffering from conditions associated with undesirable M2 polarization, and according to particular embodiment is further suffering from conditions associated with CSF-1R activity.

The invention is also more specifically directed to one method for reducing macrophage pro-tumoral functions (i.e. tumorigenicity) and/or increasing tumor suppression activity in a patient, notably by skewing their differentiation towards DCs, especially in patient suffering from conditions associated with undesirable macrophage differentiation and M2 polarization and/or from conditions associated with CSF-1R activity and/or cancer-induced immunosuppression, wherein said method comprises the step of administering to the said patient an effective amount of an antibody able to bind to CSF-1R.

According to special embodiment, the method of the invention represses at least one macrophage pro-tumoral function selected in the group consisting of tumor invasion, metastasis, tumor cell proliferation, tumor growth, tumor

survival, neo-angiogenesis, suppression of innate or adaptive immunity and extracellular matrix remodeling.

Thus, with respect to the invention, "modulating monocyte, or precursors thereof, differentiation" can further mean that the modulatory antibodies of the Invention reduce at least one macrophage pro-tumoral function (i.e. tumorigenicity) selected in the group consisting of tumor invasion, metastasis, tumor cell proliferation, tumor growth, tumor survival, neo-angiogenesis, suppression of adaptive or innate immunity and extracellular matrix remodeling.

According to special embodiment, the method of the invention inhibits MCP-1, MMP-9 and IL-6 production by differentiating monocytes, or precursors thereof

According to special embodiment, the method of the invention down regulates surface FcγRI (CD64) and FcγRIII (CD16) expression on differentiating monocytes, or precursors thereof.

According to special embodiment, the method of the invention promotes IL-12 (more particularly IL-12 P70 form) production by differentiating monocytes or precursors thereof and/or upregulates IL-12/IL-10 ratios.

According to special embodiment, the method of the invention modulates the monocyte, or precursors thereof, differentiation by means of secreted factors.

According to special embodiment, the method of the invention reduces at least one of the followings:

* TAM recruitment into tumor;
* at least one macrophage pro-tumoral functions;
* tumor angiogenesis;
* tumor invasion and metastasis;

* tumor growth;

* tumor cell proliferation

in patients, especially in patients suffering from conditions associated with undesirable M2 macrophage polarization. According to special embodiment, the said patient is further suffering from conditions associated with CSF-1R activity.

The invention is also directed to methods for driving macrophages towards dendritic cells (DCs), and optionally M1-type macrophage immune response and/or away from a M2-type (macrophage M2 polarization) immune response in patients, especially in patients suffering from conditions associated with undesirable macrophage differentiation and M2 macrophage polarization or in patient suffering from conditions associated with CSF-1R activity and/or cancer-induced immunosuppression, wherein said method comprises the step of administering to the said patient an effective amount of an antibody able to bind to CSF-1R.

The invention is further directed to methods for driving macrophages towards a Th1 immune response or away from a Th2 immune response in patients, especially in patients suffering from conditions associated with undesirable macrophage differentiation and M2 macrophage polarization or in patient suffering from conditions associated with CSF-1R activity and/or cancer-induced immunosuppression, wherein said method comprises the step of administering to the said patient an effective amount of an antibody able to bind to CSF-1R.

The invention is also directed to the use of an antibody able to bind to CSF-1R for modulating monocyte, or precursors thereof, differentiation into DCs, and optionally M1-type macrophages. The invention is also directed to the use of an antibody able to bind to CSF-1R for driving monocyte, or precursors thereof, differentiation towards DCs, and optionally M1-type macrophage to stimulate immune responses.

The invention is also directed to the use of an antibody able to bind to CSF-1R for inducing differentiating monocytes, or precursors thereof, to stimulate a Th1-type immune response. The invention is also directed to the use of compositions, such as pharmaceutical compositions, comprising an antibody able to bind CSF-1R for modulating monocyte, or precursors thereof, differentiation, for driving monocyte, or precursors thereof, differentiation towards DCs, and optionally M1-type macrophages, and/or inducing differentiating monocyte, or precursors thereof to stimulate a Th1-type immune response.

As used herein, the term "able to bind to" refers to a binding reaction which is determinative of the presence of a target protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated assay conditions, the antibody according to the invention bind preferentially to at least part of the CSF-1R and preferably do not bind in a significant amount to other components present in a test sample. Specific binding between the antibody according to the invention and the CSF-1R target means that the binding affinity is of at least 10^3 M^{-1}, and preferably 10^5 M^{-1}, 10^6 M^{-1}, 10^7 M^{-1}, 10^8 M^{-1}, 10^9 M^{-1} or 10^{10} M^{-1}.

As used herein, the term "CSF-1R" refers to the human CSF1 receptor.

As used herein, "antibody" or "Ab" is used in the broadest sense. Therefore, an "antibody" or "Ab" can be naturally occurring or man-made such as monoclonal antibodies (mAbs) produced by conventional hybridoma technology, recombinant technology and/or a functional fragment thereof. Antibodies of the present invention are preferably monoclonal antibodies (mAb).

As used herein, the term "variable region" refers to the variable region, or domain, of the light chain (VL) or heavy chain (VH) which contains the determinants for binding

recognition specificity. The variable domains are involved in antigen recognition and form the antigen binding site. The variable region of both the heavy and light chain is divided into segments comprising four framework sub-regions (FR1, FR2, FR3, and FR4), interrupted by three stretches of hypervariable sequences, or the complementary determining regions (CDR's), as defined in Kabat's database, with the CDR1 positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region as referred by others, represents the combined FR's within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FR's represents two or more of the four sub-regions constituting a framework region. The framework region of an antibody is the combined framework regions of the constituent light and heavy chains and serves to position and align the CDR's. The CDR's are primarily responsible for forming the binding site of an antibody conferring binding specificity and affinity to an epitope of an antigen. Within the variable regions of the H or L chains that provide for the antigen binding regions are smaller sequences dubbed "hypervariable" because of their extreme variability between antibodies of differing specificity. Such hypervariable regions are also referred to as "complementarity determining regions" or "CDR" regions. These CDR regions account for the basic specificity of the antibody for a particular antigenic determinant structure. The variable heavy and light chains of all antibodies each have 3 CDR regions, each non-contiguous with the others (termed L1, L2, L3, H1, H2, H3) for the respective light (L) and heavy (H) chains.

"Co-administer" means to administer in conjunction with one another, together, coordinately, including simultaneous or sequential administration of two or more agents.

"Effective amount" generally means an amount which provides the desired local or systemic effect, e.g., effective to ameliorate undesirable effects of inflammation, including modulation of polarization of macrophages, etc. For example, an effective amount is an amount sufficient to effectuate a beneficial or desired clinical result. The effective amounts can be provided all at once in a single administration or in fractional amounts that provide the effective amount in several administrations. The precise determination of what would be considered an effective amount may be based on factors individual to each subject, including their size, age, injury, and/or disease or injury being treated, and amount of time since the injury occurred or the disease began. One skilled in the art will be able to determine the effective amount for a given subject based on these considerations which are routine in the art. As used herein, "effective dose" means the same as "effective amount".

According to another embodiment, the present invention relates to the use of an antibody able to bind to human CSF-1R for reducing at least one macrophage pro-tumoral functions selected in the group consisting of tumor invasion, metastasis, tumor growth, tumor survival, neo-angiogenesis, suppression of innate or adaptive immunity and matrix remodeling (i.e. tumorigenicity)and/or increasing macrophage tumor suppression activity in patients, especially patient suffering from conditions associated with undesirable M2 polarization, and according to particular embodiment further suffering from conditions associated with CSF-1R activity.

According to another embodiment, the present invention relates to the use of an antibody able to bind to human CSF-1R for inhibiting MCP-1, MMP-9 and IL-6 production by macrophages, especially human macrophages.

According to another embodiment, the present invention relates to the use of an antibody able to bind to human CSF-1R for down regulating surface FcγRI (CD64) and/or FcγRIII (CD16) expression on macrophages, especially human macrophages.

According to another embodiment, the present invention relates to the use of an antibody able to bind to human CSF-1R for promoting IL-12 (more particularly IL-12 P70 form) production by macrophages, especially human macrophages and/or up-regulating the IL-12/IL-10 ratio.

According to another embodiment, the present invention relates to the use of an antibody able to bind to human CSF-1R for modulating the polarization state of macrophages by means of secreted factors.

According to another embodiment, the present invention relates to the use of an antibody able to bind to human CSF-1R for reducing TAM recruitment and/or tumor angiogenesis in patients, especially patient suffering from conditions associated with undesirable M2 macrophage polarization, and according to particular embodiment further suffering from conditions associated with CSF-1R activity.

The invention is also more specifically directed to the use of an antibody able to bind to human CSF-1R for reducing TAM recruitment and/or tumor invasion and metastasis in patients, especially patient suffering from conditions associated with undesirable M2 macrophage polarization, and according to particular embodiment further suffering from conditions associated with CSF-1R activity.

The invention is also more specifically directed to the use of an antibody able to bind to human CSF-1R for reducing TAM recruitment and/or tumor growth in patients, especially patient suffering from conditions associated with undesirable M2 macrophage polarization, and according to particular

embodiment further suffering from conditions associated with CSF-1R activity.

According to another embodiment, the present invention relates to the use of an antibody able to bind to human CSF-1R for driving monocyte, or precursors thereof, differentiation towards a dendritic cells (DCs), and optionally M1-type (macrophage M1 polarization), immune response and/or away from a M2-type (macrophage M2 polarization) immune response in patients, especially patient suffering from conditions associated with undesirable M2 macrophage polarization, and according to particular embodiment further suffering from conditions associated with CSF-1R activity.

According to another embodiment, the present invention relates to the use of an antibody able to bind to human CSF-1R for driving macrophages towards a Th1 immune response and/or away from a Th2 immune response in patients, especially patient suffering from conditions associated with undesirable M2 macrophage polarization, and according to particular embodiment further suffering from conditions associated with CSF-1R activity and/or cancer-induced immunosuppression.

According to preferred embodiments, the said antibody able to bind to human 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 (i.e. N-terminal part of the human domain D1). In preferred embodiment, the antibody according to the Invention binds to one epitope located between position amino acids 20 to 39 of SEQ ID N0:23 (i.e. N-terminal part of the human domain D1), to amino acids Asn72, Ser94-Ala95-Ala96, Lys102, Asp131-Pro132-Val133 and Trp159 of SEQ ID N0:23.

In another embodiment, the antibody according to the Invention binds to one epitope located between position amino acids 20 to 41 of SEQ ID N0:23 (i.e. N-terminal part of the human domain D1) and does not bind to any epitope

located between position amino acids 42 to 90, and/or between position amino acids 91 to 104, and/or between position amino acids 105 to 199, and/or between position amino acids 200 to 298 of SEQ ID N0:23. According to preferred embodiment, the antibody of the present Invention is able to recognize the minimal epitope located between position amino acids 20 to 41 of SEQ ID N0:23 (i.e. N-terminal part of the human domain D1), preferably to epitope between position amino acids 20 to 39 of SEQ ID N0:23.

In preferred embodiment, the 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. The term "does not compete with IL-34 ligand" as used herein refers to no inhibition of the IL34 ligand to its receptor CSF-1R binding.

In preferred embodiment, the 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. The term "competes partially with CSF-1 ligand" as used herein refers to an inhibition of the CSF-1 ligand to its receptor CSF-1R binding which is less than 100%, preferably less than 50%, and even more preferably less than 20%, and advantageously less than 10%. This partial inhibitor only reduces but does not totally exclude ligand binding, the inhibition is called partial inhibition. In preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that is able to partially prevent binding of CSF1 to its receptor CSF-1R, and is not able to totally inhibit said binding. More particularly, the antibodies according to the Invention are able to decrease the CSF-1 binding to CSF-1R by approximately 5 to 10%.

According to one embodiment, the said antibody able to bind to human CSF-1R is an antibody that comprises:

(i) at least one CDR wherein said CDR is comprising at least five consecutive amino acids of the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:1, of the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:1 or of the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:1;

or,

(ii) at least one CDR wherein said CDR is comprising at least five consecutive amino acids of the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:2, of the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:2 or of the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:2.

According to a preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises the following CDRs comprising at least five consecutive amino acids :

- of the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:1,
- of the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:1,
- of the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:1,
- of the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:2,
- of the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:2

- or of the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:2.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises at least one CDR selected, independently from one another, in the group of the CDR as set forth in :

- the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:1,
- the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:1,
- the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:1,
- the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:2,
- the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:2 and
- the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:2.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises the CDR as set forth in :

- the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:1,
- the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:1,
- the sequence starting in position 117 and

finishing in position 126 of SEQ ID NO:1,

- the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:2,
- the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:2 and
- the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:2.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 5, 6, 7, 8, 9 or 10.

According to a preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises the CDRs comprising amino acid sequences as set forth in SEQ ID NOs: 5, 6, 7, 8, 9 or 10.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises (i) at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 11, 12 or 13; or (ii) at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 14, 15 or 16.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises the CDR comprising amino acid sequences as set forth in SEQ ID NOs: 11, 12, 13, 14, 15 or 16.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds

specifically to human CSF-1R, and comprises (i) at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 17, 18 or 19; or (ii) at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 20, 21 or 22.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 17, 18, 19, 20, 21 or 22.

According to one preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises the CDR comprising amino acid sequences as set forth in SEQ ID NOs: 17, 18, 19, 20, 21 or 22.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises a variable region, wherein said variable region comprises the three CDRs as set forth in SEQ ID NOs: 5, 6, and 7.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises a variable region, wherein said variable region comprises the three CDRs as set forth in SEQ ID NOs: 8, 9, and 10.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises a variable region, wherein said variable region comprises the three CDRs set forth in SEQ ID NOs: 11, 12, and 13.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds

specifically to human CSF-1R, and comprises a variable region, wherein said variable region comprises the three CDRs as set forth in SEQ ID NOs: 14, 15, and 16.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises a variable region, wherein said variable region comprises the three CDRs as set forth in SEQ ID NOs: 17, 18, and 19.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises a variable region, wherein said variable region comprises the three CDRs as set forth in SEQ ID NOs: 20, 21, and 22.

According to one preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises a variable region, wherein the variable region comprises an amino acid sequence as set forth in SEQ ID NO:3.

In a more preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises a variable region, wherein the variable region is as set forth in SEQ ID NO:3.

In another preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, wherein the variable region comprises an amino acid sequence as set forth in SEQ ID NO:4.

In another more preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises a variable region, wherein the variable region is as set forth in SEQ ID NO:4.

According to a preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises :

- a variable region comprising the three CDRs as set forth in SEQ ID NOs: 5, 6, and 7, and
- a variable region comprising the three CDRs as set forth in SEQ ID NOs: 8, 9, and 10.

According to a preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises :

- a variable region comprising the three CDRs as set forth in SEQ ID NOs: 11, 12, and 13, and
- a variable region comprising the three CDRs as set forth in SEQ ID NOs: 14, 15, and 16.

According to a preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises :

- a variable region comprising the three CDRs as set forth in SEQ ID NOs: 17, 18, and 19, and
- a variable region comprising the three CDRs as set forth in SEQ ID NOs: 20, 21, and 22.

According to a preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises :

- a variable region as set forth in SEQ ID NO:3 and
- a variable region as set forth in SEQ ID

NO:4.

According to a preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises :

(i) a heavy-chain variable region comprising :

- CDR1 defined in the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:1,
- CDR2 defined in the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:1 and
- CDR3 defined in the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:1;

and

(ii) a light-chain variable region comprising :

- CDR1 defined in the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:2,
- CDR2 defined in the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:2 and
- CDR3 defined in the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:2.

Skilled man can easily define CDRs in one known antibody amino acid sequence by using well known teachings of Chothia and Lesk, 1987, J. Mol. Biol., 196, 901-917, or Kabat and Wu, 1991, J. of Immunology, 147, 1709-1719, or Lefranc and al.,

2003, Development and Comparative Immunology, 27, 55-77, or Lefranc et al, 1999, Nucleic Acid Research, 27, 209-212.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises (i) a heavy-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 5, 6, and 7, and (ii) a light-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 8, 9, and 10.

According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises (i) a heavy-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 11, 12, and 13, and (ii) a light-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 14, 15, and 16.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises (i) a heavy-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 17, 18, and 19, and (ii) a light-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 20, 21, and 22.

According to a preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, and comprises (i) a heavy-chain variable region as set forth in SEQ ID NO:3 and (ii) a light-chain variable region as set forth in SEQ ID NO:4.

According to another embodiment, the said antibody able to bind to 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.

According to another embodiment, the said antibody able to bind to 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 an amino acid sequence selected from the group consisting of: SEQ ID NO: 5, 11 and 17;

CDR2 has an amino acid sequence selected from the group consisting of: SEQ ID NO: 6, 12 and 18; and

CDR3 has an amino acid sequence selected from the group consisting of: SEQ ID NO: 7, 13 and 19;

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 an amino acid sequence selected from the group consisting of: SEQ ID NO: 8, 14 and 20;

CDR2 has an amino acid sequence selected from the group consisting of: SEQ ID NO: 9, 15 and 21; and

CDR3 has an amino acid sequence selected from the group consisting of: SEQ ID NO: 10, 16 and 22.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, comprising any one of the following (i), (ii) or (iii) :

(i)

(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 is as set forth in SEQ ID NO: 5;

CDR2 is as set forth in SEQ ID NO: 6; and

CDR3 is as set forth in SEQ ID NO: 7;

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 is as set forth in SEQ ID NO: 8;

CDR2 is as set forth in SEQ ID NO: 9; and

CDR3 is as set forth in SEQ ID NO: 10;

or

(ii) (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 is as set forth in SEQ ID NO: 11;

CDR2 is as set forth in SEQ ID NO: 12; and

CDR3 is as set forth in SEQ ID NO: 13;

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 is as set forth in SEQ ID NO: 14;

CDR2 is as set forth in SEQ ID NO: 15; and

CDR3 is as set forth in SEQ ID NO: 16;

or

(iii) (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 is as set forth in SEQ ID NO: 17;

CDR2 is as set forth in SEQ ID NO: 18; and

CDR3 is as set forth in SEQ ID NO: 19;

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 is as set forth in SEQ ID NO: 20;

CDR2 is as set forth in SEQ ID NO: 21; and

CDR3 is as set forth in SEQ ID NO: 22.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to

human CSF-1R, comprising :

- a first variable region comprising the amino acid sequence of SEQ ID NO: 3; and

- a second variable region comprising the amino acid sequence of SEQ ID NO: 4.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, comprising :

- a first variable region comprising the amino acid sequence of SEQ ID NO: 1; and

- a second variable region comprising the amino acid sequence of SEQ ID NO: 2.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, comprising :

- an heavy chain selected in the group consisting in SEQ ID N0 :24 and SEQ ID N0 :25, and

- a light chain selected in the group consisting in SEQ ID N0 :26, SEQ ID N0 :27 and SEQ ID N0 :28.

According to another embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, comprising :

- a first variable region selected in the group consisting of SEQ ID N0 :29 and SEQ ID N0 :30; and

- a second variable region selected in the group consisting of SEQ ID N0 :31, SEQ ID N0 :32 and SEQ ID N0 :33.

According to one preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R,, comprising (a) an heavy chain consisting in SEQ ID N0 :24, and (b) a light chain

consisting in SEQ ID N0 :26.

According to another preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, comprising (a) an heavy chain consisting in SEQ ID N0:25, and (b) a light chain consisting in SEQ ID N0 :27.

According to one advantageous embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, comprising (a) an heavy chain consisting in SEQ ID N0:24, and (b) a light chain consisting in SEQ ID N0:28. Example of the said monoclonal antibody is monoclonal antibody H27K15.

According to one preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R,, comprising (a) first variable region consisting in SEQ ID N0 :29, and (b) a second variable region consisting in SEQ ID N0 :31.

According to another preferred embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R,, comprising (a) first variable region consisting in SEQ ID N0 :30, and (b) a second variable region consisting in SEQ ID N0 :32.

According to one advantageous embodiment, the said antibody able to bind to CSF-1R is an antibody that binds specifically to human CSF-1R, comprising (a) first variable region consisting in SEQ ID N0 :29, and (b) a second variable region consisting in SEQ ID N0 :33. Example of the said monoclonal antibody is monoclonal antibody H27K15.

The antibody, more specifically the human antibody, according to the invention may be of different isotypes, such as IgG, IgA, IgM or IgE. In a preferred embodiment the antibody, more specifically the human antibody, according to

the invention is an IgG.

The antibody according to the invention may be glycosylated or non-glycosylated.

As used herein, the term "glycosylation" refers to the presence of carbohydrate units that are covalently attached to the antibody.

According to special embodiment, the invention is directed to methods for the generation *in vivo* or *ex vivo* of dendritic cells by exposing suitable precursor cells, usually monocytes or precursors thereof, to an effective dose of an antibody able to bind to CSF-1R. The cells may be contacted *in vivo*, or *ex vivo*.

For *ex vivo* purposes the effective dose of antibody able to bind to CSF-1R may be contacted with cells from a period of time from about 6 hours, about 12 hours, about 24 hours, about 48 hours, or more. The cells may be maintained in *ex vivo* culture for 1, 2, 3, 4, 5, 6, 7, or more days.

Precursor cells can be obtained from lymphoid tissue, including bone marrow, blood, mobilized peripheral blood, spleen, lymph node, and cord blood. The precursors, e.g. monocytes, monocyte precursors, or complex populations comprising monocytes and/or monocyte precursors can be in a variety of developmental states such as stem cells, myeloid committed progenitors, monocytes, etc.

For *in vivo* purposes the dose may vary according to the route of delivery, patient size, condition, antigen, and the like. Administration may be targeted, e.g. to infected lesions, tumors, lungs, skin, etc., or maybe systemic, e.g. intramuscular, intravenous, intraperitoneal, intradermal, oral, etc.

The administration may be combined with an antigen of interest, in a co-formulation or in separate, concurrent formulations. Administration of the antibody able to bind to CSF-1R may be repeated as necessary, for example semi-daily, daily, semi-weekly, etc., for from 1, 2, 3, 4, 5, 6, or more administrations.

DCs generated *ex vivo* by the methods of the invention can be administered to a subject in any physiologically acceptable medium, normally intravascularly, although they may also be introduced into the targeted site, where the cells home to the site of inflammation. The number of cells administered may vary widely depending on the nature of the disease being treated and/or the antigen of interest. As such, the number of DCs administered can include at least about 1 $x10^5$ DCs, at least about 1 $x10^6$ DCs, at least about 1 $x10^7$ DCs, at least about 1 $x10^8$ DCs, at least about 1 $x10^9$ DCsor more. In addition, the DCs can be administered in a single dose or at timed intervals. The cells may be introduced by injection, catheter, or the like. The cells may be frozen at liquid nitrogen temperatures and stored for long periods of time, being capable of use on thawing.

Conditions of particular interest for use with the *ex vivo* present methods involve a lack of T cell mediated response to antigen, for example chronic viral or bacterial infection, a lack of immune response to tumor antigens, and the like. In one aspect of the invention, the antigen is a tumor antigen, and is used to enhance the host immune response to tumor cells present in the body.

The methods of the invention, especially those for the generating *in vivo* dendritic cells by exposing suitable precursor cells, usually monocytes or precursors thereof, to an effective dose of an antibody able to bind to CSF-1R, are further useful in treatment of conditions associated with

undesirable macrophage differentiation and/or M2 polarization and associated with CSF-1R. The methods of the invention are useful in treatment of disease involving cancer-induced immunosuppression and associated with CSF-1R.

"Patients suffering from conditions associated with undesirable M2 macrophage polarization according to the Invention designate cancer, especially metastatic cancer, progressive fibrotic diseases such as for example idiopathic pulmonary fibrosis (IPF), hepatic fibrosis or systemic sclerosis (Wynn and Barron, 2010, Semin. Liver Dis., 30, 245), allergy and asthma, atherosclerosis and Altzheimer's disease.

According to another embodiment, the present invention relates to methods for driving monocyte, or precursors thereof, differentiation towards DCs, and optionally M1-type macrophages (M1 polarization)- to stimulate immune responses and inhibit M2-type macrophage (M2 polarization)-driven immune responses in patients suffering from cancer.

According to another embodiment, the present invention relates to methods for driving monocyte, or precursors thereof, differentiation towards DCs, and optionally M1-type macrophages (M1 polarization), to stimulate immune responses and inhibit M2-type macrophage (M2 polarization)-driven immune responses in patients suffering from progressive fibrotic diseases.

According to another embodiment, the present invention relates to methods for driving monocyte, or precursors thereof, differentiation towards DCs, and optionally M1-type macrophages (M1 polarization), to stimulate immune responses and inhibit M2-type ~~(~~macrophage (M2 polarization)-driven immune responses in patients suffering from allergy.

According to another embodiment, the present invention relates to methods for driving monocyte, or precursors thereof, differentiation towards DCs, and optionally M1-type

macrophages (M1 polarization), to stimulate immune responses and inhibit M2-type macrophage (M2 polarization)-driven immune responses in patients suffering from asthma.

As used herein, the term "cancer" refers but is not limited to adenocarcinoma, acinic cell adenocarcinoma, adrenal cortical carcinomas, alveoli cell carcinoma, anaplastic carcinoma, basaloid carcinoma, basal cell carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, renaladinol carcinoma, embryonal carcinoma, anometroid carcinoma, fibrolamolar liver cell carcinoma, follicular carcinomas, giant cell carcinomas, hepatocellular carcinoma, intraepidermal carcinoma, intraepithelial carcinoma, leptomanigio carcinoma, medullary carcinoma, melanotic carcinoma, menigual carcinoma, mesometonephric carcinoma, oat cell carcinoma, squamal cell carcinoma, sweat gland carcinoma, transitional cell carcinoma, tubular cell carcinoma, amelioblastic sarcoma, angiolithic sarcoma, botryoid sarcoma, endometrial stroma sarcoma, ewing sarcoma, fascicular sarcoma, giant cell sarcoma, granulositic sarcoma, immunoblastic sarcoma, juxaccordial osteogenic sarcoma, coppices sarcoma, leukocytic sarcoma (leukemia), lymphatic sarcoma (lympho sarcoma), medullary sarcoma, myeloid sarcoma (granulocitic sarcoma), austiogenci sarcoma, periosteal sarcoma, reticulum cell sarcoma (histiocytic lymphoma), round cell sarcoma, spindle cell sarcoma, synovial sarcoma, telangiectatic audiogenic sarcoma, Burkitt's lymphoma, NPDL, NML, NH and diffuse lymphomas. According to a preferred embodiment, the method according to the invention is directed to the treatment of metastatic cancer to bone, wherein the metastatic cancer is breast, lung, renal, multiple myeloma, thyroid, prostate, adenocarcinoma, blood cell malignancies, including leukemia and lymphoma; head and neck cancers; gastrointestinal cancers, including esophageal cancer, stomach cancer, colon

cancer, intestinal cancer, colorectal cancer, rectal cancer, pancreatic cancer, liver cancer, cancer of the bile duct or gall bladder; malignancies of the female genital tract, including ovarian carcinoma, uterine endometrial cancers, vaginal cancer, and cervical cancer; bladder cancer; brain cancer, including neuroblastoma; sarcoma, osteosarcoma; and skin cancer, including malignant melanoma or squamous cell cancer.

The present invention further concerns a method for improving the treatment of a cancer patient which is undergoing chemotherapeutic treatment with a cancer therapeutic agent, which comprises co-treatment of said patient along with a method as above disclosed.

The present invention further concerns a method for improving the treatment of a cancer patient which is undergoing immunotherapy treatment with a cancer therapeutic vaccine, which comprises co-treatment of said patient along with a method as above disclosed. According to preferred embodiment, said cancer therapeutic vaccine is a viral based therapeutic vaccine. More preferably said viral based therapeutic vaccine is a MVA based therapeutic vaccine. Even more preferably, said MVA based therapeutic vaccine is carrying and expressing human papilloma virus 16 (HPV16) E6 and E7 oncoproteins and human interleukin-2 (e.g. TG4001 product) or is expressing the Muc1 antigen and the human interleukin-2 (e.g. TG4010 product).

The invention further includes post-treatment monitoring assays, following administration to a patient of an antibody able to bind to CSF-1R to assess efficacy of said treatment, and/or to evaluate the clinical outcome of the said treatment.

The monitoring assays include, but are not limited to, assays for circulating factors expressed and/or secreted by DCs, and optionally M1-type, or M2-polarized macrophages.

Factors expressed in the macrophage M2-type polarization state include, but are not limited to IL-10, IL-6 and MCP-1. Factors expressed in the DCs, and optionally M1-type (macrophage M1 polarization), state may also be assayed, for example by measuring IL-12 levels, more particularly IL-12 P70 form levels. Driving monocyte, or precursors thereof, differentiation towards DCs, and optionally M1-type macrophages (M1 polarization), and away from M2-type macrophages (M2 polarization) to stimulate immune responses in patients can be appreciated by measuring the increase of the IL12/IL10 ratio following administration to a patient of an antibody able to bind to CSF-1R of the Invention.

These tests can be derived from the patient's serum, blood, tissue, etc.

The invention further includes post-treatment monitoring assays, following administration to a patient of an antibody able to bind to CSF-1R to monitor monocyte, or precursors thereof, differentiation and establish and/or maintain a proper dosage regimen.

In this case, it is possible to obtain a baseline levels by assaying for the presence of dendritic cells (DCs), macrophages M1 and/or M2 in tissues, either directly or by means of factors expressed and/or secreted by activated macrophages and, then, following administration of an antibody able to bind to CSF-1R during treatment, monitor one or more times for the presence of DCs and/or M1 versus M2 macrophages in tissues (tumoral or normal tissues). One could then determine the optimized dose for treatment that will result in skewing from M2-type macrophages to DCs and/or M1-type macrophages response.

The invention provides materials and methods for assessing the efficacy of a treatment involving the administration of an antibody able to bind to CSF-1R to a

patient using biological markers (biomarkers) that have been determined to be substantially reliable signature which correlates with the desired immune response. The biomarkers are present in biological samples obtained from the patient. The ability to predict the clinical outcome of a treatment, soon after its initiation, will enable clinicians and patients to identify ineffective therapy, make informed decisions regarding the course of treatment, including whether to abandon or to allow alternate therapy implementation.

The invention provides an *ex-vivo* method for assessing the efficacy of a treatment involving an antibody able to bind to CSF-1R to a patient.

According to the invention, the term "assessing" should be understood as "monitoring, modifying or adjusting" a treatment involving the administration of an antibody able to bind to CSF-1R to a patient.

In certain aspects the method includes assessing the efficacy of an antibody able to bind to CSF-1R based on the levels of interferon γ in the patient following immunotherapy treatment.

The monitoring assays include, but are not limited to, assays for circulating factors expressed and/or secreted by DCs and/or macrophages of M1 and/or M2 types.

Factors expressed in the macrophage M2-type polarization state include, but are not limited to IL-6, MMP9 and MCP-1. Factors expressed in the macrophage M1-type polarization state may also be assayed, for example by measuring IL-12 levels, more particularly IL-12 P70 form levels, or IL-12/IL-10 ratios.

In certain aspects, the method includes measuring a patient's levels of interleukin-6, interleukin-12, MMP9 and/or MCP-1 following administration into patient of an antibody

able to bind to CSF-1R; and assessing the efficacy of the treatment based on the levels of the interleukin-6, interleukin-12, MMP9 and/or MCP-1.

In certain aspects, the method includes measuring a patient's levels of interleukin-6, interleukin-12, MMP9 and/or MCP-1 at least once several weeks following administration into patient of an antibody able to bind to CSF-1R; and assessing the efficacy of the immunotherapy treatment based on the levels of the interleukin-6, interleukin-12, MMP9 and/or MCP-1.

In certain aspects, the method can further include measuring a patient's levels of interleukin-6, interleukin-12, MMP9 and/or MCP-1 prior to administration of an antibody able to bind to CSF-1R. According to preferred embodiment, the values of patient's levels of interleukin-6, interleukin-12, MMP9 and/or MCP-1 measured before said administration of an antibody able to bind to CSF-1R are the "cut-off values" according to the present invention.

The time between the administration of an antibody able to bind to CSF-1R and interleukin-6, interleukin-12, MMP9 and/or MCP-1 measurements may be 1 day to about 48 weeks or more (e.g., from about 1 day to about 1 week, from about 1 week to about 2 weeks, from about 2 weeks to about 4 weeks, from about 4 weeks to about 8 weeks, from about 8 weeks to about 12 weeks, from about 12 weeks to about 16 weeks, from about 16 weeks to about 24 weeks, from about 24 weeks to about 48 weeks, or more). In a preferred embodiment of the invention, the time interval is about 5 weeks. Similarly, additional measurements (i.e., a third, fourth, fifth, etc. measurement) may be taken at similar time intervals following the second measurement.

In related aspects the method includes determining the levels of interleukin-6, interleukin-12, MMP9 and/or MCP-1 in

a patient following administration into patient of an antibody able to bind to CSF-1R; comparing said levels to a cut-off value; and assessing the efficacy of immunotherapy treatment based on the levels of interleukin-6, interleukin-12, MMP9 and/or MCP-1 compared to the "cut-off value".

According to special embodiment, the Invention concerns a method for assessing the efficacy of a treatment involving the administration of an antibody able to bind to CSF-1R to a patient comprising:

(i) administering one or more doses of said an antibody able to bind to CSF-1R to said subject;

(ii) measuring an interleukin-6, interleukin-12, MMP9 and/or MCP-1 level in the body of said subject following at least one of the said administration.

According to alternate embodiment of the invention, the method of the invention further comprises an initial step consisting in measuring the interleukin-6, interleukin-12, MMP9 and/or MCP-1 levels in the body of the patient before administration of the antibody able to bind to CSF-1R.

According to the present invention, the levels of interleukin-6, interleukin-12, MMP9 and/or MCP-1 are measured in a biological sample obtained from the patient. Biological samples include but are not limited to blood, serum, tissue, and other liquid samples of biological origin, solid tissue samples, such as a biopsy specimen. In a preferred embodiment, the biological sample is blood, plasma or serum, in which case obtaining the samples from a patient is relatively simple and non-invasive procedure. Methods of obtaining blood or serum are well-known in the art are not part of the invention.

In addition, numerous methods for detecting and quantifying polypeptides, including the instant biomarkers,

are known. Such methods include but are not limited to antibody-based methods, more specifically monoclonal antibodies-based methods. The particular methods of detecting and quantifying the biomarkers are not important to the invention. For example the materials and methods of the present invention may be used with Luminex technology (Luminex Corporation, Austin, Tex.) or enzyme-linked immunosorbant assays (ELISA, numerous ELISA kits are commercially available e.g. by CliniScience, Diaclone, Biosource).

According to one embodiment of the Invention, the levels of interleukin-6, interleukin-12, MMP9 and/or MCP-1 are determined by using antibodies.

According to one specific embodiment of the Invention, said antibody(ies) is (are) specific of interleukin-6, interleukin-12, MMP9 or MCP-1.

According to one specific embodiment of the Invention, said antibodies are monoclonal antibodies.

According to one specific embodiment of the Invention, said antibodies are tagged for example by fluorescence, radiolabel, enzyme, biotin, or any other methods designed to render cells labelled with said antibodies detectable. These techniques are widely used and known in the art.

The immunotherapy treatment of the Invention will be considered as efficient when the levels of interleukin-6, MMP9 and/or MCP-1 measured in patient following administration of an antibody able to bind to CSF-1R is below the levels of interleukin-6 and/or MCP-1, respectively, measured in patient before said administration (i.e. cut-off value).

Alternatively, the immunotherapy treatment of the Invention will be considered as efficient when the levels of interleukin-12 measured in patient following administration of an antibody able to bind to CSF-1R is above the levels of

interleukin-12 measured in patient before said administration (i.e. cut-off value).

The invention further includes post-treatment monitoring assays, following administration to a patient of an antibody able to bind to CSF-1R to monitor macrophage polarization and establish and/or maintain a proper dosage regimen.

In this case, it is possible to obtain a baseline levels by assaying for the presence of DCs and/or M1 and/or M2 macrophages in the circulation, either directly or by means of factors expressed and/or secreted by activated macrophages and, then, following administration of an antibody able to bind to CSF-1R during treatment, monitor one or more times for the presence of the macrophages in the circulation.

One could then determine the optimized dose for treatment that will result in skewing monocyte, or precursors thereof, differentiation from M2-type macrophages to DCs and/or M1-type macrophages notably to stimulate immune responses.

The methods of the invention are useful in treatment of disease involving inflammation and associated with CSF-1R.

Figure legends

Figure 1 : H27K15 is not cytotoxic to differentiating macrophages while other anti-CD115 mAbs induce massive cell death

Cells were counted following a 6-day culture of monocyte, or precursors thereof,s with GM-CSF and CSF-1, in the presence or absence of anti-CD115 mAbs or F(ab')2 or with GW2580. Controls included cultures treated with rituximab or rituximab F(ab')2, or without any added compound. Shown are the means of cell counts in 5 microscope fields ± standard deviation obtained in each culture condition.

Figure 2 : Inhibition of CD64 (FcγRI) expression in human macrophages differentiated in the presence of monoclonal antibody H27K15

Macrophages obtained following a 6-day culture of monocyte, or precursors thereof from 3 different blood donors with GM-CSF and CSF-1 were analyzed by IC/FC for surface expression of CD64. Upper panels: CD64 staining in cultures from donor 1 treated with monoclonal antibody H27K15 (left, bold line) or with GW2580 (right, bold line) or their respective negative controls rituximab (left, thin line) or no treatment (right, thin line). Lower panel: Medians of fluorescence intensities in macrophage cultures treated with test compounds at 10, 1 or 0.1 µg/ml* were compared with those in the corresponding negative controls : H27K15 vs rituximab, H27K15-derived $F(ab')_2$ vs rituximab-derived $F(ab')_2$, mAbs 2-4A5 or 9-4D2 vs rat IgG1, GW2580 vs no treatment. Percentages of reduction in CD64 expression were calculated as: 100 - [100 x Median fluorescence intensity with test compound / Median fluorescence intensity with control]. Shown are the mean percentages of reduction in CD64 expression from the 3 blood donors. *except for the $F(ab')_2$ which were used at equimolar concentrations : 6.6; 0.6; and 0.06 µg/ml.

Figure 3: Induction of a $CD86^{bright}$ SSC^{low} macrophage population by H27K15

Upper panels : Dot plots showing CD86 staining (x-axis) and side scatter (SSC, y-axis) of macrophages from donor 3 differentiated for 6 days in the presence of H27K15 (left) or negative control rituximab (right). A gate was set on the $CD86^{bright}$ SSC^{low} cell population induced by H27K15. Lower panel : Percentages of $CD86^{bright}$ SSC^{low} cells with the test compounds at 10, 1 or 0.1 µg/ml* were compared with those in the corresponding negative controls : H27K15 vs rituximab, H27K15-derived $F(ab')_2$ vs rituximab-derived $F(ab')_2$, GW2580 vs no

treatment. Percentages of increase in the $CD86^{bright}$ SSC^{low} cell population were calculated as : 100 x percentage of $CD86^{bright}$ SSC^{low} cells with test compound / percentage of $CD86^{bright}$ SSC^{low} cells with control. Shown are the mean percentages of increase in the the $CD86^{bright}$ SSC^{low} cell population from the 3 blood donors. *except for $F(ab')_2$: 6.6; 0.6; and 0.06 µg/ml.

Figure 4: H27K15 induces IL-12p70 secretion and increases macrophage IL-12/IL-10 ratios

IL-12p70 and IL-10 were titrated in the culture supernatants from day-6 macrophages differentiated in the presence of mAb H27K15 (1 µg/ml), GW2580 (1 µM) or their respective negative controls rituximab or no treatment. Left panel : IL-12p70 and IL-10 levels (pg/ml) in macrophage cultures from 3 blood donors. Right : IL-12p70 (pg/ml) / IL-10 (pg/ml) ratios were calculated for each blood donor and each culture condition. In samples where IL-12p70 was undetectable (below the detection limit of 11 pg/m)l, its level was arbitrarily set at 1pg/ml for the calculation of IL-12p70 / IL-10 ratios.

Figure 5: H27K15 inhibits MCP-1 / CCL2 and IL-6 secretion by macrophages

MCP-1 and IL-6 were titrated in the culture supernatants from day-6 macrophages differentiated in the presence of mAb H27K15 (1 µg/ml), GW2580 (1 µM) or their respective negative controls rituximab or no treatment. Percentages of reduction in MCP-1 (upper panel) or in IL-6 production (lower panel) were calculated for the 3 blood donors as : 100 - [100 x cytokine concentration with test compound (pg/ml) / cytokine concentration with control (pg/ml)].

Figure 6. Down-regulation of MMP-9 production by mAb H27K15

Monocytes isolated from 3 different blood donors were differentiated for 6 days in the presence of GM-CSF and CSF-1, with or without mAb H27K15, Rituximab or GW2580. MAb H27K15 or Rituximab (0.1, 1 or 10 µg/ml) or equimolar concentrations of $F(ab)'_2$ derived from both mAbs were added to the cultures. MMP-9 was titrated by ELISA (R&D Systems) in day-6 culture supernatants.

Figure 7. MAb H27K15 inhibits the differentiation of $CD163^+$ M2-type macrophages.

Cells obtained after 6-day culture with GM-CSF and CSF-1 were harvested and incubated for 20 min at 4°C with PBS containing human IgG Fc fragments to saturate Fc receptors. Fluorochrome-conjugated mAbs (anti-CD14-PerCP-Cy5.5, anti-CD163-PE, anti-CD206-APC, anti-CD1a-FITC, BD Biosciences) were then incubated with each sample for 20 min at 4°C. FCM analysis was performed using a FACS LSR-II (BD biosciences) with the DIVA software. Data from 3 different blood donors are presented.

Figure 8. Upregulation of M1:M2 macrophage ratios by mAb H27K15.

Monocytes from 2 different donors were cultured with GM-CSF and CSF-1 in the presence or absence of anti-CD115 mAb H27K5 or control IgG_1 rituximab at 1 µg/ml. Ratios between M1 ($CD14^+CD163^-$) and M2 ($CD14^+CD163^+$) -type macrophages among the macrophage population were determined after 6 days of cell differentiation.

Figure 9. MAb H27K15 skews monocyte differentiation towards DCs instead of macrophages.

Cells obtained after 6-day culture with GM-CSF and CSF-1 were harvested and incubated for 20 min at 4°C with PBS containing human IgG Fc fragments to saturate Fc receptors. Fluorochrome-conjugated mAbs (anti-CD14-PerCP-Cy5.5, anti-

CD163-PE, anti-CD206-APC, anti-CD1a-FITC, BD Biosciences) were then incubated with each sample for 20 min at 4°C. FCM analysis was performed using a FACS LSR-II (BD biosciences) with the DIVA software. Data from 6 different blood donors are presented.

Figure 10. H27K15 treatment increases the immunostimulatory capacity of monocytes differentiated with GM-CSF and CSF-1.

MLR between allogeneic PBMCs and cells from day-6 monocytes differentiated with GM-CSF and CSF-1 and treated with mAb H27K15 or rituximab at 4 different concentrations. T(-): unstimulated PBMCs. T(+): PBMC stimulated with anti-CD3 mAb (BD Pharmingen) and IL-2 (125 UI/ml), corresponding to 100% proliferation. Percentages of proliferating PBMCs were calculated as follows : % CFSE-low cells with test mAb x 100 / % CFSE-low cells with T(+).

Figure 11. Monoclonal antibody H27K15 inhibits MCP-1/CCL2 production by monocytes cultured with either CSF-1 or IL-34

Figure 11A: MCP-1/CCL2 was titrated in culture supernatants from monocyte-derived cells after a 6-day culture in 48-well plates with CSF-1 or IL-34 in the presence or absence of monoclonal antobody H27K15 or isotype control rituximab (both at 1 µg/ml). The result shown was obtained using cells from one blood donor representative of 3 tested.

Figure 11B: Cell viability was assessed in parallel, using cells from the same donor cultured in 96-well plates with similar conditions. Viability was only marginally affected by H27K15 antibody.

Examples

The following commercial monoclonal antibodies were used throughout the study: anti-human CD115 mAb 2-4A5-4 (rat $IgG_{1,K}$,

Santa Cruz), 9-4D2 (rat IgG1, Biolegend) and isotype control rat IgG_1 (R&D Systems). mAb 1.2 SM is anti-CD115 mAb of sequence 1.2 SM published in patent application WO 2009/026303. Rituximab was obtained from Roche. F(ab')2 were produced at Transgene by pepsine digestion of the monoclonal antibodies, followed by purification by gel filtration.

Human macrophage differentiation assay: protocol

Buffy coats were provided by the Etablissement Français du Sang (EFS, Strasbourg). Peripheral blood mononucleated cells (PBMC) were obtained by centrifugation on a ficoll gradient. Monocytes were purified by immunomagnetic cell sorting, using CD14-antibody-coated beads (Miltenyii). Enriched monocyte suspensions were more than 95% pure. Monocytes were differentiated for 6 days in 48-well plates (3 x 10^5 cells/well) in RPMI-Glutamax™ medium supplemented with 10% heat inactivated fetal calf serum and 1% tri-antibiotic mixture (penicillin, streptomycin, neomycin). GM-CSF (10 ng/ml) was added in the cell culture medium from day 0 to day 3. H27K15, other antibodies or internal control GW2580 (LC Labs) were added at day 0. On day 3 post-isolation, monocytes were washed with PBS and further cultivated in medium supplemented with CSF-1 (10 ng/ml) and GM-CSF (2 ng/ml), in the presence or absence of antibodies or GW2580. On day 6, supernatants were collected and stored at -20°C. Cells were detached from the plastic and pools of triplicates were analyzed by IC/FC (immunocytochemistry / flow cytometry) for cell sizes and expression of FcγR and CD86. Cytokines and chemokines were quantified in the culture supernatants by multiplex (Bioplex, Bio-Rad) or by ELISA.

Results

Anti-CD115 mAb H27K15 is not cytotoxic to macrophages but polarizes their differentiation towards the M1 type

To study whether mAb H27K15 could affect macrophage differentiation, purified $CD14^+$ monocytes were cultured for 7 days in the presence of GM-CSF and CSF-1, known to induce respectively M1- and M2-type macrophages (Akagawa K.S., 2002; Verreck F.A. *et al.*, 2004). Three different doses of mab H27K15 or isotype control rituximab (0.1 ; 1 or 10 µg/ml) were added to the culture medium at the beginning of the culture and again 3 days later. F(ab')2 generated from H27K15 or from rituximab were tested in parallel at equivalent molar concentrations. F(ab')2 generated from mAb 1.2 SM (WO 2009/026303) was tested for comparison with the F(ab')2 derived from H27K15 or rituximab. The known blocking mAb to human CD115, 2-4A5, or the non-blocking mAb 9-4D2 (Sherr C.J. *et al.*, 1989) were assayed and compared with isotype control rat IgG1. As another control, the small molecule CD115 tyrosine kinase inhibitor GW2580 was added to some of the cultures at 1 µM, a concentration previously shown to inhibit the CSF-1-dependent proliferation of human monocytes and the differentiation of murine macrophages *in vitro* (Conway J.G. *et al.*, 2005; Paniagua R.T. *et al.*, 2010).

Microscopical observation of day-6 cultures showed no obvious differences between wells treated with H27K15, Rituximab, mAb 9-4D2, IgG1, H27K15 F(ab')2 or Rituximab F(ab')2, compared with untreated cells, independently of the blood donor. For the wells treated with mAb SM1.2 F(ab')2, full cytotoxicity was observed for all donors for the 3 doses evaluated (0.066, 0.66 and 6.6 µg/ml). For the wells treated with mAb 2-4A5, full cytotoxicity was observed for all donors at the 2 highest doses tested (1 and 10 µg/ml). For the lowest dose (0.1 µg/ml), cytotoxicity was only partial. Altogether, those results did not reveal any toxicity of any antibody except for mAb SM1.2 F(ab')2 and mAb 2-4A5. For the cells with the control GW2580 at 1 µM, depending on the microscope field, a relative cytotoxicity was visualized as debris and less

density of cells was observed in all blood donors. Cell viability was analyzed at day 6 by counting 5 microscope fields in each well of the plate (one in the center of the well and four fields at mid-distance between the center and the side of the well). Based on the above observations, numeration was done also for wells treated with compounds exhibiting partial or full cytotoxicity (mAb SM1.2 F(ab')2, mAb 2-4A5 and GW2580). Figure 1 shows the means of 5 fields counted by individual well ± standard deviation. Some antibodies exhibited partial or full cytotoxicity. Thus, the F(ab')2 derived from mAb SM1.2 had induced massive cell death at all concentrations and mAb 2-4A5 also dramatically reduced macrophage numbers, in comparison with their respective controls. In the presence of GW2580, approximately 70% of the macrophage number remained at day 6 compared with untreated cultures.

Day 6 cultures were analyzed by IC/FC for surface expression of the activating FcγR CD64 (FcγRI) and of the activation marker CD86. As shown on Figure 2, surface expression of CD64 was drastically reduced upon treatment with H27K15 or with GW2580. H27K15 almost totally inhibited CD64 expression at all doses tested. Rat anti-human CD115 mAb 2-4A5 also decreased surface CD64 expression, but in a dose-dependent fashion. The non-CD115 blocking mAb 9-4D2 did not modify CD64 expression level. Interestingly, $F(ab')_2$ derived from H27K15 also down-regulated CD64 expression, but less potently than the full mAb and only when tested at 1 or 10 µg/ml, suggesting that the effect of H27K15 was partially Fc-dependent.

When the expression of the activation marker / co-stimulatory molecule CD86 was analyzed in day-6 macrophages, we found that a sub-population of cells characterized by the $CD86^{bright}$ SSC^{low} phenotype had appeared in the cultures treated with either mAb H27K15 or with GW2580 (Figure 3). Induction of this population

of round-shaped cells expressing high levels of CD86 was not observed with H27K15-derived $F(ab')_2$, suggesting a role for H27K15 Fc region in this phenomenon. Gating on this population showed that $CD86^{bright}$ SSC^{low} cells were mainly CD64 ^{low} to CD64-negative (data not shown).

IL-12p70 and IL-10 were titrated in day-6 culture supernatants. Macrophages from the 3 donors tested did not produce any detectable IL-12p70 following culture with human IgG_1 rituximab or no reagent. Culture in the presence of mAb H27K15 induced IL-12p70 secretion by macrophages from 2 of the 3 blood donors. In contrast, IL-12p70 was not detectable after treatment with GW2580 (Figure 4). IL-10, which was produced by resting macrophages from all donors, was up-regulated by H27K15 in macrophages from donor 1 but not in donor 2 and was only weakly increased in donor 3. As a result, IL-12p70 / IL-10 ratios were up-regulated in the 3 blood donors tested (Figure 4, right panel). Small molecule GW2580 also increased IL-12p70 / IL-10 ratios, but rather due to the inhibition of IL-10 production.

These results show that targeting CD115 with mAb H27K15 on differentiating macrophages not only dramatically down-regulates the expression of CD64 / FcγRI, but also induces a population of SSC^{low} cells expressing high levels of the CD86 activation marker. In addition, H27K15 can induce IL-12p70 production and upregulates IL-12p70 / IL-10 ratios in all donors, indicative of macrophage polarization towards M1-type.

Strikingly, production of the chemokine MCP-1 / CCL2 was found to be almost totally suppressed when macrophages were differentiated in the presence of mAb H27K15 or GW2580 (Figure 5, upper panel). Inhibition of MCP-1 secretion by H27K15 was effective in the 3 donors tested and ranged from 74 % to 99 %. Small molecule GW2580 was as efficacious as H27K15 in suppressing MCP-1 production. Levels of IL-6 in day-6

macrophage culture supernatants were also reduced by either H27K15 or GW2580 in all donors (Figure 5, lower panel). Inhibition of IL-6 production was less drastic with H27K15 (from 27 % to 70 %) than with GW2580 (from 75 to 96%). H27K15-mediated inhibition of macrophage MCP-1 and IL-6 production (Roca H. *et al.*, 2009), two soluble factors implicated in M2 macrophage polarization, is another piece of evidence indicating that the anti-CD115 mAb repolarizes macrophages towards M1.

Anti-CD115 mAb H27K15 inhibits MMP-9 production by monocytes cultured with GM-CSF and CSF-1

Tumor-associated macrophages are known to produce MMP-9 (matrix-metalloprotease 9), which promotes both tumor cell metastasis by degrading the extracellular matrix and neoangiogenesis by inducing VEGF release in the tumor microenvironment. MMP-9 produced by macrophages is a major regulator of the angiogenic switch in tumors.

$CD14^+$ monocytes from 3 different donors were allowed to differentiate in the presence of both GM-CSF and CSF-1, known to induce macrophage differentiation towards respectively the M1- and M2-types. MAb H27K15 or Rituximab (used as a negative control) were added to the cultures at 0.1, 1 or 10 µg/ml. Equimolar concentrations of $F(ab)'_2$ derived from both mAbs were assayed in parallel. The tyrosine kinase inhibitor GW2580, previously shown to inhibit the CSF-1-dependent proliferation of human monocytes and the differentiation of murine macrophages *in vitro* was tested in the same assay. After 6 days of culture, MMP-9 concentrations were measured in the supernatants by ELISA (Figure 6). In 2 out of 3 donors tested, there was a dose-dependent decrease in MMP-9 production when cultures were treated with mAb H27K15 or with GW2580. With $F(ab)'_2$ derived from H27K15, there was an inhibitory effect in the same 2 donors. Thus, mAb H27K15 is

capable of decreasing MMP-9 secretion by differentiating M2 macrophages.

These observations in macrophage cultures suggest that H27K15 administered to cancer patients may down-regulate MMP-9 concentration in the tumor microenvironment.

MAb H27K15 inhibits the differentiation of CD163-positive M2-type macrophages

The hemoglobin scavenger receptor (CD163) has been identified as a marker of M2-polarized macrophages which is expressed by TAMs, notably in breast cancer. The surface expression of CD163 was analyzed by flow cytometry in day-6 macrophages derived from human monocytes cultured with GM-CSF and CSF-1. Figure 7 shows the percentages of CD163-positive cells in differentiated cultures. Culture with mAb H27K15 inhibited the differentiation of the $CD163^+$ macrophage population in all 3 donors tested. In the presence of 1 µg/ml H27K15, percentages of CD163-positive cells decreased from 2.5 to 4 folds compared with control cultures treated with rituximab. GW2580 had the same effect in only 2/3 donors. $F(ab)'_2$ derived from H27K15 had weak or no effect on CD163 expression, indicating that the Fc region of the anti-CD115 mAb was involved in its mode of action.

As evidenced by these changes in surface CD163 expression and in agreement with the previous results, targeting CD115 with mAb H27K15 inhibits the differentiation of M2-type macrophages.

MAb H27K15 skews monocyte differentiation from M2 to M1 macrophages

The ratios between M1 and M2 macrophages were analyzed in cells derived from monocytes cultured with GM-CSF and CSF-1, in the presence or absence of mAb H27K15 or rituximab. After a

6-days culture of cells from 3 different blood donors, M1 ($CD14^+CD163^-$) versus M2 ($CD14^+CD163^+$) macrophages were quantified by flow cytometry. Figure 8 shows the M1/M2 macrophage ratios calculated among the $CD14^+$ macrophage population for each culture condition. MAb H27K15 at 1 µg/ml increased M1/M2 ratios in macrophages from the 3 donors tested, compared to control IgG_1 rituximab at the same concentration or to untreated cultures.

MAb H27K15 skews monocyte differentiation from M2 macrophages to dendritic cells (DCs)

The phenotypes of monocytes-derived cells obtained after 6 days of culture in the presence of GM-CSF and CSF-1 were analyzed by FC for expression of the monocyte-macrophage marker CD14 and of CD163 and CD206, common markers of M2-type macrophages (Porcheray *et al.* 2005, *Clin Exp Immunol.*;142(3):481-489. ; Schmieder *et al.* 2012. *Semin Cancer Biol.*;22(4):289-297). Because it has been reported that CSF-1, together with IL-6, inhibits the differentiation of DCs (Menetrier-Caux *et al.* 1998, *Blood.*;92(12):4778-4791), cultures were analyzed for cell-surface expression of the DC marker CD1a. Figure 9A shows that while $CD14^+CD163^+CD206^+$ M2-macrophages had disappeared in cultures treated with H27K15, the population of $CD1a^+CD14^-$ cells was concomitantly induced. Figure 9B shows the percentages of the $CD14^+CD1a^-$ versus the $CD14^-CD1a^+$ populations, measured at day 6 in cultures from 3 different blood donors. In 3/3 donors, $CD1a^+CD14^-$ dendritic cells replaced $CD1a^-CD14^+$ macrophages after monocyte differentiation with mAb H27K15.

These results show that mAb H27K15 can skew the differentiation of M2-polarized macrophages towards DCs.

Monocytes differentiated with GM-CSF and CSF-1 in the presence of mAb H27K15 have increased immunostimulatory capacity

Monocytes cultured for 6 days with GM-CSF and CSF-1 in the presence of mAb H27K15 gave rise to a cell population devoid of M2-type macrophages but comprising DCs. DCs or M1-type macrophages are known to be more potent than M2-polarized macrophages in stimulating T cell responses (Chomarat *et al.* 2000, *Nat Immunol.*;1(6):510-514; Duluc *et al.* 2007 *Blood.*;110(13):4319-4330). We therefore tested the capacity of day-6 cultures to stimulate allogeneic lymphocyte proliferation in a Mixed Lymphocyte Reaction (MLR). Monocyte-derived cells obtained after culture with mAb H27K15 or Rituximab were stimulated with LPS for 48 h, harvested and co-cultured for 5 days with CFSE-labelled allogeneic PBMCs. The percentages of PBMCs which had proliferated in the co-cultures were measured using FC, by gating on CFSE-low cells. Figure 10 shows that the percentages of proliferating PBMCs were increased upon co-culture with monocytes differentiated in the presence of H27K15, compared with Rituximab.

This result shows that monocytes differentiated with GM-CSF and CSF-1 in the presence of mAb H27K15 have an increased capacity to induce lymphocyte proliferation, correlating with the inhibition of M2-type macrophages and the induction of DCs. These data suggest that mAb H27K15 may facilitate immune responses in treated patients.

MAb H27K15 inhibits MCP-1/CCL2 production by monocytes cultured with either CSF-1 or IL-34

Interleukin-34 (IL-34) has been identified as another CD115 ligand, with biological effects comparable to those of CSF-1 (Droin and Solary, 2010, J; Leukoc Biol 87:745-7). Purified $CD14^+$ monocytes were cultured in 48-well or 96-well plates in medium supplemented with either CSF-1 or IL-34 (50 ng/ml, Peprotech). MAb H27K15 or rituximab were added to some of the wells on day 0 and day 3. On day 6, culture supernatants from 48-well plates were sampled and the chemokine MCP-1/CCL2 was

titrated by ELISA (R&D Systems). Cell viability was measured in 96-well plates using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega).

Figure 11A shows that IL-34 as well as CSF-1 was able to induce MCP-1/CCL2 production by monocytes. MAb H27K15 drastically reduced the chemokine levels in both culture conditions, compared with isotype control (rituximab) or untreated cultures. This result shows that mAb H27K15 can inhibit CD115 stimulation by either CSF-1 or IL-34, and potently down-regulate the production of MCP-1/CCL2. This chemokine is known to play a main role in the recruitment of M2-polarized TAM (Sica *et al.* 2006, Eur J Cancer 42:717-27).

This inhibition of MCP-1/CCL2 production was not due to cytotoxicity, as shown by cell viability measurement (Figure 11B). As shown previously (Figures 1 and 5) for monocytes differentiated in presence of GM-CSF and CSF-1, mAb H27K15 down-regulates MCP-1 production without depleting monocytes cultured with CSF-1 or IL-34 alone.

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CLAIMS

1. 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, 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 NO:23, and wherein said antibody comprises (a) heavy variable region consisting in SEQ ID NO: 29, and (b) a light variable region consisting in SEQ ID NO: 33.

2. The method of claim 1, wherein said method decreases the macrophage M2 pool.

3. The method of claim 1 or 2, wherein said method further increases M1-type macrophage pool.

4. The method of any one of the claims 1 to 3, wherein said method inhibits macrophage MCP-1, IL10 and IL-6 production.

5. The method of any one of the claims 1 to 4, wherein said method down regulates surface FcγRI (CD64) and FcγRIII (CD16) expression on macrophages.

6. The method of any one of the claims 1 to 5, wherein said method up-regulates IL-12/IL10 ratio in patient samples, e.g blood samples.

7. The method of any one of the claims 1 to 6, wherein the dendritic cells generated ex vivo are suitable for reducing 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

8. The method of any one of the claims 1 to 7, wherein the dendritic cells generated ex vivo are suitable to be used for treatment of a patient suffering from conditions associated with undesirable M2 polarization is selected in the group consisting of cancer, asthma, allergy and progressive fibrosis diseases.

9. The method of any one of the claims 1 to 8, 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 NO:23, to amino acids Asn72, Ser94-Ala95-Ala96, Lys102, Asp131-Pro132-Val133 and Trp159 of SEQ ID NO:23.

10. The method of any one of the claims 1 to 9, 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.

11. The method of any one of the claims 1 to 10, 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.

12. The method of any one of the claims 1 to 11, 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.

13. The method of any one of the claims 1 to 12, wherein said antibody able to bind to CSF-1R is comprising (a) an heavy chain consisting in SEQ ID NO:24, and (b) a light chain consisting in SEQ ID NO:28.