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(54) Title: PROGNOSIS OF DEMYELINATING DISEASES PATIENTS AND TREATMENT THEREOF

(57) Abstract: The present invention relates to an in vitro method for the prognostic of a subject affected by a demyelinating disease comprising detecting and quantifying the level of at least one of CCL19, IL-15, ENA78 and/or TRAIL in a biological fluid from the subject. The invention also relates to the treatment of a subject affected by a demyelinating disease comprising the use of at least one modulator of CCL19, IL-15, ENA78 and/or TRAIL.



PROGNOSIS OF DEMYELINATING DISEASES PATIENTS AND TREATMENT THEREOF

FIELD OF INVENTION

- 5 The present invention relates to the field of demyelinating diseases, to methods for prognosing demyelinating diseases and to methods for treating thereof.

BACKGROUND OF INVENTION

10 Myelin destruction leads to irreversible neurological disabilities in patients affected by demyelinating diseases such as multiple sclerosis (MS). MS is an auto-immune disease of the central nervous system (CNS) resulting in the destruction of myelin formed by oligodendrocytes (OL).

Remarkably, myelin repair can occur in some patients, and then not only elicits axonal conduction restoration but also functional improvement. In MS, successful remyelination
15 is correlated with a decrease of disease severity. Therefore, remyelination-stimulating processes have been investigated in order to treat patients affected by demyelinating diseases such as MS.

Recent studies report that the extent of remyelination is highly variable among patients and is correlated neither with the disease duration nor with the different clinical forms of
20 the disease: relapsing-remitting (RRMS) or progressive (primary (PPMS)) or secondary (SPMS). The remyelination index is inversely correlated with clinical disability, thus confirming that myelin repair is essential to slower disease evolution.

The most accepted hypothesis to account for remyelination failure relates to a so-called “hostile” environment that presumably prevents the repair process (Franklin RJM. Nat.
25 Rev. Neurosci. 2002;3(9):705–714).

These data suggest that the control of the local inflammatory environment is a key component of a successful remyelination.

The inventors identified among demyelinating disease patients such as MS patients that some patients present a local environment proper for remyelination *in vivo* (high remyelination profile group) and other patients present a local environment hostile for remyelination (low remyelination profile group). The inventors then determined
5 discriminating factors among these two groups of patients comprising factors in the group of CCL19, SDF-1, ENA78, IL-15, TRAIL and LIF.

The state of the art remains unclear about the function of some chemokines/cytokines in the progression and development of demyelinating diseases such as MS. On one hand, some studies have demonstrated an up regulation of CCL19 transcripts in the central
10 nervous system of MS patients (Krumbholz M *et al.*, 2007 J. Neuroimmunol. Oct; 190 (1-2): 72-9). On the other hand, Chen *et al.* (J Immunol. 2002 Feb 1;168(3):1009-17) observed that CCL19 expression induced a significant inflammatory response in the CNS and transgenic mice expressing CCL19 in the CNS developed normally.

Studies also disclose that IL-15 is up-regulated in the central nervous system of MS
15 patients without distinguishing any subgroup of patients (Broux B *et al.*, J Immunol. 2015 Mar 1;194(5):2099-109). Meanwhile, Romme Christensen *et al.*, (J Neuroinflammation. 2012 Sep 14;9:215) seems to suggest that IL-15 expression is increased in RRMS patients in remission.

So far CCL19 and IL-15 were suggested to mediate inflammatory responses and to
20 promote pathogen and debris clearance which consequently damage tissue and enhance cell death. In particular, CCL19 was considered as recruiter of lymphocytes that destroy the myelin (Gomez-Nicola D *et al.*, Exp Neurol. 2010 Apr; 222(2):235-42. Kim CH *et al.*, J Immunol 1998; 160 (5): 2418-2424).

Based on these factors that have a different expression profile between the group of
25 patients having a high remyelination profile and the group of patients having a low remyelination profile, the present invention thus aims to provide (i) a method for discriminating demyelinating diseases patients into one of these two groups and (ii) a method for treating those patients, in particular the patients classified in the low remyelination profile group.

SUMMARY

The present invention relates to an *in vitro* method for the prognostic of a subject affected by a demyelinating disease comprising detecting and quantifying the level of at least one of CCL19, IL-15, ENA78 and/or TRAIL in a biological fluid from the subject.

- 5 In one embodiment, the *in vitro* method further comprises detecting and quantifying the level of at least one of LIF and SDF-1.

In one embodiment, the level of the at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL allows classification of the subject in a group having a low remyelination profile or in a group having a high remyelination profile.

- 10 In one embodiment, the level of the at least one of CCL19, IL-15, ENA78, and/or TRAIL and optionally the level of at least one of LIF and SDF-1 allows classification of the subject in a group having a low remyelination profile or in a group having a high remyelination profile.

- In one embodiment, the measured levels of CCL19, IL-15, ENA78, LIF, SDF-1, and/or
15 TRAIL is/are compared to reference values. Preferably, said reference values correspond respectively to the median levels of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL in a group of patients with a demyelinating disease.

- The present invention also relates to a composition for use in the treatment of a demyelinating disease comprising at least one modulator of CCL19, IL-15,
20 ENA78 and/or TRAIL.

In one embodiment, the composition for use in the treatment of a demyelinating disease further comprises at least one modulator of LIF and/or SDF-1.

- In one embodiment, the at least one modulator of CCL19, IL-15, ENA78, TRAIL, LIF and/or SDF-1 is an antibody or fragment or mimetic thereof, an aptamer, a small
25 molecule, a peptide mimetic, a siRNA, an asRNA, an antagonist, an agonist or an inverse agonist.

In one embodiment, the composition for use in the treatment of a demyelinating disease comprises at least one inhibitor of CCL19, ENA78 and/or SDF-1 and/or at least one activator of IL-15, LIF and/or TRAIL.

5 In one embodiment, the composition for use in the treatment of a demyelinating disease comprises at least one of (i) an inhibitor of CCL19, (ii) an inhibitor of ENA78, (iii) an activator of IL-15 and (iv) an activator of TRAIL, and optionally at least one of an inhibitor of SDF-1 and an activator of LIF.

10 In another embodiment, the composition for use in the treatment of a demyelinating disease comprises at least one antagonist or at least one inverse agonist of CCR7, CXCR2 and/or CXCR4; and/or at least one agonist of IL-15R, LIFR, TRAIL-RI and/or TRAIL-RII.

15 In one embodiment, the composition for use in the treatment of a demyelinating disease comprises at least one of (i) an antagonist of inverse agonist of CCR7, (ii) an antagonist of inverse agonist of CXCR2, (iii) an agonist of IL-15R and (iv) an agonist of TRAIL-RI or TRAIL-RII, and optionally at least one of an antagonist of inverse agonist of CXCR4 and an agonist of LIFR.

20 In one embodiment, the composition is for use in the treatment of a demyelinating disease selected from the group comprising inflammatory demyelinating disorders, multiple sclerosis, acute disseminated encephalomyelitis, transverse myelitis, acute transverse myelitis, Guillain-Barre syndrome, brainstem encephalitis, optic neuritis, neuromyelitis optica, leukodystrophy, adrenoleukodystrophy, adrenomyeloma neuropathy, idiopathic inflammatory demyelinating disease, central pontine myelinolysis, optic neuritis, aquaporin 4 antibody-negative neuromyelitis optica, progressive multifocal leukoencephalopathy, periventricular leukomalacia, Vitamin B12 deficiency, Wernicke's
25 encephalopathy, osmotic demyelination syndrome, Leigh's disease.

In one embodiment, the modulator is to be administered in a subject having a low remyelination profile.

DEFINITIONS

In the present invention, the following terms have the following meanings:

- **“About”**: preceding a figure means plus or less 10% of the value of said figure.
- **“Ligand of CCL19, IL-15, ENA78, LIF, SDF-1, or TRAIL”** refers to a natural or synthetic compound that forms a complex with the chemokines/cytokines CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL. Typical ligands include, but are not limited to, polypeptides and proteins. As used herein, a polypeptide refers to a linear polymer of amino acids (preferably at least 50 amino acids) linked together by peptide bonds. A protein specifically refers to a functional entity formed of one or more polypeptides, and optionally of non-polypeptides cofactors. The ligand can either be an activator, an agonist, an antagonist or an inhibitor of the chemokines/cytokines CCL19, IL-15, ENA78, LIF, SDF-1 or TRAIL.
- **“Positive remyelination profile group”** or **“high remyelination profile group”** refer to subjects affected by a demyelinating disease able to enhance an endogenous remyelination process. In one embodiment, subjects of the positive remyelination profile group or high remyelination profile group show a cytokine/chemokine's profile exhibiting a lower level of CCL19, ENA78, and/or SDF-1 compared to a reference value and/or a higher level of IL-15, LIF, and/or TRAIL compared to a reference value. In another embodiment, T lymphocytes of subjects of the positive remyelination profile group or high remyelination profile group induce *in vitro* a percentage of 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase) positive cells higher than a reference value, and thereby a good oligodendrocyte precursor cells (OPC) differentiation. The enhancement of a remyelination process involves a pro-regenerative microglia (MIG) activation (that lead to a proper OPC differentiation, in particular increase in CNPase⁺ cells which correlate to myelin-producing oligodendrocytes compared to a reference value of a cohort of demyelinating disease subjects, preferably MS subjects). The reference value as used herein may in particular correspond to the average level of cytokines/chemokines described herein among the subjects from the low remyelination profile group.

- **“Negative remyelination profile group”** or **“low remyelination profile group”**
refer to subjects affected by a demyelinating disorder unable to enhance an endogenous remyelination process. In one embodiment, subjects of the negative remyelination profile group or low remyelination profile group show a
5 cytokine/chemokine’s profile exhibiting a higher level of CCL19, ENA78, and/or SDF-1 compared to a reference value and/or a lower level of IL-15, LIF, and/or TRAIL compared to a reference value. In another embodiment, T lymphocytes of subjects of the negative remyelination profile group or low remyelination profile group induce *in vitro* a percentage of CNPase positive cells lower than a reference
10 value, and thereby an impaired OPC differentiation. The reference value as used herein may in particular correspond to the average level of cytokines/chemokines described herein among the subjects from the high remyelination profile group.
- **“Modulator of a cytokine”** or **“modulator of a cytokine pathway”** (both expression may be used interchangeably), in particular a modulator of CCL19, ENA78, SDF-1,
15 IL-15, LIF, or TRAIL refer to a natural or synthetic compound modulating (*i.e.*, activating or inhibiting) the pathway associated with said cytokine. In one embodiment, a modulator of a cytokine may thus present an inhibitory, or activating effect on the expression of the gene encoding said cytokine; and/or on the expression of the cytokine. In another embodiment, a modulator of the cytokine may present an
20 inhibitory or activating effect on the biological activity of said cytokine; and/or on the release/secretion of said cytokine. In one embodiment, a modulator of a cytokine present an inhibitory or activating effect on the downstream biological effect of said cytokine. In particular, a modulator of a cytokine may be an antagonist, an inverse agonist or an agonist of the receptor of said cytokine. As used herein, the term
25 “inhibitor” may thus refer to a natural or synthetic compound that has a biological effect to inhibit or significantly reduce or down-regulate the expression of a gene and/or a protein or that has a biological effect to inhibit or significantly reduce the biological activity of a protein or inhibit downstream biological effects. As used herein, the term “activator” may refer to a natural or synthetic compound that has a
30 biological effect to activate or significantly increase or up-regulate the expression of

a gene and/or a protein or that has a biological effect to activate or significantly increase the biological activity of a protein or activate downstream biological effects.

- As used herein, the term “**consisting essentially of**”, with reference to a composition, pharmaceutical composition or medicament, means that the at least one modulator of the invention is the only one therapeutic agent or agent with a biologic activity within said composition, pharmaceutical composition or medicament.
- “**Pharmaceutically acceptable excipient**” refers to an excipient that does not produce any adverse, allergic or other unwanted reactions when administered to an animal, preferably a human. It includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. For human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by regulatory offices, such as, for example, FDA Office or EMA.
- “**Subject**” refers to a mammal, preferably a human. In one embodiment, the subject is a female or a male, an adult or a child. In one embodiment, a subject may be a “patient”, *i.e.*, a subject who/which is awaiting the receipt of, or is receiving medical care or was/is/will be the object of a medical procedure, or is monitored for the development of a disease.
- “**Therapeutically effective amount**” refers to a level or amount of agent that is aimed at, without causing significant negative or adverse side effects to the target, (1) delaying or preventing the onset of a demyelination disease; (2) slowing down or stopping the progression, aggravation, or deterioration of one or more symptoms of a demyelination disease; (3) bringing about ameliorations of the symptoms of a demyelination disease; (4) reducing the severity or incidence of a demyelination disease; or (5) curing a demyelination disease. A therapeutically effective amount may be administered prior to the onset of a demyelination disease, for a prophylactic or preventive action. Alternatively or additionally, the therapeutically effective amount may be administered after the onset of a demyelination disease, for a therapeutic action. In one embodiment, a therapeutically effective amount of the

modulator is an amount that is effective in reducing at least one symptom of a demyelination disease.

- **“Treating”** or **“treatment”** or **“alleviation”** refers to both therapeutic treatment and prophylactic or preventative measures; wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. A subject or mammal is successfully “treated” for the targeted pathologic disorder if, after receiving a therapeutic amount of the composition of the present invention, the patient shows observable effects on one or more of the followings; (i) promotion of neuronal cell survival; (ii) increase in remyelination; (iii) relief to some extent of one or more of the symptoms associated with the specific disorder or condition; (iv) reduced morbidity and mortality, and (v) improvement in quality of life issues. The above parameters for assessing successful treatment and improvement in the disorder are readily measurable by routine procedures familiar to a physician.
- **“CCL19”** (chemokine (C-C motif) ligand 19) is a T cells attractive chemokine and has a major role in balancing immunity and tolerance through its receptor C-C chemokine receptor type 7 (CCR7).
- **“IL-15”** (interleukin 15) is a proinflammatory cytokine. It is produced by activated blood monocytes, macrophages, dendritic cells, and activated glial cells. It promotes T-cell proliferation, induction of cytolytic effector cells including natural killer and cytotoxic cells and stimulates B-cell to proliferate and secrete immunoglobulins, upon binding to its receptor, IL-15R.
- **“ENA78”** (Epithelial-derived neutrophil-activating peptide 78) also named C-X-C motif chemokine 5 (CXCL5) stimulates the chemotaxis of neutrophils possessing angiogenic properties by interacting with its receptor, CXCR2. It also plays a role in reducing sensitivity to sunburn pain in some subjects, and is a potential target which can be utilized to understand more about pain in other inflammatory conditions like arthritis and cystitis.

- “**LIF**” (leukemia inhibitory factor) is an interleukin 6 class cytokine that affects cell growth by inhibiting differentiation. It binds to the specific LIF receptor, LIFR, which forms a heterodimer with the GP130 signal transducing subunit.
- “**SDF-1**” (stromal cell-derived factor 1) or CXCL12 is strongly chemotactic for lymphocytes. SDF-1 also plays an important role in angiogenesis by recruiting endothelial progenitor cells (EPCs) from the bone marrow through binding to its receptor CXCR4.
- “**TRAIL**” (TNF-related apoptosis-inducing ligand) is a protein functioning as a ligand that induces the process of cell death called apoptosis. It also binds to the death receptors DR4 (TRAIL-RI) and DR5 (TRAIL-RII).

DETAILED DESCRIPTION

In the present invention, the applicant identified two subgroups of MS patients showing strong differences in their ability to induce an endogenous remyelination process. Subjects of the “high remyelination profile group” correspond to subjects that can induce an endogenous remyelination process, whereas subjects of the “low remyelination profile group” cannot induce an endogenous remyelination process or induce a poor endogenous remyelination process. The applicant provided evidence of the heterogeneous cytokine/chemokine profile within both groups of MS subjects (see examples).

Both subgroups differ in particular by their cytokine secretion profile in a biological fluid, in particular by the secretion of chemokine (C-C motif) ligand 19 (CCL19), epithelial-derived neutrophil-activating peptide 78 (ENA78) and/or stromal cell-derived factor 1 (SDF-1) (which are higher in the low remyelination profile group than in the high remyelination profile group) and by the secretion of interleukin 15 (IL-15), leukemia inhibitory factor (LIF) and/or TNF-related apoptosis-inducing ligand (TRAIL) (which are lower in the low remyelination profile group than in the high remyelination profile group).

Moreover, the applicant demonstrated that molecules secreted by lymphocytes obtained for patients of the low remyelination group maintain microglial cells in a pro-inflammatory state within the local environment of demyelinating lesions. In particular,

CCL19 was shown to trigger a shift in M1/M2 balance toward M1-polarized macrophage, as evidenced by the high ratio of inducible Nitric Oxide Synthase (iNOS⁺) to insulin growth factor 1 (IGF-1⁺) expressing cells. Likewise, the applicant shown that CCL19 also chiefly control the remyelination process, notably by regulating the differentiation of oligodendrocyte precursor cells (OPC), as revealed by the low percentage of CNPase⁺ OPC observed within demyelinating lesions.

The identification of this dichotomy in MS patients may thus be used in prognostic methods as well as in methods of treatment, wherein the aim is to restore a secretion profile of CCL19, ENA78 and/or SDF-1 on one hand and IL-15, LIF and/or TRAIL on another hand to levels that can be observed in the high remyelination profile group.

The present invention thus relates to a method for classifying a patient with a demyelinating disease (preferably a MS patient) within the group having a high remyelination profile or within the group having a low remyelination profile. In one embodiment, the method of the invention comprises detecting and quantifying the level of at least one of CCL19, IL-15, ENA78, and/or TRAIL in a biological fluid obtained from the subject. Preferably, the subject affected by a demyelinating disease is a MS subject.

Another object of the present invention is thus an *in vitro* method for the prognostic of a subject affected by a demyelinating disease (*i.e.* for determining if the patient presents a positive or a low remyelination profile), wherein said method comprises detecting and quantifying the level of at least one of CCL19, IL-15, ENA78, and/or TRAIL in a biological fluid obtained from the subject. Preferably, the subject affected by a demyelinating disease is a MS subject.

In one embodiment, the method of the invention comprises detecting and quantifying the level of at least one of CCL19, IL-15, and/or TRAIL in a biological fluid obtained from the subject.

In one embodiment, the method does not consist in detecting and quantifying the level of ENA78 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of CCL19 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of IL-15 in a biological fluid obtained from the subject.

- 5 In one embodiment, said method comprises detecting and quantifying the level of TRAIL in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of ENA78 in a biological fluid obtained from the subject.

- 10 In one embodiment, said method comprises detecting and quantifying the level of CCL19 and IL-15 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of CCL19 and TRAIL in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of CCL19 and ENA78 in a biological fluid obtained from the subject.

- 15 In one embodiment, said method comprises detecting and quantifying the level of IL-15 and TRAIL in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of IL-15 and ENA78 in a biological fluid obtained from the subject.

- 20 In one embodiment, said method comprises detecting and quantifying the level of ENA78 and TRAIL in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of CCL19, IL-15 and TRAIL in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of CCL19, IL-15 and ENA78 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of CCL19, ENA78 and TRAIL in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of IL-15, ENA78 and TRAIL in a biological fluid obtained from the subject.

- 5 In one embodiment, said method comprises detecting and quantifying the level of CCL19, IL-15, ENA78 and TRAIL in a biological fluid obtained from the subject.

In one embodiment, the measured level of the at least one of CCL19, IL-15, ENA78, and/or TRAIL is further compared to a reference value. In a preferred embodiment, the reference value is a median value, measured in a group of demyelinating disease subjects,
10 preferably MS subjects.

In one embodiment, the method further comprises detecting and quantifying the level of at least one of LIF and/or SDF-1, preferably LIF.

In one embodiment, the method does not consist in detecting and quantifying the levels of ENA78 and SDF1 in a biological fluid obtained from the subject.

- 15 In one embodiment, the measured level of the at least one of LIF and/or SDF-1 is further compared to a reference value. Preferably, the reference value is a median value, measured in a group of demyelinating disease subjects, preferably MS subjects.

In one embodiment, the *in vitro* method for the prognostic of a subject affected by a demyelinating disease (*i.e.* for determining if the patient presents a high or a low remyelination profile), comprises detecting and quantifying the level of at least one of
20 CCL19, IL-15, ENA78 and/or TRAIL, preferably CCL19, IL-15 and/or TRAIL and the level of at least one of LIF and/or SDF-1, preferably LIF in a biological fluid obtained from the subject.

In one embodiment, the measured level of the at least one of CCL19, IL-15, ENA78, and/or TRAIL and the measured level of the at least one of LIF and/or SDF-1 are further compared to reference values. Preferably, the reference values are median values, measured in a group of demyelinating disease subjects, preferably MS subjects.
25

In one embodiment, the *in vitro* method for the prognostic of a subject affected by a demyelinating disease (*i.e.* for determining if the patient presents a positive or a low remyelination profile), comprises detecting and quantifying the level of at least one of CCL19, IL-15, ENA78, LIF, SDF-1 and/or TRAIL, preferably at least one of CCL19, IL-15, LIF, and/or TRAIL in a biological fluid obtained from the subject.

In one embodiment, the measured level of the at least one of CCL19, IL-15, ENA78, LIF, SDF-1 and/or TRAIL is further compared to a reference value. Preferably, the reference value is a median value, measured in a group of demyelinating disease subjects, preferably MS subjects.

10 In one embodiment, said method comprises detecting and quantifying the level of (i) CCL19 and LIF, (ii) CCL19 and SDF-1 or (iii) CCL19, LIF and SDF1 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of (i) IL-15 and LIF, (ii) IL-15 and SDF-1 or (iii) IL-15, LIF and SDF1 in a biological fluid
15 obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of (i) TRAIL and LIF, (ii) TRAIL and SDF-1 or (iii) TRAIL, LIF and SDF1 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of (i)
20 ENA78 and LIF, (ii) ENA78 and SDF-1 or (iii) ENA78, LIF and SDF1 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of (i) CCL19, IL-15 and LIF, (ii) CCL19, IL-15 and SDF-1 or (iii) CCL19, IL-15, LIF and SDF1 in a biological fluid obtained from the subject.

25 In one embodiment, said method comprises detecting and quantifying the level of (i) CCL19, TRAIL and LIF, (ii) CCL19, TRAIL and SDF-1 or (iii) CCL19, TRAIL, LIF and SDF1 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of (i) CCL19, ENA78 and LIF, (ii) CCL19, ENA78 and SDF-1 or (iii) CCL19, ENA78, LIF and SDF1 in a biological fluid obtained from the subject.

5 In one embodiment, said method comprises detecting and quantifying the level of (i) IL-15, TRAIL and LIF, (ii) IL-15, TRAIL and SDF-1 or (iii) IL-15, TRAIL, LIF and SDF1 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of (i) IL-15, ENA78 and LIF, (ii) IL-15, ENA78 and SDF-1 or (iii) IL-15, ENA78, LIF and SDF1 in a biological fluid obtained from the subject.

10 In one embodiment, said method comprises detecting and quantifying the level of (i) ENA78, TRAIL and LIF, (ii) ENA78, TRAIL and SDF-1 or (iii) ENA78, TRAIL, LIF and SDF1 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of (i) CCL19, IL-15, TRAIL and LIF, (ii) CCL19, IL-15, TRAIL and SDF-1 or (iii) CCL19,
15 IL-15, TRAIL, LIF and SDF1 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of (i) CCL19, IL-15, ENA78 and LIF, (ii) CCL19, IL-15, ENA78 and SDF-1 or (iii) CCL19, IL-15, ENA78, LIF and SDF1 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of (i)
20 CCL19, ENA78, TRAIL and LIF, (ii) CCL19, ENA78, TRAIL and SDF-1 or (iii) CCL19, ENA78, TRAIL, LIF and SDF1 in a biological fluid obtained from the subject.

In one embodiment, said method comprises detecting and quantifying the level of (i) IL-15, ENA78, TRAIL and LIF, (ii) ENA78, IL-15, TRAIL and SDF-1 or (iii) ENA78, IL-15, TRAIL, LIF and SDF1 in a biological fluid obtained from the subject.

25 In one embodiment, said method comprises detecting and quantifying the level of (i) CCL19, IL-15, ENA78, TRAIL and LIF, (ii) CCL19, IL-15, ENA78, TRAIL and SDF-1

or (iii) CCL19, IL-15, ENA78, TRAIL, LIF and SDF1 in a biological fluid obtained from the subject.

A reference value can be relative to an expression value derived from population studies, including without limitation, such subjects having similar age range, subjects in the same
5 or similar ethnic group, similar demyelinating disease history and the like.

In one embodiment, the reference value is constructed using algorithms and other methods of statistical and structural classification.

In one embodiment, the reference value is derived from the measurement of the level of a cytokine in a biological sample derived from one or more subjects of the reference
10 population. Preferably, the reference population comprises at least 100, more preferably at least 250, more preferably at least 500 subjects.

In one embodiment, the reference value corresponds to the mean level of cytokine measured in the reference population. In another embodiment of the invention, the reference value corresponds to the median level of a cytokine measured in the reference
15 population.

In one embodiment, the reference value corresponds to the level of a cytokine measured in a reference population. In one embodiment, the reference value was measured in a reference population comprising substantially healthy subjects. In another embodiment, the reference value was measured in a reference population comprising demyelinating
20 disease subjects, preferably MS subjects. In one embodiment, the demyelinating disease subjects of the reference population present a low remyelination profile. In another embodiment, the demyelinating disease subjects of the reference population present a high remyelination profile.

As used herein, the term “substantially healthy” means that the subject has not been
25 previously diagnosed or identified as having or suffering from a demyelinating disease (preferably MS), or that has not developed a demyelinating disease (preferably MS).

In one embodiment, a subject with a lower level of at least one of CCL19, ENA78, and/or SDF-1, preferably CCL19, compared to the reference value can be classified in the group

having a high remyelination profile. According to this embodiment, the reference value is preferably derived from a population of demyelinating disease subjects, more preferably of demyelinating disease subjects with a low remyelination profile.

5 In another embodiment, a subject with a higher level of at least one of CCL19, ENA78, and/or SDF-1, preferably CCL19, compared to the reference value can be classified in the group having a low remyelination profile. According to this embodiment, the reference value is preferably derived from a population of demyelinating disease subjects, more preferably of demyelinating disease subjects with a high remyelination profile.

10 In another embodiment, a subject with a higher level of at least one of IL-15, LIF, and/or TRAIL compared to the reference value can be classified in the group having a high remyelination profile. According to this embodiment, the reference value is preferably derived from a population of demyelinating disease subjects, more preferably of demyelinating disease subjects with a low remyelination profile.

15 In another embodiment, a subject with a lower level of at least one of IL-15, LIF, and/or TRAIL compared to the reference value can be classified in the group having a low remyelination profile. According to this embodiment, the reference value is preferably derived from a population of demyelinating disease subjects, more preferably of demyelinating disease subjects with a high remyelination profile.

20 In one embodiment, the term “higher level” refers to an increased level as compared to a reference value, such as, for example, an level superior or equal to 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 300%, 400%, 500%, 600%, 700%, 800% 900% or 1000% or more of the reference value.

25 In another embodiment, the term “lower level” refers to a decreased expression level as compared to a reference expression level, such as, for example, a level inferior or equal to 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, or less of the reference value.

Examples of biological fluids include without limitation, a bodily fluid, whole blood, blood serum, blood plasma, cerebrospinal fluid, urine, mucous, saliva, suspended fecal material, menstrual fluid, perspiration or seminal fluid.

In one embodiment, the biological fluid is whole blood, blood serum or blood plasma.

In one embodiment, the biological fluid is whole blood or blood serum.

In one embodiment, the *in vitro* method for the prognostic of a subject affected by a demyelinating disease comprises the steps of:

- 5 1. obtaining peripheral blood mononuclear cells (PBMC) and/or lymphocytes from a biological fluid sample from said subject;
 2. contacting said PBMC and/or lymphocytes with at least one TCR activating agent;
 3. detecting and quantifying the level of at least one of CCL19, IL-15, ENA78 and/or TRAIL, preferably at least one of CCL19, IL-15 and/or TRAIL.
- 10 In one embodiment, the level of at least one of CCL19, IL-15, ENA78 and/or TRAIL is measured in the culture supernatant.

According to one embodiment, the *in vitro* method further comprises in step 3), detecting and quantifying the level of at least one of LIF and/or SDF-1, preferably LIF.

- According to one embodiment, peripheral blood mononuclear cells (PBMC) or
15 lymphocytes are obtained from a biological fluid of the subject, preferably by centrifugation on a Ficoll gradient.

- In one embodiment, PBMC or lymphocytes are activated in the presence of one or more TCR activating agents. In one embodiment, the TCR activating agents are polyclonal activators of T lymphocytes such as anti-CD3 + anti-CD28 antibodies or Interleukin-2,
20 PMA + ionomycin.

In another embodiment, the TCR activating agent is an antigen presented by an antigen presenting cell. Preferably the cells are activated in the presence of anti-CD2/anti-CD3/anti-CD28 antibodies that can be conjugated to beads.

- Cells are activated in the presence of one or more TCR activating agents from 36h to 96h.
25 Medium is then harvested at about 36h, 48h, 72h or 96h and cytokines production measured by methods well known in the art.

Methods to determine the level of cytokines/chemokines, preferably CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, are well-known to the skilled artisan, and include, without limitation, determining the transcriptome (in an embodiment relating to the transcription level of a cytokine/chemokine), and/or the proteome (in an embodiment relating to the translation level of a cytokine/chemokine).

Methods to determine the level of cytokines/chemokines, preferably CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL include without limitation, the determination of the level of these cytokines/chemokines at the transcription level or at the translation level or at the secretion/release level of said cytokines/chemokines.

10 In one embodiment of the invention, the level of cytokines/chemokines, preferably CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, is assessed at the transcription level (*i.e.*, at the mRNA level).

In vitro methods for assessing the transcription level of a cytokine/chemokine are well known in the prior art. Examples of such methods include, but are not limited to, RT-PCR, RT-qPCR, Northern Blot, hybridization techniques such as, for example, use of microarrays, and combination thereof including but not limited to, hybridization of amplicons obtained by RT-PCR, sequencing such as, for example, next-generation DNA sequencing (NGS) or RNA-seq (also known as “Whole Transcriptome Shotgun Sequencing”) and the like.

20 In one embodiment of the invention, the level of cytokines/chemokines, preferably CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, is assessed at the translation level (*i.e.*, at the protein level).

In vitro methods for assessing the translation level of a cytokine/chemokine are well-known in the art. Examples of such methods include, but are not limited to, immunohistochemistry, Multiplex methods (Luminex), western blot, enzyme-linked immunosorbent assay (ELISA), sandwich ELISA, fluorescent-linked immunosorbent assay (FLISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), flow cytometry (FACS) and the like.

In one embodiment of the invention, determining the level of a cytokine/chemokine, preferably CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL specifically corresponds to the detection and quantification of said cytokines secreted in the biological fluid.

In vitro methods for determining a protein secreted in a biological fluid are well-known in the art. Examples of such methods include, but are not limited to, immunohistochemistry, Multiplex methods (Luminex), western blot, enzyme-linked immunosorbent assay (ELISA), sandwich ELISA, fluorescent-linked immunosorbent assay (FLISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), flow cytometry (FACS) and the like.

10 The present application also relates to the *in vitro* use of means for measuring the level of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL as described here above for the prognostic of a demyelinating disease.

Examples of means for measuring the level of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL include without limitation, specific primers of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL or ligands of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, such as, for example, antibodies anti-CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL.

In one embodiment, said means for measuring the level of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL is a ligand, preferably an antibody directed to CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL.

20 Examples of human anti-CCL19 antibodies include without limitation, recombinant human anti-CCL19 (1ng/mL, R&D systems), mouse monoclonal CCL19/MIP-3 beta Antibody, clone 2A12 (Novus Biologicals), rabbit anti human MIP-3 BETA AHP789 (Biorad), Rabbit Polyclonal Anti-CCL19 / MIP3-Beta Antibody LS-C104606-100 (LifeSpan BioSciences), Goat Polyclonal Anti-CCL19 / MIP3-Beta Antibody AF361
25 (R&D Systems) or from multiplex kit. Other examples of antibodies directed to human CCL19 include without limitation, monoclonal 3E9 (SAB1402911 from Sigma), monoclonal YNR-HMIP3b (ANT-213, Prospec protein specialists), monoclonal 54909 (MAB361; R&D systems) anti CCL19/MIP3beta antibody (LS-C10460650; Lifespan Biosciences).

Examples of human anti-IL-15 antibodies include without limitation, Rabbit Polyclonal IL-15 Antibody NBP1-58976 (Novus Biologicals), mouse monoclonal antibody clone ct2nu (eBioscience), human IL-15 monoclonal antibody (Clone 34505) (R&D Biosystems), anti-IL-15, clone 1H3 (Merck Millipore) or from multiplex kit. Other
5 examples of antibodies directed to human IL-15 include without limitation, human anti IL-15 antibody 146b7, human anti-IL-15 from Pepro Tech Co./catalog No. 200-15, antibodies described in US patent 6,165,466 (which is incorporated herein by reference and describes an IL-15 specific monoclonal antibody directed against the epitopes containing Asp56/Gln156 and preventing signal transduction via the IL-15 receptor),
10 antibodies described in US patent 6,013,480 or 6,177,079 (which are incorporated herein by reference); or antibodies described in US patent 5,795,966 (which is incorporated herein by reference).

Examples of human anti-ENA78 antibodies include without limitation, recombinant hENA-78 (peprotech ref 500-P91), TA590149 (Acris-Origene), MBS535839
15 (mybiosource) or from multiplex kit. Other examples of antibodies directed to human ENA78 include without limitation, polyclonal rabbit anti-human ENA-78 sera produced by immunization of rabbits with ENA-78, (R & D Systems) in multiple intradermal sites with Freund's complete adjuvant (Arenberg DA, et al J Clin Invest. 1996;97(12):2792-2802), polyclonal anti-human 500-P91 (Preprotech), clone 33170 (Novus Biologicals).

20 Examples of human anti-LIF antibodies include without limitation, anti-human LIF mouse monoclonal antibody (clone 1F10) 100 µg (eBioscience), human LIF goat polyclonal antibody AF-250-NA (R&D Systems) or from multiplex kit.

Examples of human anti-SDF-1 antibodies include without limitation, CXCL12/SDF-1 Antibody mouse monoclonal (MM0211-9N26) (Novus Biologicals), rabbit polyclonal
25 antibody PAB9508 (Abnova Corporation) or from multiplex kit.

Examples of human anti-TRAIL antibodies include without limitation, mouse monoclonal TRAIL/TNFSF10 antibody 55B709.3(Novus Biologicals), TRAIL rabbit monoclonal antibody (clone C92B9) (Cell signaling) or from multiplex kit.

In one embodiment, said ligand of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL is tagged.

An additional polypeptide ("tag") can be added on the ligand (preferably on the antibody) for the purpose of purifying or identifying or detecting or quantifying a complex formed between said ligand and CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL. Protein tags make it possible, for example, for the polypeptides to be adsorbed, with high affinity, to a matrix, and for the matrix then to be washed stringently with suitable buffers without the complex being eluted to any significant extent, and for the adsorbed complex subsequently to be eluted selectively. Examples of protein tags which are known to the skilled person are a (His)₆ tag, a Myc tag, a FLAG tag, a hemagglutinin tag, a glutathione transferase (GST) tag, intein having an affinity chitin-binding tag or maltose-binding protein (MBP) tag. These protein tags can be located N-terminally, C-terminally and/or internally.

In one embodiment, said ligand of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL is fused to a detectable molecule such as, for example, a fluorescent agent (*e.g.*, GFP protein, Cyanine dye, Alexa dye, Quantum dye, etc) or a Fc fragment.

Examples of demyelinating diseases include without limitation, multiple sclerosis, inflammatory demyelinating disorders, acute disseminated encephalomyelitis, transverse myelitis, acute transverse myelitis, Guillain-Barre syndrome, brainstem encephalitis, optic neuritis, neuromyelitis optica, leukodystrophy, adrenoleukodystrophy, adrenomyeloma neuropathy, idiopathic inflammatory demyelinating disease, central pontine myelinolysis, optic neuritis, aquaporin 4 antibody-negative neuromyelitis optica, progressive multifocal leukoencephalopathy, periventricular leukomalacia, Vitamin B12 deficiency, Wernicke's encephalopathy, osmotic demyelination syndrome, Leigh's disease.

In one embodiment, said demyelinating disease is not optic neuritis. Examples of optic neuritis include, but are not limited to, idiopathic demyelinating optic neuritis and neuromyelitis optica.

In one embodiment, said demyelinating disease is multiple sclerosis. In one embodiment, said multiple sclerosis is relapsing-remitting multiple sclerosis (RRMS), progressive primary multiple sclerosis (PPMS) or secondary multiple sclerosis (SPMS).

Another object of the present invention is a kit for implementing the method of the invention, wherein said kit comprises means for measuring the level of at least one of
5 CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL.

In one embodiment, the level of at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL is assessed at the RNA level, and the kit of the invention may comprise means for total RNA extraction, means for reverse transcription of total RNA, and means for
10 quantifying the expression of RNA of at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL. In one embodiment, the means for quantifying the expression of RNA of at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL are PCR or qPCR primers specific for said cytokines. In one embodiment, the kit also comprises reagents for carrying out a quantitative PCR (such as, for example, buffers, enzyme, and
15 the like). In one embodiment, the kit of the invention may also comprise means for detecting the level of at least one normalization gene at the RNA level.

In another embodiment, the expression level of at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL is assessed at the protein level, and the kit of the invention may comprise means for detecting the at least one cytokine. In one embodiment, said
20 means for detecting the at least one cytokine is an antibody specific of said at least one cytokine. In one embodiment, the kit of the invention may also comprise means for detecting the level of at least one normalization protein or of a protein allowing monitoring protein expression.

By “kit” is intended any manufacture (e.g., a package or a container) comprising at least
25 one reagent for specifically detecting the level of at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL. The kit may be promoted, distributed, or sold as a unit for performing the methods of the present invention. Furthermore, any or all of the kit reagents may be provided within containers that protect them from the external

environment, such as in sealed and sterile containers. The kits may also contain a package insert describing the kit and methods for its use.

Another object of the present invention is a composition comprising, consisting of or consisting essentially of at least one modulator of CCL19, IL-15, ENA78, and/or TRAIL,
5 preferably at least one modulator of CCL19, IL-15, and/or TRAIL.

In one embodiment, the composition of the invention does not consist essentially of a modulator of ENA78.

In one embodiment, said composition comprises, consists of or consists essentially of a modulator of CCL19.

10 In one embodiment, said composition comprises, consists of or consists essentially of a modulator of IL-15.

In one embodiment, said composition comprises, consists of or consists essentially of a modulator of ENA78.

In one embodiment, said composition comprises, consists of or consists essentially of a
15 modulator of TRAIL.

In one embodiment, said composition comprises, consists of or consists essentially of a modulator of CCL19 and a modulator of IL-15.

In one embodiment, said composition comprises, consists of or consists essentially of a modulator of CCL19 and a modulator of ENA78.

20 In one embodiment, said composition comprises, consists of or consists essentially of a modulator of CCL19 and a modulator of TRAIL.

In one embodiment, said composition comprises, consists of or consists essentially of a modulator of IL-15 and a modulator of ENA78.

In one embodiment, said composition comprises, consists of or consists essentially of a
25 modulator of IL-15 and a modulator of TRAIL.

In one embodiment, said composition comprises, consists of or consists essentially of a modulator of ENA78 and a modulator of TRAIL.

In one embodiment, said composition comprises, consists of or consists essentially of a modulator of CCL19, a modulator of IL-15 and a modulator of ENA78.

- 5 In one embodiment, said composition comprises, consists of or consists essentially of a modulator of CCL19, a modulator of ENA78 and a modulator of TRAIL.

In one embodiment, said composition comprises, consists of or consists essentially of a modulator of CCL19, a modulator of IL-15 and a modulator of TRAIL.

- 10 In one embodiment, said composition comprises, consists of or consists essentially of a modulator of IL-15, a modulator of ENA78 and a modulator of TRAIL.

In one embodiment, said composition comprises, consists of or consists essentially of a modulator of CCL19, a modulator of IL-15, a modulator of ENA78 and a modulator of TRAIL.

- 15 In one embodiment, the composition further comprises at least one modulator of LIF and/or SDF-1.

In one embodiment, the composition of the invention does not consist essentially of a modulator of ENA78 and a modulator of LIF. In one embodiment, the composition of the invention does not consist essentially of a modulator of ENA78 and a modulator of SDF1.

- 20 In one embodiment, the composition of the invention does not consist essentially of a modulator of ENA78, a modulator of SDF-1 and a modulator of LIF.

In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of CCL19 and a modulator of LIF, (ii) a modulator of CCL19 and a modulator of SDF-1 or (iii) a modulator of CCL19, a modulator of LIF and a modulator of SDF1.

In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of IL-15 and a modulator of LIF, (ii) a modulator of IL-15 and a modulator of SDF-1 or (iii) a modulator of IL-15, a modulator of LIF and a modulator of SDF1.

5 In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of ENA78 and a modulator of LIF, (ii) a modulator of ENA78 and a modulator of SDF-1 or (iii) a modulator of ENA78, a modulator of LIF and a modulator of SDF1.

10 In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of TRAIL and a modulator of LIF, (ii) a modulator of TRAIL and a modulator of SDF-1 or (iii) a modulator of TRAIL, a modulator of LIF and a modulator of SDF1.

15 In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of CCL19, a modulator of IL-15 and a modulator of LIF, (ii) a modulator of CCL19, a modulator of IL-15 and a modulator of SDF-1 or (iii) a modulator of CCL19, a modulator of IL-15, a modulator of LIF and a modulator of SDF1.

In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of CCL19, a modulator of ENA78 and a modulator of LIF, (ii) a modulator of CCL19, a modulator of ENA78 and a modulator of SDF-1 or (iii) a modulator of CCL19, a modulator of ENA78, a modulator of LIF and a modulator of SDF1.

20 In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of CCL19, a modulator of TRAIL and a modulator of LIF, (ii) a modulator of CCL19, a modulator of TRAIL and a modulator of SDF-1 or (iii) a modulator of CCL19, a modulator of TRAIL, a modulator of LIF and a modulator of SDF1.

25 In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of IL-15, a modulator of ENA78 and a modulator of LIF, (ii) a modulator of IL-15, a modulator of ENA78 and a modulator of SDF-1 or (iii) a modulator of IL-15, a modulator of ENA78, a modulator of LIF and a modulator of SDF1.

In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of IL-15, a modulator of TRAIL and a modulator of LIF, (ii) a modulator of IL-15, a modulator of TRAIL and a modulator of SDF-1 or (iii) a modulator of IL-15, a modulator of TRAIL, a modulator of LIF and a modulator of SDF1.

- 5 In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of ENA78, a modulator of TRAIL and a modulator of LIF, (ii) a modulator of ENA78, a modulator of TRAIL and a modulator of SDF-1 or (iii) a modulator of ENA78, a modulator of TRAIL, a modulator of LIF and a modulator of SDF1.

- 10 In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of CCL19, a modulator of IL-15, a modulator of ENA78 and a modulator of LIF, (ii) a modulator of CCL19, a modulator of IL-15, a modulator of ENA78 and a modulator of SDF-1 or (iii) a modulator of CCL19, a modulator of IL-15, a modulator of ENA78, a modulator of LIF and a modulator of SDF1.

- 15 In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of CCL19, a modulator of ENA78, a modulator of TRAIL and a modulator of LIF, (ii) a modulator of CCL19, a modulator of ENA78, a modulator of TRAIL and a modulator of SDF-1 or (iii) a modulator of CCL19, a modulator of ENA78, a modulator of TRAIL, a modulator of LIF and a modulator of SDF1.

- 20 In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of CCL19, a modulator of IL-15, a modulator of TRAIL and a modulator of LIF, (ii) a modulator of CCL19, a modulator of IL-15, a modulator of TRAIL and a modulator of SDF-1 or (iii) a modulator of CCL19, a modulator of IL-15, a modulator of TRAIL, a modulator of LIF and a modulator of SDF1.

- 25 In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of IL-15, a modulator of ENA78, a modulator of TRAIL and a modulator of LIF, (ii) a modulator of IL-15, a modulator of ENA78, a modulator of TRAIL and a modulator of SDF-1 or (iii) a modulator of IL-15, a modulator of ENA78, a modulator of TRAIL, a modulator of LIF and a modulator of SDF1.

In one embodiment, said composition comprises, consists of or consists essentially of (i) a modulator of CCL19, a modulator of IL-15, a modulator of ENA78, a modulator of TRAIL and a modulator of LIF, (ii) a modulator of CCL19, a modulator of IL-15, a modulator of ENA78, a modulator of TRAIL and a modulator of SDF-1 or (iii) a
5 modulator of CCL19, a modulator of IL-15, a modulator of ENA78, a modulator of TRAIL, a modulator of LIF and a modulator of SDF1.

Another object of the present invention is thus a composition comprising, consisting of or consisting essentially of at least one modulator of CCL19, IL-15, ENA78, and/or TRAIL, and optionally at least one of a modulator of LIF and a modulator of SDF-1.

10 Another object of the present invention is thus a composition comprising, consisting of or consisting essentially of at least one modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, preferably at least one modulator of CCL19, IL-15 and/or TRAIL.

This invention relates in particular to a composition comprising, consisting of or consisting essentially of at least one modulator of CCL19, IL-15, ENA78, and/or TRAIL,
15 and optionally at least one of a modulator of LIF and a modulator of SDF-1, wherein said at least one modulator activates or stimulates remyelination.

This invention relates in particular to a composition comprising, consisting of or consisting essentially of at least one modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, preferably at least one modulator of CCL19, IL-15 and/or TRAIL,
20 wherein said at least one modulator activates or stimulates remyelination.

Examples of modulators include, but are not limited to, ligands, antibodies or fragment or mimetic thereof, aptamers, peptide mimetics, small molecules, antagonists, agonists, inverse agonists, small interfering RNA (siRNA) and antisense (asRNA).

In one embodiment, said modulator is a ligand of CCL19, IL-15, ENA78, LIF, SDF-1,
25 and/or TRAIL, preferably a ligand of CCL19, IL-15 and/or TRAIL.

In another embodiment, said modulator is a ligand of CCL19 receptor, IL-15 receptor, ENA78 receptor, LIF receptor, SDF-1 receptor, and/or TRAIL receptor, preferably a ligand of CCL19 receptor, IL-15 receptor and/or TRAIL receptor.

In one embodiment, said modulator is an antibody directed to CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL.

As used herein, the term “antibody” is selected from the group consisting of a whole antibody, a humanized antibody, a single chain antibody, a dimeric single chain antibody, a Fv, a Fab, a F(ab')₂, a scFv, a defucosylated antibody, a bi-specific antibody, a minibody, a diabody, a triabody, a tetrabody.

Antibodies directed against said cytokines can be raised according to known methods by administering the appropriate antigen or epitope to a host animal selected, *e.g.*, from pigs, cows, horses, rabbits, goats, sheep, and mice, among others. Various adjuvants known in the art can be used to enhance antibody production. Although antibodies useful in practicing the invention can be polyclonal, monoclonal antibodies are preferred. Monoclonal antibodies against said cytokines/chemokines can be prepared and isolated using any technique that provides for the production of antibody molecules by continuous cell lines in culture. Techniques for production and isolation include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975); the human B-cell hybridoma technique (Cote *et al.*, 1983); and the EBV-hybridoma technique (Cole *et al.*, 1985). Alternatively, techniques described for the production of single chain antibodies (see *e.g.* U.S. Pat. No. 4,946,778) can be adapted to produce anti-cytokine, or anti-cytokine ligands single chain antibodies. Cytokine ligand useful in practicing the present invention also include antibody fragments including but not limited to F(ab')₂ fragments, which can be generated by pepsin digestion of an intact antibody molecule, and Fab fragments, which can be generated by reducing the disulphide bridges of the F(ab')₂ fragments. Alternatively, Fab and/or scFv expression libraries can be constructed to allow rapid identification of fragments having the desired specificity to said cytokines/chemokines.

Technics to produce minibodies or bi-specific antibodies which are engineered antibodies similar to F(ab')₂ fragments (V_L-V_H-C_H3,) with bivalent binding are described in Hu S *et al.*, Cancer Res. 1996 Jul 1;56(13):3055-61.

In another embodiment, said antibody fragment is selected from the group consisting of a unibody, a domain antibody, and a nanobody.

In another embodiment, said antibody mimetic is selected from the group consisting of an affibody, an affilin, an affitin, an adnectin, an atrimer, an evasin, a DARPin, an
5 anticalin, an avimer, a fynomer, a versabody and a duocalin.

A domain antibody is well known in the art and refers to the smallest functional binding units of antibodies, corresponding to the variable regions of either the heavy or light chains of antibodies.

A nanobody is well known in the art and refers to an antibody-derived therapeutic protein
10 that contains the unique structural and functional properties of naturally-occurring heavy chain antibodies. These heavy chain antibodies contain a single variable domain (VHH) and two constant domains (CH2 and CH3). A unibody is well known in the art and refers to an antibody fragment lacking the hinge region of IgG4 antibodies. The deletion of the hinge region results in a molecule that is essentially half the size of traditional IgG4
15 antibodies and has a univalent binding region rather than the bivalent binding region of IgG4 antibodies. An affibody is well known in the art and refers to affinity proteins based on a 58 amino acid residue protein domain, derived from one of the IgG binding domain of staphylococcal protein A. DARPins (Designed Ankyrin Repeat Proteins) are well known in the art and refer to an antibody mimetic DRP (designed repeat protein)
20 technology developed to exploit the binding abilities of non-antibody polypeptides. Anticalins are well known in the art and refer to another antibody mimetic technology, wherein the binding specificity is derived from lipocalins. Anticalins may also be formatted as dual targeting protein, called Duocalins. Avimers are well known in the art and refer to another antibody mimetic technology. Versabodies are well known in the art
25 and refer to another antibody mimetic technology. They are small proteins of 3-5 kDa with >15% cysteines, which form a high disulfide density scaffold, replacing the hydrophobic core the typical proteins have.

In one embodiment, the modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL is a monoclonal antibody directed to CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL.

In another embodiment, said modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL is a polyclonal antibody directed to CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL.

In one embodiment, said modulator of CCL19, ENA78, and/or SDF-1 is a neutralizing
5 antibody directed to CCL19, ENA78, and/or SDF-1.

In another embodiment, said modulator of IL-15, LIF, and/or TRAIL is not a neutralizing antibody directed to IL-15, LIF, and/or TRAIL.

In one embodiment, the antibody anti-IL-15, LIF, and/or TRAIL is activating IL-15, LIF, and/or TRAIL signaling pathway and downstream biological effects.

10 In another embodiment, the antibody anti-CCL19, ENA78, and/or SDF-1 is inhibiting CCL19, ENA78, and/or SDF-1 signaling pathway and downstream biological effects.

In one embodiment, said modulator is an aptamer directed to CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL.

Aptamers are a class of molecules that represents an alternative to antibodies in term of
15 molecular recognition. Aptamers are oligonucleotide or oligopeptide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity. Such ligands may be isolated through Systematic Evolution of Ligands by EXponential enrichment (SELEX) of a random sequence library, as described in Tuerk C. and Gold L., 1990. The random sequence library is obtainable by combinatorial chemical
20 synthesis of DNA. In this library, each member is a linear oligomer, eventually chemically modified, of a unique sequence. Possible modifications, uses and advantages of this class of molecules have been reviewed in Jayasena S.D., 1999. Peptide aptamers consist of a conformational constrained antibody variable region displayed by a platform protein, such as *E. coli* Thioredoxin A that are selected from combinatorial libraries by
25 two hybrid methods (Colas *et al.*, 1996).

In another embodiment, the modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL may be an isostere of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, an isomer of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, a tautomer of CCL19, IL-

15, ENA78, LIF, SDF-1, and/or TRAIL, a zwitterion of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, an enantiomer of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, a diastereomer of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, a racemate of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, or stereochemical mixture thereof.

- 5 The term “isosteres” as used herein broadly refers to elements, functional groups, substituents, molecules, or ions having different molecular formulae but exhibiting similar or identical physical properties. For example, tetrazole is an isostere of carboxylic acid because it mimics the properties of carboxylic acid even though they both have different molecular formulae. Typically, two isosteric molecules have similar or identical
- 10 volumes and shapes. Other physical properties that isosteric compounds usually share include boiling point, density, viscosity, and thermal conductivity. However, certain properties are usually different: dipolar moments, polarity, polarization, size, and shape since the external orbitals may be hybridized differently. The term “isomers” as used herein refers broadly to compounds having the same number and kind of atoms, and hence
- 15 the same molecular weight, but differing with respect to the arrangement or configuration of the atoms in space. Additionally, the term “isomers” includes stereoisomers and geometric isomers. The terms “stereoisomer” or “optical isomer” as used herein refer to a stable isomer that has at least one chiral atom or restricted rotation giving rise to perpendicular dissymmetric planes (*e.g.*, certain biphenyls, allenes, and spiro compounds)
- 20 and can rotate plane-polarized light. Because asymmetric centers and other chemical structure can exist in some of the compounds of the present invention, which may give rise to stereoisomerism, the invention contemplates stereoisomers and mixtures thereof. The compounds of the present invention and their salts can include asymmetric carbon atoms and may therefore exist as single stereoisomers, racemates, and as mixtures of
- 25 enantiomers and diastereomers. Typically, such compounds will be prepared as a racemic mixture. Such compounds can also be prepared or isolated as pure stereoisomers, *i.e.*, as individual enantiomers or diastereomers, or as stereoisomer-enriched mixtures. Tautomers are readily inter-convertible constitutional isomers and there is a change in connectivity of a ligand, as in the keto and enol forms of ethyl acetoacetate (including
- 30 tautomers of any said compounds.) Zwitterions are inner salts or dipolar compounds

possessing acidic and basic groups in the same molecule. At neutral pH, the cation and anion of most zwitterions are equally ionized.

In another embodiment, the modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL may be a nucleic acid molecule, preferably an RNA molecule, more preferably
5 an RNAi agent.

As used herein, the term “RNAi” refers to modulation of a target nucleic acid or target protein through RISC. As used herein, the term “RISC” refers to the RNA induced silencing complex.

In one embodiment, the modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL
10 is thus an RNAi agent targeting CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL mRNA.

As used herein, the term “RNAi agent” refers to any molecule that activates the RISC pathway. RNAi agents include, but are not limited to siRNA and asRNA.

As used herein, the term “small interfering RNA” or “siRNA” refers to an RNAi agent
15 that is a double stranded oligonucleotide. In a typical siRNA, the two strands usually have 19 nucleotides complementary to each other thereby creating a double strand that is 19 nucleotides long and each strand having a 3'-end of two overhanging nucleotides. This is not a strict definition of siRNA, which may be slightly longer or shorter, and with or without overhangs. In siRNA, one strand is guiding and complementary to the target RNA
20 (antisense strand), and the other strand (sense strand) has the same sequence as the target RNA and hence is complementary to the guiding/antisense strand. Herein, regulatory RNAs such as “micro RNA” (miRNA) and “short RNA” (shRNA) and a variety of structural RNAs such as “transfer RNA” (tRNA), “small nuclear RNA” (snRNA), “small conditional RNA” (scRNA) or “ribosomal RNA” (rRNA) are used interchangeably with
25 the term “siRNA”.

As used herein, the term “antisense RNA” or “asRNA” refers to an RNAi agent that is a single stranded oligonucleotide. In a typical asRNA, the single strand is complementary to all or a part of the target mRNA. The complementarity of an asRNA may be with any

part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-translated sequence, introns, or the coding sequence.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an inhibitor of CCL19, an inhibitor of ENA78, and/or an inhibitor of SDF-1.

In one embodiment, the composition of the invention does not consist essentially of an inhibitor of ENA78. In one embodiment, the composition of the invention does not consist essentially of an inhibitor of ENA78 and an inhibitor of SDF-1.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an inhibitor of CCL19.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an inhibitor of ENA78.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an inhibitor of SDF-1.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an inhibitor of CCL19 and an inhibitor of ENA78.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an inhibitor of CCL19 and an inhibitor of SDF-1.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an inhibitor of ENA78 and an inhibitor of SDF-1.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an inhibitor of CCL19, an inhibitor of ENA78 and an inhibitor of SDF-1.

Inhibitors of chemokines/cytokines as used herein refer to a natural or synthetic compound which binds to the CCL19, ENA78, and/or SDF-1 and inhibits the biological activity of the CCL19, ENA78, and/or SDF-1, and thereby the action of the said chemokine/cytokine. A CCL19, ENA78, and/or SDF-1 inhibitor includes any chemical

entity that, upon administration to a patient or a cell expressing a functional CCL19, ENA78, and/or SDF-1 signaling pathway, i.e. expressing CCL19, ENA78, and/or SDF-1 and its respective receptors, results in the inhibition of a biological activity of CCL19, ENA78, and/or SDF-1 in said subject or said cell, including any of the downstream
5 biological effects otherwise resulting from the binding of CCL19, ENA78, and/or SDF-1 to its receptor. In one embodiment, CCL19, ENA78, or SDF-1 inhibitor includes any agent that can inhibit CCL19, ENA78, and/or SDF-1 activity or release/secretion or any of the downstream biological effects of CCL19, ENA78, and/or SDF-1. In one embodiment, CCL19, ENA78, or SDF-1 inhibitor includes aptamers, isosteres or RNAi
10 as described hereinabove.

Inhibitors can take several forms. They may be monoclonal antibodies. They may be a monoclonal antibody fragment. They may take the form of a soluble receptor to that cytokine. Soluble receptors freely circulate in the body. When they encounter their target (chemokine/cytokine), they bind to it, effectively inactivating the chemokine/cytokine,
15 since the chemokine/cytokine is then no longer able to bind with its biologic target in the body. An even more potent inhibitor consists of two soluble receptors fused together to a specific portion of an immunoglobulin molecule (Fc fragment). This produces a dimer composed of two soluble receptors which have a high affinity for the target, and a prolonged half-life. This molecule is called a Fc fusion protein.

20 Examples of inhibitor of CCL19 include without limitation, anti-CCL19 antibodies; soluble CCR7, fragments and derivatives thereof which competitively bind to CCL19; Prostaglandin E₂ (PGE₂) (Muthuswamy R *et al.*, Blood, 2010 Sep 2; 116(9):1454-1459 incorporated herein by reference).

Anti-CCL19 antibodies encompass any immunoglobulin molecule that specifically binds
25 to an epitope of CCL19. As used herein, "epitope" refers to a region of the CCL19 protein that is recognized by the isolated antibody and involved in mediating the binding interaction between CCL19 and CCR7, or involved in mediating the downstream molecular signaling pathway triggered by the CCR7-CCL19 binding interaction. In a preferred embodiment, the anti-CCL19 antibody has antigen specificity to the
30 extracellular domain of CCL19.

Examples of inhibitor of ENA78 or CXCL5 include without limitation, anti-ENA78 antibodies; soluble CXCR2, fragments and derivatives thereof which competitively bind to ENA78; Piroxicam (Pfizer), a non-selective COX inhibitor which attenuates the expression of ENA78 (Dawes JM *et al.* Sci Transl Med 2011 Jul 6; 3:90 incorporated
5 herein by reference); peptides comprising the sequences ALWPPNLHAWVP (SEQ ID NO: 1) or AHSVNSDVLGI (SEQ ID NO: 2) which are described in WO2014173967 incorporated herein by reference.

Anti-ENA78 antibodies encompass any immunoglobulin molecule that specifically binds to an epitope of ENA78. As used herein, "epitope" refers to a region of the ENA78 protein
10 that is recognized by the isolated antibody and involved in mediating the binding interaction between ENA78 and CXCR2, or involved in mediating the downstream molecular signaling pathway triggered by the CXCR2-ENA78 binding interaction. In a preferred embodiment, the anti-ENA78 antibody has antigen specificity to the extracellular domain of ENA78.

15 Examples of inhibitor of SDF-1 include without limitation, anti-SDF-1 antibodies; soluble CXCR4, fragments and derivatives thereof which competitively bind to SDF-1; NOX-A12 (Noxxon Pharma).

Anti-SDF-1 antibodies encompass any immunoglobulin molecule that specifically binds to an epitope of SDF-1. As used herein, "epitope" refers to a region of the SDF-1 protein
20 that is recognized by the isolated antibody and involved in mediating the binding interaction between SDF-1 and CXCR4, or involved in mediating the downstream molecular signaling pathway triggered by the CXCR4-SDF-1 binding interaction. In a preferred embodiment, the anti-SDF-1 antibody has antigen specificity to the extracellular domain of SDF-1.

25 In one embodiment, the composition of the invention comprises, consists of or consists essentially of an activator of IL-15, an activator of LIF and/or an activator of TRAIL.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an activator of IL-15.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an activator of LIF.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an activator of TRAIL.

- 5 In one embodiment, the composition of the invention comprises, consists of or consists essentially of an activator of IL-15 and an activator of LIF.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an activator of IL-15 and an activator of TRAIL.

- 10 In one embodiment, the composition of the invention comprises, consists of or consists essentially of an activator of LIF and an activator of TRAIL.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an activator of IL-15, an activator of LIF and an activator of TRAIL.

- 15 Activators of chemokines/cytokines as used herein refer to a natural or synthetic IL-15, LIF, and/or TRAIL compound or to a natural or synthetic compound which binds to the IL-15, LIF, and/or TRAIL and amplify or stimulate the biological activity of the IL-15, LIF, and/or TRAIL, and thereby the action of the said chemokine/cytokine. A IL-15, LIF and/or TRAIL activator includes any chemical entity that, upon administration to a subject or to a cell expressing a functional IL-15, LIF, and/or TRAIL signaling pathway, (*i.e.* expressing for example IL-15, LIF, and/or TRAIL and their respective receptors),
20 results in the amplification or stimulation of a biological activity of IL-15, LIF and/or TRAIL in said subject or said cell, including any of the downstream biological effects otherwise resulting from the binding of IL-15, LIF and/or TRAIL to its receptor. In one embodiment, IL-15, LIF and/or TRAIL activator include any agent that can amplify or stimulate IL-15, LIF and/or TRAIL activity or release/secretion or any of the downstream
25 biological effects of IL-15, LIF and/or TRAIL. An activator of cytokine can take the form of a partial or full activator.

Activators can take several forms. They may be monoclonal antibodies. They may be a monoclonal antibody fragment. They may take the form of a soluble receptor to that

cytokine. Soluble receptors freely circulate in the body. When they encounter their target (chemokine/cytokine), they bind to it, effectively activating or amplifying the biological activity of the chemokine/cytokine. An even more potent activator consists of two soluble receptors fused together to a specific portion of an immunoglobulin molecule (Fc
5 fragment). This produces a dimer composed of two soluble receptors which have a high affinity for the target, and a prolonged half-life. This molecule is called a Fc fusion protein.

Examples of activator of IL-15 include without limitation, anti-IL-15 activating antibodies; IL-15:sIL-15R α complex; fusion polypeptide of IL-15 and IL-15R α sushi
10 domain.

Examples of activator of LIF include without limitation, anti-LIF activating antibodies.

Examples of activator of TRAIL include without limitation, anti-TRAIL activating antibodies.

In another embodiment, the composition of the invention comprises, consists of or
15 consists essentially of at least one of (i) an inhibitor of CCL19, (ii) an inhibitor of ENA78, (iii) an activator of IL-15 and (iv) an activator of TRAIL, and optionally at least one of an inhibitor of SDF-1 and an activator of LIF.

In another embodiment, the composition of the invention comprises at least one inhibitor of CCL19, ENA78, and/or SDF-1 and at least one activator of IL-15, LIF and/or TRAIL,
20 preferably at least one inhibitor of CCL19 and at least one activator of IL-15 and/or TRAIL.

In one embodiment, the composition of the invention does not consist essentially of an inhibitor of ENA78. In one embodiment, the composition of the invention does not consist essentially of an inhibitor of ENA78 and an activator of LIF. In one embodiment,
25 the composition of the invention does not consist essentially of an inhibitor of ENA78 and an inhibitor of SDF1. In one embodiment, the composition of the invention does not consist essentially of an inhibitor of ENA78, an inhibitor of SDF-1 and an activator of LIF.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one inhibitor of CCL19, an activator of IL-15, an inhibitor of ENA78, and/or an activator of TRAIL.

5 In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one inhibitor of CCL19.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one activator of IL-15.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one inhibitor of ENA78.

10 In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one activator of TRAIL.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one inhibitor of CCL19 and at least one activator of IL-15.

15 In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one inhibitor of CCL19 and at least one inhibitor of ENA78.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one inhibitor of CCL19 and at least one activator of TRAIL.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one activator of IL-15 and at least one inhibitor of ENA78.

20 In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one activator of IL-15 and at least one activator of TRAIL.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one inhibitor of ENA78 and at least one activator of TRAIL.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one inhibitor of CCL19, at least one activator of IL-15 and at least one inhibitor of ENA78.

5 In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one inhibitor of CCL19, at least one inhibitor of ENA78 and at least one activator of TRAIL.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one inhibitor of CCL19, at least one activator of IL-15 and at least one activator of TRAIL.

10 In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one activator of IL-15, at least one inhibitor of ENA78 and at least one activator of TRAIL.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one inhibitor of CCL19, at least one activator of IL-15, at
15 least one inhibitor of ENA78 and at least one activator of TRAIL.

In another embodiment, the composition of the invention further comprises at least one activator of LIF and/or at least one inhibitor of SDF-1.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one inhibitor of CCL19 and at least one activator of LIF,
20 (ii) at least one inhibitor of CCL19 and at least one inhibitor of SDF-1 or (iii) at least one inhibitor of CCL19, at least one activator of LIF and at least one inhibitor of SDF1.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one activator of IL-15 and at least one activator of LIF,
(ii) at least one activator of IL-15 and at least one inhibitor of SDF-1 or (iii) at least one
25 activator of IL-15, at least one activator of LIF and at least one inhibitor of SDF1.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one inhibitor of ENA78 and at least one activator of LIF,

(ii) at least one inhibitor of ENA78 and at least one inhibitor of SDF-1 or (iii) at least one inhibitor of ENA78, at least one activator of LIF and at least one inhibitor of SDF1.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one activator of TRAIL and at least one activator of LIF,
5 (ii) at least one activator of TRAIL and at least one inhibitor of SDF-1 or (iii) at least one activator of TRAIL, at least one activator of LIF and at least one inhibitor of SDF1.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one inhibitor of CCL19, at least one activator of IL-15 and at least one activator of LIF, (ii) at least one inhibitor of CCL19, at least one activator
10 of IL15 and at least one inhibitor of SDF-1 or (iii) at least one inhibitor of CCL19, at least one activator of IL-15, at least one activator of LIF and at least one inhibitor of SDF1.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one inhibitor of CCL19, at least one inhibitor of ENA78 and at least one activator of LIF, (ii) at least one inhibitor of CCL19, at least one inhibitor
15 of ENA78 and at least one inhibitor of SDF-1 or (iii) at least one inhibitor of CCL19, at least one inhibitor of ENA78, at least one activator of LIF and at least one inhibitor of SDF1.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one inhibitor of CCL19, at least one activator of TRAIL
20 and at least one activator of LIF, (ii) at least one inhibitor of CCL19, at least one activator of TRAIL and at least one inhibitor of SDF-1 or (iii) at least one inhibitor of CCL19, at least one activator of TRAIL, at least one activator of LIF and at least one inhibitor of SDF1.

In another embodiment, the composition of the invention comprises, consists of or
25 consists essentially of (i) at least one activator of IL-15, at least one inhibitor of ENA78 and at least one activator of LIF, (ii) at least one activator of IL-15, at least one inhibitor of ENA78 and at least one inhibitor of SDF-1 or (iii) at least one activator of IL-15, at least one inhibitor of ENA78, at least one activator of LIF and at least one inhibitor of SDF1.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one activator of IL-15, at least one activator of TRAIL and at least one activator of LIF, (ii) at least one activator of IL-15, at least one activator of TRAIL and at least one inhibitor of SDF-1 or (iii) at least one activator of IL-15, at
5 least one activator of TRAIL, at least one activator of LIF and at least one inhibitor of SDF1.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one inhibitor of ENA78, at least one activator of TRAIL and at least one activator of LIF, (ii) at least one inhibitor of ENA78, at least one activator
10 of TRAIL and at least one inhibitor of SDF-1 or (iii) at least one inhibitor of ENA78, at least one activator of TRAIL, at least one activator of LIF and at least one inhibitor of SDF1.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one inhibitor of CCL19, at least one activator of IL-15,
15 at least one inhibitor of ENA78 and at least one activator of LIF, (ii) at least one inhibitor of CCL19, at least one activator of IL-15, at least one inhibitor of ENA78 and at least one inhibitor of SDF-1 or (iii) at least one inhibitor of CCL19, at least one activator of IL-15, at least one inhibitor of ENA78, at least one activator of LIF and at least one inhibitor of SDF1.

20 In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one inhibitor of CCL19, at least one inhibitor of ENA78, at least one activator of TRAIL and at least one activator of LIF, (ii) at least one inhibitor of CCL19, at least one inhibitor of ENA78, at least one activator of TRAIL and at least
25 one inhibitor of SDF-1 or (iii) at least one inhibitor of CCL19, at least one inhibitor of ENA78, at least one activator of TRAIL, at least one activator of LIF and at least one inhibitor of SDF1.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one inhibitor of CCL19, at least one activator of IL-15, at least one activator of TRAIL and at least one activator of LIF, (ii) at least one inhibitor

of CCL19, at least one activator of IL-15, at least one activator of TRAIL and at least one inhibitor of SDF-1 or (iii) at least one inhibitor of CCL19, at least one activator of IL-15, at least one activator of TRAIL, at least one activator of LIF and at least one inhibitor of SDF1.

- 5 In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one activator of IL-15, at least one inhibitor of ENA78, at least one activator of TRAIL and at least one activator of LIF, (ii) at least one activator of IL-15, at least one inhibitor of ENA78, at least one activator of TRAIL and at least one inhibitor of SDF-1 or (iii) at least one activator of IL-15, at least one inhibitor of ENA78,
10 at least one activator of TRAIL, at least one activator of LIF and at least one inhibitor of SDF1.

- In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one inhibitor of CCL19, at least one activator of IL-15, at least one inhibitor of ENA78, at least one activator of TRAIL and at least one activator
15 of LIF, (ii) at least one inhibitor of CCL19, at least one activator of IL-15, at least one inhibitor of ENA78, at least one activator of TRAIL and at least one inhibitor of SDF-1 or (iii) at least one inhibitor of CCL19, at least one activator of IL-15, at least one inhibitor of ENA78, at least one activator of TRAIL, at least one activator of LIF and at least one inhibitor of SDF1.

- 20 In one embodiment, the modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL may be an agonist, antagonist or inverse agonist specific towards their receptor.

- As used herein, the receptor of CCL19 is C-C chemokine receptor type 7 (CCR7). As used herein, the receptor of IL-15 is IL-15R. As used herein, the receptor of ENA78 is C-X-C motif chemokine receptor 2 (CXCR2). As used herein, the receptor of LIF is the
25 specific LIF receptor, LIFR. As used herein, the receptor of SDF-1 is C-X-C motif chemokine receptor 4 (CXCR4). As used herein, the receptors of TRAIL are the death receptors DR4 (TRAIL-RI) and DR5 (TRAIL-RII).

In one embodiment, said modulator is an antagonist of the CCL19 receptor, ENA78 receptor, and/or SDF-1 receptor.

In one embodiment, said modulator is an antagonist of the CCL19 receptor.

In one embodiment, said modulator is an antagonist of the ENA78 receptor.

In one embodiment, said modulator is an antagonist of the SDF-1 receptor.

In one embodiment, said modulator is an antagonist of the CCL19 receptor and an
5 antagonist of the ENA78 receptor.

In one embodiment, said modulator is an antagonist of the CCL19 receptor and an
antagonist of the SDF-1 receptor.

In one embodiment, said modulator is an antagonist of the ENA78 receptor and an
antagonist of the SDF-1 receptor.

10 In one embodiment, said modulator is an antagonist of the CCL19 receptor, an antagonist
of the ENA78 receptor and an antagonist of SDF-1.

Antagonists of chemokines/cytokines as used herein refer to a natural or synthetic
compound which binds to the CCL19 receptor, ENA78 receptor, and/or SDF-1 receptor
and inhibits the biological activity of the CCL19 receptor, ENA78 receptor, and/or
15 SDF-1 receptors, i.e., CCR7, CXCR2 and/or CXCR4, and thereby the action of the said
chemokine/cytokine.

As used herein, “a CCR7, CXCR2 and/or CXCR4 antagonist” includes any chemical
entity that, upon administration to a subject or to a cell expressing a functional CCR7,
CXCR2 and/or CXCR4 signaling pathway, (*i.e.*, expressing for example CCR7, CXCR2
20 and/or CXCR4 and their respective ligands), results in the inhibition of a biological
activity associated with the down-regulation of CCR7, CXCR2 and/or CXCR4 in said
subject or said cell, including any of the downstream biological effects otherwise
resulting from the binding of CCL19, ENA78, and/or SDF-1 to CCR7, CXCR2 and/or
CXCR4, respectively. In one embodiment, CCR7, CXCR2 and/or CXCR4 antagonists
25 include any agent that can inhibit CCR7, CXCR2 and/or CXCR4 activity or any of the
downstream biological effects of CCR7, CXCR2 and/or CXCR4. An antagonist of
cytokine receptors can take the form of a competitive, non-competitive or uncompetitive

antagonist. An antagonist of cytokine receptors can be an orthosteric antagonist or an allosteric antagonist. An orthosteric antagonist binds at the binding site of a receptor, while an allosteric antagonist binds to regulatory sites at the protein surface, and allosterically changes the conformation of the receptor binding site. Orthosteric binding is thus mutually exclusive, while allosteric ligand can bind to a receptor at the same time as an orthosteric ligand.

Examples of antagonists of CCR7 include, without limitation, anti-CCR7 antibodies; soluble CCL19 fragment which competitively binds to CXCL12 (e.g., CCL19 (8-83), described in Pilkington KR *et al.*, 2004 incorporated herein by reference); thiadiazoledioxides and thiadiazoleoxides (See e.g. US Patent 7,691,856 incorporated herein by reference); a tertiary amine containing a multiplicity of heteroaromatic substituents (See e.g. US Patents 6,835,731 and 6,864,265 both incorporated herein by reference); a piperazinyloxy derivative (See e.g. US Patent 7,678,798 incorporated herein by reference).

Anti-CCR7 antibodies encompass any immunoglobulin molecule that specifically binds to an epitope of CCR7. As used herein, "epitope" refers to a region of the CCR7 protein that is recognized by the isolated antibody and involved in mediating the binding interaction between CCL19 and CCR7, or involved in mediating the downstream molecular signaling pathway triggered by the CCR7-CCL19 binding interaction. In a preferred embodiment, the anti-CCR7 antibody has antigen specificity to the extracellular domain of CCR7. Suitable CCR7 antibodies and methods of making the same are disclosed in WO2004/104574 and WO2007/003216, which are hereby incorporated by reference in their entirety.

Examples of antagonists of CXCR2 include, without limitation, anti-CXCR2 antibodies; soluble ENA78 fragment which competitively binds to CXCR2; AZD5069 (AstraZeneca); CXCL8(3-74)^{K11R/G31P}; GSK1325756/Danirixin; Ladarixin; Repertaxin; SB225002; SB272844; SB265610; SB656933/Elubrixin; SCH479833; SCH-527123/Navarixin; thiazolopyrimidinone derivatives (See e.g. WO2001025242 incorporated herein by reference); 5-sulfanylmethyl-[1,2,4]triazol[1,5-a]pyrimidin-7-ol derivatives (See e.g. WO2008061741 incorporated herein by reference);

5-sulfanylmethyl-pyrazolo[1,5-a]pyrimidin-7-ol derivatives (See e.g. WO2008062026 incorporated herein by reference); n-(4-chloro-2-hydroxy-3-((3s)-3-piperidinylsulfonyl)phenyl-n'-(3-fluoro-2-methylphenyl)urea derivatives (See e.g. WO2015071235 incorporated herein by reference); other examples are disclosed in
5 US7,132,445 and WO2002083624, which are hereby incorporated by reference.

Anti-CXCR2 antibodies encompass any immunoglobulin molecule that specifically binds to an epitope of CXCR2. As used herein, “epitope” refers to a region of the CXCR2 protein that is recognized by the isolated antibody and involved in mediating the binding interaction between ENA78 and CXCR2, or involved in mediating the downstream
10 molecular signaling pathway triggered by the CXCR2-ENA78 binding interaction. In a preferred embodiment, the anti-CXCR2 antibody has antigen specificity to the extracellular domain of CXCR2.

Examples of antagonists of CXCR4 include, without limitation, anti-CXCR4 antibodies; soluble SDF-1 fragment which competitively binds to CXCR4; AMD3100/Plerixafor;
15 AMD070; AMD11070; AMD3465; BL-8040 (BioLineRx); BMS-936564/MDX-1338 (Bristol-Meyer Squibb); CS3955; CTCE-9908 (Tocris Bioscience); EPI-X4; FC131 (Tocris Bioscience); KRH-1120; KRH-1636; KRH-2731; KRH-3955; MSX-122 (Metastatix Inc.); POL5551; POL6326 (Polyphor); TG-0054; TN14003; bicyclam derivatives; tetrahydroquinoline derivatives; cyclic peptides; para-xylyl-enediamine-
20 based derivatives; isothiourea derivatives.

Anti-CXCR4 antibodies encompass any immunoglobulin molecule that specifically binds to an epitope of CXCR4. As used herein, “epitope” refers to a region of the CXCR4 protein that is recognized by the isolated antibody and involved in mediating the binding interaction between SDF-1 and CXCR4, or involved in mediating the downstream
25 molecular signaling pathway triggered by the CXCR4-SDF-1 binding interaction. In a preferred embodiment, the anti-CXCR4 antibody has antigen specificity to the extracellular domain of CXCR4.

In one embodiment, said modulator is an inverse agonist of the CCL19 receptor, ENA78 receptor, and/or SDF-1 receptor.

In one embodiment, said modulator is an inverse agonist of the CCL19 receptor.

In one embodiment, said modulator is an inverse agonist of the ENA78 receptor.

In one embodiment, said modulator is an inverse agonist of the SDF-1 receptor.

In one embodiment, said modulator is an inverse agonist of the CCL19 receptor and an
5 inverse agonist of the ENA78 receptor.

In one embodiment, said modulator is an inverse agonist of the CCL19 receptor and an
inverse agonist of the SDF-1 receptor.

In one embodiment, said modulator is an inverse agonist of the ENA78 receptor and an
inverse agonist of the SDF-1 receptor.

10 In one embodiment, said modulator is an inverse agonist of the CCL19 receptor, an
inverse agonist of the ENA78 receptor and an inverse agonist of the SDF-1 receptor.

Inverse agonists of chemokines/cytokines as used herein refer to a natural or synthetic
compound which binds to the CCL19 receptor, ENA78 receptor, and/or SDF-1 receptor
and inhibits the biological activity of the CCL19 receptor, ENA78 receptor, and/or
15 SDF-1 receptor, i.e., CCR7, CXCR2 and/or CXCR4, and thereby the action of the said
chemokine/cytokine.

As used herein, “a CCR7, CXCR2 and/or CXCR4 inverse agonist” includes any chemical
entity that, upon administration to a subject or to a cell expressing a functional CCR7,
CXCR2 and/or CXCR4 signaling pathway (*i.e.*, expressing for example CCR7, CXCR2
20 and/or CXCR4 and their respective ligands), results in the inhibition of a biological
activity associated with the down-regulation of CCR7, CXCR2 and/or CXCR4 in said
subject or said cell, including any of the downstream biological effects otherwise
resulting from the binding of CCL19, ENA78, and/or SDF-1 to CCR7, CXCR2 and/or
CXCR4, respectively; or from the constitutive (*i.e.*, intrinsic or basal) level activity of
25 CCR7, CXCR2 and/or CXCR4 in absence of ligand. In one embodiment, CCR7, CXCR2
and/or CXCR4 inverse agonist include any agent that can inhibit CCR7, CXCR2 and/or
CXCR4 activity or any of the downstream biological effects of CCR7, CXCR2 and/or

CXCR4. An inverse agonist of cytokine receptors can take the form of a partial, full or super inverse agonist. An inverse agonist of cytokine receptors can be an orthosteric inverse agonist or an allosteric inverse agonist.

In one embodiment, said modulator is an agonist of the IL-15 receptor, LIF receptor,
5 and/or TRAIL receptor.

In one embodiment, said modulator is an agonist of the IL-15 receptor.

In one embodiment, said modulator is an agonist of the LIF receptor.

In one embodiment, said modulator is an agonist of the TRAIL receptor.

In one embodiment, said modulator is an agonist of the IL-15 receptor and an agonist of
10 LIF receptor.

In one embodiment, said modulator is an agonist of the IL-15 receptor and an agonist of TRAIL receptor.

In one embodiment, said modulator is an agonist of the LIF receptor and an agonist of TRAIL receptor.

15 In one embodiment, said modulator is an agonist of the IL-15 receptor, an agonist of the LIF receptor and an agonist of TRAIL receptor.

Agonists of chemokines/cytokines as used herein refer to a natural or synthetic compound which binds to the IL-15 receptor, LIF receptor, and/or TRAIL receptor and amplify or stimulate the biological activity of the IL-15 receptor, LIF receptor, and/or TRAIL
20 receptor, i.e., IL-15R, LIFR, TRAIL-RI and/or TRAIL-RII, and thereby the action of the said chemokine/cytokine.

As used herein, “a IL-15R, LIFR, TRAIL-RI and/or TRAIL-RII agonist” includes any chemical entity that, upon administration to a subject or to a cell expressing a functional IL-15R, LIFR, TRAIL-RI and/or TRAIL-RII signaling pathway (*i.e.*, expressing for
25 example IL-15R, LIFR, TRAIL-RI and/or TRAIL-RII and their respective ligands), results in the amplification or stimulation of a biological activity associated with the up-

regulation of IL-15R, LIFR, TRAIL-RI and/or TRAIL-RII in said subject or said cell, including any of the downstream biological effects otherwise resulting from the binding of IL-15, LIF and/or TRAIL to IL-15R, LIFR, and/or TRAIL-RI or TRAIL-RII, respectively. In one embodiment, IL-15R, LIFR, TRAIL-RI and/or TRAIL-RII agonist
5 include any agent that can amplify or stimulate IL-15R, LIFR, TRAIL-RI and/or TRAIL-RII activity or any of the downstream biological effects of IL-15R, LIFR, TRAIL-RI and/or TRAIL-RII. An agonist of cytokine receptors can take the form of a partial, full or super agonist. An agonist of cytokine receptors can be an orthosteric agonist or an allosteric agonist.

10 Examples of agonists of IL-15R include, without limitation, recombinant human IL-15, fragments and derivatives thereof; ALT-803, which is a combined IL-15^{N72D} mutant with the soluble domain of IL-15R α ; anti-IL-15R activating antibodies.

Examples of agonists of LIFR include, without limitation, recombinant human LIF, fragments and derivatives thereof; oncostatin M (Walker E *et al.*, J Clin Invest. 2010
15 Feb;120(2):582-92 incorporated herein by reference); anti-LIFR activating antibodies.

Examples of agonists of TRAIL-RI and/or TRAIL-RII include, without limitation, recombinant human TRAIL, fragments and derivatives thereof; anti-TRAIL-RI activating antibodies; anti-TRAIL-RII activating antibodies; recombinant Fas protein and fragments thereof; recombinant TNF protein and fragments thereof; AMG 655 (conatumumab); CS-
20 1008 (tigatuzumab); HGS-ETR1 (mapatumumab); HGS-ETR2 (lexatumumab); dulanermin.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of an antagonist of CCR7, CXCR2 and/or CXCR4, preferably of CCR7 and/or CXCR4. In another embodiment, the composition of the invention comprises, consists of
25 or consists essentially of an agonist of IL-15R, LIFR, TRAIL-RI and/or TRAIL-RII, preferably IL-15R, TRAIL-RI and/or TRAIL-RII. In another embodiment, the composition of the invention comprises, consists of or consists essentially of an antagonist of CCR7, CXCR2 and/or CXCR4, preferably of CCR7 and/or CXCR4, and an

agonist of IL-15R, LIFR, TRAIL-RI and/or TRAIL-RII, preferably of IL-15R, TRAIL-RI and/or TRAIL-RII.

In one embodiment, the composition of the invention comprises, consists of or consists essentially of at least one of (i) an antagonist or an inverse agonist of CCR7, (ii) an
5 antagonist or an inverse agonist of CXCR2, (iii) an agonist of IL-15R and (iv) an agonist of TRAIL-RI or TRAIL-RII, and optionally at least one of an antagonist or an inverse agonist of CXCR4 and an agonist of LIFR.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one antagonist or inverse agonist of CCR7, at least one
10 antagonist or an inverse agonist of CXCR2, and/or at least one antagonist or inverse agonist of CXCR4 and at least one agonist of IL-15R, at least one agonist of LIFR and/or at least one agonist of TRAIL-RI or TRAIL-RII, preferably at least one antagonist or inverse agonist of CCR7 and at least one agonist of IL-15R and/or TRAIL-RI or TRAIL-RII.

15 In one embodiment, the composition of the invention does not consist essentially of an antagonist or an inverse agonist of CXCR2. In one embodiment, the composition of the invention does not consist essentially of an antagonist or an inverse agonist of CXCR2 and an agonist of LIFR. In one embodiment, the composition of the invention does not consist essentially of an antagonist or an inverse agonist of CXCR2 and an antagonist or
20 an inverse agonist of CXCR4. In one embodiment, the composition of the invention does not consist essentially of an antagonist or an inverse agonist of CXCR2, an antagonist or an inverse agonist of CXCR4 and an agonist of LIFR.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one antagonist or inverse agonist of CCR7, an agonist of
25 IL-15R, an antagonist or inverse agonist of CXCR2, and/or an agonist of TRAIL-RI or TRAIL-RII.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one antagonist or inverse agonist of CCR7.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one agonist of IL-15R.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one antagonist or inverse agonist of CXCR2.

- 5 In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one agonist of TRAIL-RI or TRAIL-RII.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one antagonist or inverse agonist of CCR7 and at least one agonist of IL-15R.

- 10 In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one antagonist or inverse agonist of CCR7 and at least one antagonist or inverse agonist of CXCR2.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one antagonist or inverse agonist of CCR7 and at least one
15 agonist of TRAIL-RI or TRAIL-RII.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one agonist of IL-15R and at least one antagonist or inverse agonist of CXCR2.

- In another embodiment, the composition of the invention comprises, consists of or
20 consists essentially of at least one agonist of IL-15R and at least one agonist of TRAIL-RI or TRAIL-RII.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one antagonist or inverse agonist of CXCR2 and at least one agonist of TRAIL-RI or TRAIL-RII.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R and at least one antagonist or inverse agonist of CXCR2.

5 In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one antagonist or inverse agonist of CCR7, at least one antagonist or inverse agonist of CXCR2 and at least one agonist of TRAIL-RI or TRAIL-RII.

10 In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R and at least one agonist of TRAIL-RI or TRAIL-RII.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one agonist of IL-15R, at least one antagonist or inverse agonist of CXCR2 and at least one agonist of TRAIL-RI or TRAIL-RII.

15 In another embodiment, the composition of the invention comprises, consists of or consists essentially of at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R, at least one antagonist or inverse agonist of CXCR2 and at least one agonist of TRAIL-RI or TRAIL-RII.

In another embodiment, the composition of the invention further comprises at least one agonist of LIFR and/or at least one antagonist or inverse agonist of CXCR4.

20 In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one antagonist or inverse agonist of CCR7 and at least one agonist of LIFR, (ii) at least one antagonist or inverse agonist of CCR7 and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one antagonist or inverse agonist of CCR7, at least one agonist of LIFR and at least one antagonist or inverse
25 agonist of CXCR4.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one agonist of IL-15R and at least one agonist of LIFR, (ii) at least one agonist of IL-15R and at least one antagonist or inverse agonist of CXCR4

or (iii) at least one agonist of IL-15R, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one antagonist or inverse agonist of CXCR2 and at least one agonist of LIFR, (ii) at least one antagonist or inverse agonist of CXCR2 and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one antagonist or inverse agonist of CXCR2, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one agonist of TRAIL-RI or TRAIL-RII and at least one agonist of LIFR, (ii) at least one agonist of TRAIL-RI or TRAIL-RII and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one agonist of TRAIL-RI or TRAIL-RII, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R and at least one agonist of LIFR, (ii) at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one antagonist or inverse agonist of CCR7, at least one antagonist or inverse agonist of CXCR2 and at least one agonist of LIFR, (ii) at least one antagonist or inverse agonist of CCR7, at least one antagonist or inverse agonist of CXCR2 and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one antagonist or inverse agonist of CCR7, at least one antagonist or inverse agonist of CXCR2, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one antagonist or inverse agonist of CCR7, at least one agonist of TRAIL-RI or TRAIL-RII and at least one agonist of LIFR, (ii) at least one antagonist or inverse agonist of CCR7, at least one agonist of TRAIL-RI or TRAIL-RII
5 and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one antagonist or inverse agonist of CCR7, at least one agonist of TRAIL-RI or TRAIL-RII, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one agonist of IL-15R, at least one antagonist or inverse
10 agonist of CXCR2 and at least one agonist of LIFR, (ii) at least one agonist of IL-15R, at least one antagonist or inverse agonist of CXCR2 and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one agonist of IL-15R, at least one antagonist or inverse agonist of CXCR2, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

15 In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one agonist of IL-15R, at least one agonist of TRAIL-RI or TRAIL-RII and at least one agonist of LIFR, (ii) at least one agonist of IL-15R, at least one agonist of TRAIL-RI or TRAIL-RII and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one agonist of IL-15R, at least one agonist of TRAIL-
20 RI or TRAIL-RII, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one antagonist or inverse agonist of CXCR2, at least one agonist of TRAIL-RI or TRAIL-RII and at least one agonist of LIFR, (ii) at least one
25 antagonist or inverse agonist of CXCR2, at least one agonist of TRAIL-RI or TRAIL-RII and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one antagonist or inverse agonist of CXCR2, at least one agonist of TRAIL-RI or TRAIL-RII, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R, at least one antagonist or inverse agonist of CXCR2 and at least one agonist of LIFR, (ii) at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R, at least one antagonist or inverse agonist of CXCR2 and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R, at least one antagonist or inverse agonist of CXCR2, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

10 In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one antagonist or inverse agonist of CCR7, at least one inhibitor of ENA78, at least one agonist of TRAIL-RI or TRAIL-RII and at least one agonist of LIFR, (ii) at least one antagonist or inverse agonist of CCR7, at least one antagonist or inverse agonist of CXCR2, at least one agonist of TRAIL-RI or TRAIL-RII
15 and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one antagonist or inverse agonist of CCR7, at least one antagonist or inverse agonist of CXCR2, at least one agonist of TRAIL-RI or TRAIL-RII, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

In another embodiment, the composition of the invention comprises, consists of or
20 consists essentially of (i) at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R, at least one agonist of TRAIL-RI or TRAIL-RII and at least one agonist of LIFR, (ii) at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R, at least one agonist of TRAIL-RI or TRAIL-RII and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one antagonist or inverse agonist
25 of CCR7, at least one agonist of IL-15R, at least one agonist of TRAIL-RI or TRAIL-RII, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one agonist of IL-15R, at least one antagonist or inverse agonist of CXCR2, at least one agonist of TRAIL-RI or TRAIL-RII and at least one
30 agonist of LIFR, (ii) at least one agonist of IL-15R, at least one antagonist or inverse

agonist of CXCR2, at least one agonist of TRAIL-RI or TRAIL-RII and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one agonist of IL-15R, at least one antagonist or inverse agonist of CXCR2, at least one agonist of TRAIL-RI or TRAIL-RII, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.

- 5 In another embodiment, the composition of the invention comprises, consists of or consists essentially of (i) at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R, at least one antagonist or inverse agonist of CXCR2, at least one agonist of TRAIL-RI or TRAIL-RII and at least one agonist of LIFR, (ii) at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R, at least one
10 antagonist or inverse agonist of CXCR2, at least one agonist of TRAIL-RI or TRAIL-RII and at least one antagonist or inverse agonist of CXCR4 or (iii) at least one antagonist or inverse agonist of CCR7, at least one agonist of IL-15R, at least one antagonist or inverse agonist of CXCR2, at least one agonist of TRAIL-RI or TRAIL-RII, at least one agonist of LIFR and at least one antagonist or inverse agonist of CXCR4.
- 15 In one embodiment, the composition of the invention is a pharmaceutical composition and further comprises at least one pharmaceutically acceptable excipient.

In one embodiment, the composition of the invention is a medicament.

- Another object of the present invention is a composition as described hereinabove for treating or for use in treating a demyelinating disease, wherein said composition
20 comprises at least one modulator of CCL19, IL-15, ENA78, and/or TRAIL, preferably at least one modulator of CCL19, IL-15, and/or TRAIL.

In one embodiment, the composition further comprises at least one modulator of LIF and/or SDF-1.

- Another object of the present invention is a composition for treating or for use in treating
25 a demyelinating disease comprising at least one modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, preferably at least one modulator of CCL19, IL-15 and/or TRAIL.

This invention relates in particular to a composition comprising at least one modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, preferably at least one modulator of CCL19, IL-15 and/or TRAIL, wherein said modulator activates or stimulates remyelination.

- 5 Another object of the invention is a pharmaceutical composition as described hereinabove for treating or for use in treating a demyelinating disease comprising or consisting of or consisting essentially of at least one modulator of CCL19, IL-15, ENA78, and/or TRAIL, preferably at least one modulator of CCL19, IL-15 and/or TRAIL as described here above and at least one pharmaceutically acceptable excipient.
- 10 Another object of the invention is a pharmaceutical composition for treating or for use in treating a demyelinating disease comprising or consisting of or consisting essentially of at least one modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, preferably at least one modulator of CCL19, IL-15 and/or TRAIL, as described here above and at least one pharmaceutically acceptable excipient.
- 15 Another object of the invention is a medicament for treating or for use in treating a demyelinating disease comprising or consisting of or consisting essentially of at least one modulator of CCL19, IL-15, ENA78, and/or TRAIL as described here above.

Another object of the invention is a medicament for treating or for use in treating a demyelinating disease comprising or consisting of or consisting essentially of at least one
20 modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, preferably at least one modulator of CCL19, IL-15, ENA78, SDF-1, and/or TRAIL as described here above.

Pharmaceutically acceptable excipients include, without limitation water, saline, Ringer's solution, dextrose solution, and solutions of ethanol, glucose, sucrose, dextran, mannose, mannitol, sorbitol, polyethylene glycol (PEG), phosphate, acetate, gelatin, collagen,
25 Carbopol®, vegetable oils, and the like. One may additionally include suitable preservatives, stabilizers, antioxidants, antimicrobials, and buffering agents, such as, for example, BHA, BHT, citric acid, ascorbic acid, tetracycline, and the like.

- Other examples of pharmaceutically acceptable excipients that may be used in the composition of the invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene- block polymers, polyethylene glycol and wool fat.
- 10 In addition, pharmaceutically acceptable excipients may comprise some excipients, such as, for example, surfactants (e.g. hydroxypropylcellulose); suitable carriers, such as, for example, solvents and dispersion media containing, for example, water, ethanol, polyol (e.g. glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils, such as, for example, peanut oil and sesame oil;
- 15 isotonic agents, such as, for example, sugars or sodium chloride; coating agents, such as, for example, lecithin; agents delaying absorption, such as, for example, aluminum monostearate and gelatin; preservatives, such as, for example, benzalkonium chloride, benzethonium chloride, chlorobutanol, thimerosal and the like; buffers, such as, for example, boric acid, sodium and potassium bicarbonate, sodium and potassium borates,
- 20 sodium and potassium carbonate, sodium acetate, sodium biphosphate and the like; tonicity agents, such as, for example, dextrose, potassium chloride, propylene glycol, sodium chloride; antioxidants and stabilizers, such as, for example, sodium bisulfite, sodium metabisulfite, sodium thiosulfite, thiourea and the like; nonionic wetting or clarifying agents, such as, for example, polysorbate 80, polysorbate 20, poloxamer 282
- 25 and tyloxapol; viscosity modifying agents, such as, for example dextran 40, dextran 70, gelatin, glycerin, hydroxyethylcellulose, hydroxymethylpropylcellulose, lanolin, methylcellulose, petrolatum, polyethylene glycol, polyvinyl alcohol, polyvinylpyrrolidone, carboxymethylcellulose; and the like.

The present invention further relates to a method for treating a demyelinating disease

30 (preferably MS) in a subject in need thereof, wherein said method comprises restoring the

level of at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, preferably at least one of CCL19, IL-15, and/or TRAIL to a level measured in a subject of the high remyelination profile group.

In one embodiment, the method of the invention does not consist in restoring the level of
5 ENA78 to a level measured in a subject of the high remyelination profile group. In one embodiment, the method of the invention does not consist in restoring the levels of ENA78 and of LIF to a level measured in a subject of the high remyelination profile group. In one embodiment, the method of the invention does not consist in restoring the levels of ENA78 and of SDF-1 to a level measured in a subject of the high remyelination
10 profile group. In one embodiment, the method of the invention does not consist in restoring the levels of ENA78, of LIF and of SDF-1 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of CCL19 to a level measured in a subject of the high remyelination profile group.

15 Indeed, the Applicant suggests that CCL19 is an interesting target, in view of its role on remyelination but also in view of its chemoattractive role on B cells and on pro-inflammatory macrophages. Without willing to be bound to any theory, the Applicant suggests that inhibiting CCL19 in patients of the low remyelination profile group may both enhance remyelination and avoid the invasion of the central nervous system by B
20 cells and pro-inflammatory macrophages that are involved in the pathogenesis of the demyelinating disease.

In one embodiment, said method comprises or consists in restoring the level of IL-15 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of ENA78
25 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of TRAIL to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of CCL19 and IL-15 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of CCL19 and ENA78 to a level measured in a subject of the high remyelination profile group.

- 5 In one embodiment, said method comprises or consists in restoring the level of CCL19 and TRAIL to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of IL-15 and ENA78 to a level measured in a subject of the high remyelination profile group.

- 10 In one embodiment, said method comprises or consists in restoring the level of IL-15 and TRAIL to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of ENA78 and TRAIL to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of CCL19, IL-15 and ENA78 to a level measured in a subject of the high remyelination profile group.

- 15 In one embodiment, said method comprises or consists in restoring the level of CCL19, ENA78 and TRAIL to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of CCL19, IL-15 and TRAIL to a level measured in a subject of the high remyelination profile group.

- 20 In one embodiment, said method comprises or consists in restoring the level of IL-15, ENA78 and TRAIL to a level measured in a subject of the high remyelination profile group.

- 25 In one embodiment, said method comprises or consists in restoring the level of CCL19, IL-15, ENA78 and TRAIL to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of (i) CCL19 and LIF, (ii) CCL19 and SDF-1 or (iii) CCL19, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

5 In one embodiment, said method comprises or consists in restoring the level of (i) IL-15 and LIF, (ii) IL-15 and SDF-1 or (iii) IL-15, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of (i) ENA78 and LIF, (ii) ENA78 and SDF-1 or (iii) ENA78, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

10 In one embodiment, said method comprises or consists in restoring the level of (i) TRAIL and LIF, (ii) TRAIL and SDF-1 or (iii) TRAIL, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of (i) CCL19, IL-15 and LIF, (ii) CCL19, IL-15 and SDF-1 or (iii) CCL19, IL-15, LIF and SDF1 to a
15 level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of (i) CCL19, ENA78 and LIF, (ii) CCL19, ENA78 and SDF-1 or (iii) CCL19, ENA78, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of (i) CCL19, TRAIL and LIF, (ii) CCL19, TRAIL and SDF-1 or (iii) CCL19, TRAIL, LIF and SDF1
20 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of (i) IL-15, ENA78 and LIF, (ii) IL-15, ENA78 and SDF-1 or (iii) IL-15, ENA78, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

25 In one embodiment, said method comprises or consists in restoring the level of (i) IL-15, TRAIL and LIF, (ii) IL-15, TRAIL and SDF-1 or (iii) IL-15, TRAIL, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of (i) ENA78, TRAIL and LIF, (ii) ENA78, TRAIL and SDF-1 or (iii) ENA78, TRAIL, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of (i) CCL19, IL-15, ENA78 and LIF, (ii) CCL19, IL-15, ENA78 and SDF-1 or (iii) CCL19, IL-15, ENA78, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of (i) CCL19, ENA78, TRAIL and LIF, (ii) CCL19, ENA78, TRAIL and SDF-1 or (iii) CCL19, ENA78, TRAIL, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of (i) CCL19, IL-15, TRAIL and LIF, (ii) CCL19, IL-15, TRAIL and SDF-1 or (iii) CCL19, IL-15, TRAIL, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of (i) IL-15, ENA78, TRAIL and LIF, (ii) IL-15, ENA78, TRAIL and SDF-1 or (iii) IL-15, ENA78, TRAIL, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

In one embodiment, said method comprises or consists in restoring the level of (i) CCL9, IL-15, ENA78, TRAIL and LIF, (ii) CCL9, IL-15, ENA78, TRAIL and SDF-1 or (iii) CCL9, IL-15, ENA78, TRAIL, LIF and SDF1 to a level measured in a subject of the high remyelination profile group.

In one embodiment, the method for treating a demyelinating disease of the invention comprises administering to the subject the composition described here above, the pharmaceutical composition or the medicament of the invention.

In one embodiment, the method of the invention comprises administering at least one inhibitor of CCL19, ENA78, and/or SDF-1, preferably at least one inhibitor of CCL19, to the subject in need thereof.

5 In another embodiment, the method of the invention comprises administering at least one activator of IL-15, LIF and/or TRAIL, preferably at least one IL-15 and/or TRAIL to the subject in need thereof.

In one embodiment, the method of the invention comprises administering at least one antagonist of CCL19 receptor, ENA78 receptor, and/or SDF-1 receptor, preferably at least one antagonist of CCL19 receptor to the subject in need thereof.

10 In one embodiment, the method of the invention comprises administering at least one inverse agonist of CCL19 receptor, ENA78 receptor, and/or SDF-1 receptor, preferably at least one inverse agonist of CCL19 receptor to the subject in need thereof.

In another embodiment, the method of the invention comprises administering at least one agonist of IL-15 receptor, LIF receptor and/or TRAIL receptor, preferably of at least one
15 agonist of IL-15 receptor and/or TRAIL receptor to the subject in need thereof.

The present invention further relates to a method for treating a demyelinating disease in a subject with a low remyelination profile, wherein said method comprises restoring the level of at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, preferably of at least one of CCL19, IL-15 and/or TRAIL to a normal level, preferably to a level
20 measured in a subject of the high remyelination profile group. Indeed, the Applicant demonstrated that MS patients with a low remyelination profile present an increased level of CCL19, ENA78, and/or SDF-1 and a decreased level of IL-15, LIF and/or TRAIL as compared to patients with a high remyelination profile.

Therefore, in one embodiment, the method of the invention comprises administering at
25 least one inhibitor of CCL19, ENA78, and/or SDF-1, preferably at least one inhibitor of CCL19 and/or administering at least one of IL-15, LIF and/or TRAIL or at least one activator of IL-15, LIF and/or TRAIL, preferably at least one of IL-15 and/or TRAIL or at least one activator of IL-15 and/or TRAIL.

Another object of the present invention is a method for treating a demyelinating disease wherein the method comprises administering to a subject in need thereof an effective amount of at least one modulator of CCL19, IL-15, ENA78, and/or TRAIL, preferably at least one modulator of CCL19, IL-15 and/or TRAIL.

- 5 In one embodiment, the method of the invention does not consist in administering to the subject a modulator of ENA78.

In one embodiment, the method for treating a demyelinating disease further comprises administering to a subject in need thereof an effective amount of at least one modulator of LIF and/or SDF-1.

- 10 In one embodiment, the method of the invention does not consist in administering to the subject a modulator of ENA78 and a modulator of LIF. In one embodiment, the method of the invention does not consist in administering to the subject a modulator of ENA78 and a modulator of SDF1. In one embodiment, the method of the invention does not consist in administering to the subject a modulator of ENA78, a modulator of LIF and a
15 modulator of SDF-1.

In a preferred embodiment, the present invention relates thus to a method for treating a demyelinating disease wherein the method comprises administering to a subject in need thereof an effective amount of at least one modulator of CCL19, IL-15, ENA78, LIF, SDF-1 and/or TRAIL, preferably at least one modulator of CCL19, IL-15 and/or TRAIL.

- 20 In one embodiment, the method of the invention comprises administering to the subject a composition as described hereinabove.

In one embodiment, the method of the invention comprises the steps of:

- obtaining PBMC, preferably lymphocytes, from the subject to be treated;
- *ex vivo* modifying the PBMC, preferably lymphocytes, in order to restore the level of
25 at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, preferably at least one of CCL19, IL-15, and/or TRAIL to a level measured in a subject of the high remyelination profile group; and
- injecting the modified PBMC, preferably lymphocytes, thus obtained to the subject.

In one embodiment, the step of restoring a level of at least one cytokine to a level measured in a subject of the high remyelination group comprises contacting the PBMC, preferably the lymphocyte, with at least one modulator as described herein.

5 In another embodiment the step of restoring a level of at least one cytokine to a level measured in a subject of the high remyelination group comprises inhibiting the expression of at least one of CCL19, ENA78, and/or SDF-1. Examples of methods for inhibiting the expression of a gene are well known from the skilled artisan and include, without limitation, knocking down the CCL19, ENA78, and/or SDF-1 gene using, for example, siRNA, shRNA, clustered regularly interspaced short palindromic repeats (CRISPR)
10 transcription-activator like effector nuclease (TALEN), zinc finger endonuclease (ZFN), meganuclease (mn, also known as homing endonuclease), or megaTAL (combining a TAL effector with a mn cleavage domain).

In another embodiment the step of restoring a level of at least one cytokine to a level measured in a subject of the high remyelination group comprises increasing the
15 expression of at least one of IL-15, LIF and/or TRAIL. Examples of methods for increasing the expression of a gene are well known from the skilled artisan and include, without limitation, transduction with a polynucleotide encoding IL-15, LIF and/or TRAIL (associated with elements required for its transcription, such as, for example, a promoter sequence, a polyadenylation sequences, a terminator and optionally regulatory elements),
20 CRISPR/Cas9 system or a derivative thereof, and the like.

Physical methods for introducing a polynucleotide into a cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like.

Biological methods for introducing a polynucleotide of interest into a cell include the use
25 of DNA and RNA vectors, plasmids, phagemids, phages derivative, animal viruses and cosmids. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, *e.g.*, human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like.

Chemical means for introducing a polynucleotide into a cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (e.g., an artificial membrane vesicle).

The present invention thus further relates to a modified PBMC (preferably a modified lymphocyte) for use in treating a demyelinating disease, wherein said PBMC or lymphocyte presents a level of expression of at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL substantially equivalent (*e.g.* not statistically different) to the level measured in a subject of the high remyelination profile group.

In one embodiment, said PBMC or lymphocyte is modified to express a level of at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL, preferably at least one of CCL19, IL-15, and/or TRAIL substantially equivalent to the level measured in a subject of the high remyelination profile group. Examples of methods for restoring the level of at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL to the level measured in a subject of the high remyelination profile group are listed hereinabove.

In one embodiment, said PBMC or lymphocyte is allogenic. The term “allogeneic” refers to any material derived from a different individual of the same specie as the individual to whom the material is introduced.

In one embodiment, said PBMC or lymphocyte is autologous. The term “autologous” refers to any material derived from the same individual to whom it is later to be re-introduced.

In one embodiment of the invention, the composition described here above, the pharmaceutical composition, the medicament of the invention is to be administered at a therapeutically effective amount.

In one embodiment, the composition described here above or the pharmaceutical composition or the medicament of the invention is to be administered at a dose determined by the skilled artisan and personally adapted to each subject.

It will be understood that the usage of the composition described here above, the pharmaceutical composition, the medicament of the invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective amount for any particular patient will depend upon a variety of factors including the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and like factors well known in the medical arts.

In one embodiment, the composition described here above, the pharmaceutical composition or the medicament of the invention is to be administered systemically or locally, by injection, orally, topically, nasally, by inhalation, buccally, rectally, intratracheally, by endoscopy, transmucosally, by percutaneous administration or by perispinal administration.

In one embodiment, the composition described here above, the pharmaceutical composition, the medicament of the invention is to be administered by injection, preferably is to be systemically injected. Examples of formulations adapted to systemic injections include, but are not limited to: liquid solutions or suspensions, solid forms suitable for solution in, or suspension in, liquid prior to injection. Examples of systemic injections include, but are not limited to, intravenous, subcutaneous, intramuscular, intradermal, intravitreal, and intraperitoneal injection, or perfusion. In another embodiment, when injected, the composition, the pharmaceutical composition or the medicament of the invention is sterile. Methods for obtaining a sterile pharmaceutical composition include, but are not limited to, GMP synthesis (GMP stands for “Good manufacturing practice”).

In one embodiment, the composition described here above, the pharmaceutical composition, the medicament of the invention is to be orally administered. Examples of formulations adapted to oral administration include, but are not limited to: solid forms, liquid forms and gels. Examples of solid forms adapted to oral administration include, but are not limited to, pill, tablet, capsule, soft gelatin capsule, hard gelatin capsule, caplet, compressed tablet, cachet, wafer, sugar-coated pill, sugar coated tablet, or dispersing/or disintegrating tablet, powder, solid forms suitable for solution in, or suspension in, liquid

prior to oral administration and effervescent tablet. Examples of liquid forms adapted to oral administration include, but are not limited to, solutions, suspensions, drinkable solutions, elixirs, sealed phial, potion, drench, syrup and liquor.

5 In another embodiment, the composition described here above, the pharmaceutical composition, the medicament of the invention is to be topically administered. Examples of formulations adapted to topical administration include, but are not limited to, sticks, waxes, creams, lotions, ointments, balms, gels, masks, leave-on washes and/or the like.

10 In one embodiment, the composition described here above, the pharmaceutical composition or the medicament of the invention is to be administered in a sustained-release form. In another embodiment, the composition, the pharmaceutical composition or the medicament of the invention comprises a delivery system that controls the release of the agent.

15 In one embodiment, the composition described here above, the pharmaceutical composition or the medicament of the invention is to be administered perispinally or intranasally in improved delivery, either by local diffusion; by improved transport into the cerebrospinal fluid (CSF); or by direct transport into the CNS.

In one embodiment, a therapeutically effective amount of the composition described here above, the pharmaceutical composition or the medicament of the invention is administered at least once a day, twice a day, or at least three times a day.

20 In another embodiment, a therapeutically effective amount of the composition described here above, the pharmaceutical composition, medicament of the invention is administered every day, or every two, three, four, five, or six days.

25 In another embodiment, a therapeutically effective amount of the composition described here above, the pharmaceutical composition, medicament of the invention is administered twice a week, every week, every two weeks, or once a month.

In another embodiment, a therapeutically effective amount of the composition described here above or the pharmaceutical composition of the invention, the medicament of the

invention is administered every month for a period at least 2; 3; 4; 5; 6 months or for the rest of the life of the subject.

In another embodiment, a therapeutically effective amount of the composition described here above or the pharmaceutical composition or the medicament of the invention of the
5 invention ranges from about 1 μ g to about 100 g, preferably from about 100 mg to about 10 g, more preferably from about 1 mg to about 1 g.

In another embodiment, a therapeutically effective amount of the composition described here above, the pharmaceutical composition or the medicament of the invention ranges from about 0.1 μ g/kg to about 1 g/kg of body weight, preferably from about 0.1 mg/kg of
10 body weight to about 0.1 g/kg, more preferably from about 1.5 mg/kg to about 0.15 g/kg of body weight.

In another embodiment, a therapeutically effective amount of the composition described here above, the pharmaceutical composition or the medicament of the invention ranges from about 0.5 mg/kg of body weight to about 50 mg/kg of body weight, preferably from
15 about 1 mg/kg to about 25 mg/kg.

In one embodiment, the subject is affected, preferably is diagnosed with a demyelinating disease (preferably MS). In another embodiment, the subject of the invention is at risk of developing a demyelinating disease (preferably MS). Examples of risk factors include, but are not limited to, genetic factors, smoking, stress, diet and hormone intake (such as
20 for example fat intake, deficiencies in fish oil and vitamin D), infectious agents such as for example microbes, viruses and in particular herpes virus, Epstein Barr virus).

In one embodiment, the subject affected by a demyelinating disease has been classified in a group having a low remyelination profile.

In one embodiment, the subject affected by a demyelinating disease has been classified
25 in a group having a high remyelination profile.

Another object of the present invention is a method for activating myelination in a subject in need thereof wherein the method comprises administering to said subject in need

thereof an effective amount of at least one modulator of CCL19, IL-15, ENA78, and/or TRAIL, preferably at least one modulator of CCL19, IL-15 and/or TRAIL.

In one embodiment, the method for activating myelination further comprises administering to said subject in need thereof an effective amount of at least one modulator
5 of LIF and/or SDF-1.

In a preferred embodiment, the present invention relates thus to a method for activating myelination in a subject in need thereof wherein the method comprises administering to said subject in need thereof an effective amount of at least one modulator of CCL19, IL-15, ENA78, LIF, SDF-1 and/or TRAIL, preferably at least one modulator of CCL19,
10 IL-15 and/or TRAIL.

In one embodiment, the method of the invention comprises administering to the subject a composition, pharmaceutical composition or medicament as described hereinabove.

Technics to detect myelination process *in vivo* are well known to the skilled artisan and include performing magnetic resonance imaging (MRI) or positon electron tomography
15 (PET) scan on the subject.

Technics to detect myelination process *in vitro* are well known to the skilled artisan and include performing immunohistochemistry on a subject's sample using at least one of the following molecular markers: Olig2 (expressed in the oligodendroglial lineage), CC1 (expressed in mature stages of the lineage), Ki67 (expressed in proliferating cells), O4
20 (expressed in immature oligodendrocytes), Galc (expressed in pre-oligodendrocytes) and CNPase (expressed in mature oligodendrocytes).

Technics to detect microglial cells activation *in vivo* are well known to the skilled artisan and include using magnetic resonance imaging (MRI) or positon electron tomography (PET) and a ligand with relative selectivity for activated microglia on the subject.

25 Another object of the present invention is a method for recruiting (in particular at the demyelinated area), inducing proliferation and/or differentiating oligodendrocyte precursor cells (OPC) in a subject in need thereof comprising administering an effective amount of at least one modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL.

In one embodiment, the method for recruiting, inducing proliferation and/or differentiating oligodendrocyte precursor cells (OPC) in a subject in need thereof further comprises administering to a subject in need thereof an effective amount of IL-15, LIF, , and/or TRAIL.

- 5 Another object of the present invention is a method for modulating the inflammatory state of microglial cells in a subject in need thereof comprising administering an effective amount of at least one modulator of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL.

Technics to detect the inflammatory state of microglial cells *in vitro* are well known to the skilled artisan and include performing immunohistochemistry on a subject's sample
10 using at least one of the following molecular markers: iNOS (expressed in M1 macrophage) and IGF-1 (expressed in M2 macrophage).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a set of diagram and histograms showing that multiple sclerosis patients LT
15 impede OL remyelination. (A) Schematic of human LT influence on remyelination assay. 48h after chemically induced demyelination in the dorsal spinal cord of nude mice, human LT were grafted at the lesion site. Blood was collected from mice 8 to 12 days post lesion, and analyzed with flow cytometry to identify LT based on HLA-DR and hCD4 expression (B) (here is shown one representative experiment out of
20 6 performed). The presence of human lymphocytes within the lesion was revealed by HLA staining, 21 days post grafting (data not shown). For the remyelination assay HD or multiple sclerosis patient activated LT were grafted. After 21 days, OL differentiation was assessed using the combination Olig2/CC1 to distinguish between mature OL and immature cells. The number of Olig2⁺ cells and the percentage Olig2⁺CC1⁺ cells over
25 Olig2⁺ cells (C) were evaluated in the HD (n = 8 individuals) and multiple sclerosis (n = 9 individuals, 3–5 grafted mice per individual) conditions. **p < 0.01, unpaired, two-tailed Student's t-test. A second set of mice was processed for electron microscopy. Beside the mice grafted with the LT of the HD subgroup, two subgroups of multiple sclerosis patients were considered (D): multiple sclerosis patient with a Low (light grey

- triangle – patients 7 to 9) % of Olig-2⁺CC1⁺ and patient with a High (dark grey triangle – patients 1 to 5) % of Olig-2⁺CC1⁺. Toluidine blue stained semi-thin section of lesion reveals the lesion area (data not shown). Ultrastructure analysis was used to highlight axons remyelinated by OL, by SC or non remyelinated in lesion grafted with LT from the multiple sclerosis Low subgroup (n = 3) compared to those grafted with LT from HD (n = 6) or the multiple sclerosis High subgroup (n = 5). 3-5 mice were grafted for each individual. Remyelination was evaluated by measuring the total area of remyelination conducted by OL (**D**). *p < 0.05, **p < 0.01, 1-way ANOVA and Tukey's multiple comparison test.
- 10 **Figure 2** is a set of diagram, and histograms showing that multiple sclerosis patients LT supernatants induce an increase in the M1/M2 ratio of microglia. Schematic of the MIG activation assay in response to LT supernatants (**A**). HD or multiple sclerosis LT were activated during 72h with anti CD2/CD3/CD28 antibodies and the supernatants were collected. Murine microglia cells were next exposed to culture media or multiple sclerosis patient LT supernatants during 24h. M1 and M2 cells were labelled using respectively iNOS and IGF-1. The iNOS⁺ cells / IGF-1⁺ cells ratio was calculated for the CT (n = 7), HD (n = 11) and multiple sclerosis (n = 26) groups (**B**). Each experiment was performed in triplicates. *p < 0.05, ***p < 0.001, 1-way ANOVA and Tukey's multiple comparison test.
- 20 **Figure 3** is a diagram, images and a graph showing that the conditioned media of MIG pre-exposed to multiple sclerosis patients LT supernatant induces an increase in OPC proliferation and a decrease of differentiation. Schematic of the OPC proliferation and differentiation assay in response to MIG conditioned media (**A**). Murine OPCs were exposed during 24h (proliferation) or 72h (differentiation) to the microglia conditioned media that were incubated with fresh culture media (CT) (**B**), to HD (**C**) or to multiple sclerosis patient (**D**) LT supernatants. Proliferating OPCs were highlighted using Olig2 and Ki67 (**B-D**). The percentage of Olig2⁺/Ki-67⁺ proliferating OPCs (white arrowheads) amount the total oligodendroglial cells Olig2⁺ was calculated in the CT (n = 6), HD (n = 9) and multiple sclerosis (n = 27) conditions (**E**). Each experiment was performed in quadruplicates. For the differentiation assay, the state of maturation of

oligodendrocyte was analyzed using chronologically expressed markers: O4, GalC and CNPase. The percentage of positive cells for each of the marker was calculated in CT (n = 8 to 12), HD (n = 11) and multiple sclerosis (n = 27) conditions (**F, G, H**). Each experiment was performed in triplicates. *p < 0.05, #p < 0.05 **p < 0.01. ***p < 0.001, 1-way ANOVA and Tukey's multiple comparison test. Scale bar 100 μ m.

Figure 4 is a set of four histograms showing that multiple sclerosis patient and HD LT have a different secretory pattern. After *in vitro* activation of multiple sclerosis patients (n = 27) or HD (n = 12) LT with anti-CD2/CD3/CD28 antibodies during 72h, supernatants were collected. The proportion of different subtypes of T helper (Th1, Treg and Th17) and B cells was assessed by flow cytometry (data not shown) and the level of expression of 72 cytokines was evaluated by Luminex (67 cytokines were detectable). Mean values for each tested cytokine were calculated. Four cytokines were evidenced as differentially expressed between multiple sclerosis and HD. *p < 0.05, **p < 0.01, unpaired, two-tailed student's t-test.

Figure 5 is a set of ten histograms representing the capacity of multiple sclerosis patients to induce a proper inflammatory context for remyelination is not dependant on the age, sex, treatment status, disease form or duration. The M1/M2 ratio (**A, C, E-G**) and the percentage of CNPase⁺ cells (**B, D, H-J**) were calculated as previously described (Fig. 2 and 3). Correlations between the age of individuals (patients or HD) and the M1/M2 ratio (**A**) or the % of CNPase (**B**) that their LT supernatants were inducing were calculated. Patients (Female n=18, males n=8) and HD (females n=6, males n=5) were also stratified according to their sex (**C, D**). To analyze the influence of disease characteristics on the M1/M2 ratio or the % of CNPase⁺ cells, patients were next stratified according to their disease form (**E, H**): relapsing-remitting (RR, n=17), secondary progressive (SP, n = 7) or primary progressive (PP, n = 3), to their disease duration (**F, I**): under 10 years (n = 9), between 10 and 20 years (n = 10) and above 20 years (n = 8), or to their treatment status (**G, J**) : either treated (n = 18) or not (n = 9). On graphs E-J, the mean value in HD conditions is represented by a dashed line. Two-tailed unpaired student's t-test or 1-way ANOVA and Tukey's multiple comparison test.

Figure 6 is a set of two diagrams, three histograms and a table showing that MS patients LT secretory patterns analysis revealed the cytokine correlated to OPC differentiation. All individuals used for the study were clustered in 3 subgroups: HD and multiple sclerosis patients inducing an *in vitro* CNPase expression either above (HIGH, N = 13) or below (LOW, n = 13) the median value. A barycentric discriminant analysis was performed (**A**) to define which variables were implementing variability in our data set. A Partial least square regression (PLS) was performed on the data set of patients according to a linear model (**B**) to determine which of the 3 blocks (LT composition, Cytokines expression level and MIG activation) were correlated and could predict the value of the percentage of CNPase⁺ cells. Correlations between the different blocks and the percentage of CNPase⁺ cells were calculated (**C**). Bootstrap ratios were calculated to evaluate the significance of each variable of each block allowing to highlight cytokines that were not correlated (grey bars), significantly positively (black bars) or significantly negatively (black bars) correlated with the percentage of CNPase⁺ cells. From top to bottom: CCL19; ENA78; SDF-1; PDGF-BB; Eoxatin-2; MCP-2; PDGF-AA; CXCL6; IL-29; MCP-4; MCP-3; X6Ckine; RANTES; GRO; IL-6; MCP-1b; FGF-2; EGF; CCL14; Eoxatin; CCL20; IP-10; CXCL9; G-CSF; IL-1B; IL-8; IL-20; BCA-1; MCP-1a; IFN- γ ; CXCL11; M-CSF; XCL1; IL-12p70; CTACK; IL-17A; MCP-1; VEGF; IL-4; TGF α ; TNF α ; fractalkine; I-309; sCD40L; IL-16; MDC; IL-12p40; IL-2; IL-7; IL-5; IL-1RA; MIP-1D; TARC; GM-CSF; IL-1A; IL-3; TNF β ; IL-10; IL-9; CXCL7; N-a2; FLT3; IL-23; IL-13; IL-15; TRAIL; LIF (**D**). The effect of CCL19 on OPC differentiation was next evaluated in patients: MIG were exposed to LT supernatant (as described in Fig. 2) from LOW or HIGH with or without human recombinant CCL19 at 1 ng/mL. The M1/M2 ratio was next calculated (**E**). Microglia conditioned media pre-exposed to LT supernatant coming from LOW or HIGH which were supplemented or not with CCL19 at 1 ng/mL was put on OPC (as described in Fig. 3). The proportion of CNPase⁺ cells was evaluated 72h later (**F**). Each experiment was performed in quadruplicates. *p < 0.05, **p < 0.01, ***p < 0.001. Bootstrap ratio (PLS) or paired two-tailed student's t-test.

Figure 7 is a schematic representation of MS patient and HD LT influence on MIG activation, OPC behavior and remyelination. *In vivo* OPC remyelination (but not OPC

recruitment) were impeded after graft of MS patients with Low repair capacities compared to HD and MS patients with High repair capacities. *In vitro*, the overexpression of CCL19 and SDF-1 in the LT supernatant of patients with LOW OPC differentiation capacities was strongly correlated with pro-inflammatory MIG activation and a decreased
5 OPC differentiation.

Figure 8 is a set of three histograms showing the patients follow-up across experiments. Patients were ranked (from 1 to 9, from dark to light) according to the percentage of mature oligodendrocytes that their LT were inducing 3 weeks post grafting in the focal demyelinated lesion of nude mice spinal cords (**A**). Patients were next followed in the
10 remyelination assay evaluated by electron microscopy (**B**) and in the OPC differentiation in vitro assay (**C**). In each graph, the mean value in HD is represented by a dashed line.

Figure 9 is a set of four histograms showing the replication of the effect of multiple sclerosis LT, HD LT and CCL19 on MIG activation and OPC differentiation with an independent cohort of HD and treatment-free multiple sclerosis patients. The MIG
15 activation (**A**), the OPC differentiation assay (**B**) and the test of the effect of CCL19 on MIG activation (**C**) and OPC differentiation (**D**) were replicated on independent cohorts of HD (n=5) and of treatment-free multiple sclerosis (n=8) patients. HIGH and LOW patients were stratified in two groups according to the median value of the % of CNPase.
*p < 0.05, **p < 0.01. Unpaired, two-tailed Student's t-test.

20

EXAMPLES

The present invention is further illustrated by the following examples.

Abbreviations:

HD: healthy donors;
25 LT: lymphocyte;
MIG: microglial cells;
MS: multiple sclerosis;
OL: oligodendrocyte;
OPC: oligodendrocyte precursor cell.

Example 1: Influence of human immune cells on endogenous remyelination and established new in vitro and in vivo experimental paradigm to evaluate how human LT gear microglia (MIG) polarization and the consequences of this crosstalk on the remyelination process

5 *In vivo evaluation of human LT effect on remyelination*

In Nude mice, spontaneous remyelination occurs after focal demyelination from lysophosphatidylcholine (LPC) injection and is completed within 4 weeks (Jeffery and Blakemore, 1995). To evaluate how LT influence remyelination, we combined LPC induced demyelination in the spinal cord of Nude mice and the graft within the lesion 48h after demyelination of 10⁵ activated LT isolated from blood samples of 6 HD and 7 multiple sclerosis patients (**Table 1**) (**Fig. 1A**).

Characteristics	ALL MS	RRMS	SPMS	PPMS	HD
Number	26	16	7	3	14
Female/male	19/8	13/4	4/3	2/1	6/8
Mean Age	46.9	43.2	47.9	65.7	46.6
Range [IQR]	(25-72) [15,5]	(27-57) [12]	(25-67) [11.5]	(59-72) [6.5]	(28-65) [11.75]
Mean Age of disease onset	30.4	29.3	23.9	51.7	
Range [IQR]	(17/57) [13]	(17-44) [12]	(20-31) [5.5]	(48-57) [4.5]	
Mean disease duration	16.4	14.0	24.0	12.7	
Range [IQR]	(4-47) [13]	(4-29) [11]	(5-47) [11.5]	(7-16) [0.5]	
Mean EDSS	3.6	2.6	5.8	4.0	
Range [IQR]	(0-7) [3.75]	(0-6.5) [2]	(4-7) [1.75]	(3.5-4.5) [0.5]	
Mean MSSS	4.0	3.0	6.2	4.8	
Range [IQR]	(0.04-8.83) [4.355]	(0.04-8.5) [2.88]	(1.69-8.83) [1.855]	(4.14-5.75) [0.805]	
Under treatment/ treatment free	18/9	16/2	3/4	0/3	

Table 1. Characteristics of MS patients' cohort. (MSSS (EDSS related to disease duration): multiple sclerosis severity score. PPMS: primary progressive multiple sclerosis. RRMS: relapsing remitting multiple sclerosis. SPMS: secondary progressive multiple sclerosis. HD: healthy donor. IQR: interquartile range.

To follow LT fate, we detected in blood samples of grafted mice the presence of HLA⁺ ($1.13\% \pm 0.25$) and human cluster of differentiation 4⁺ hCD4⁺ ($0.56\% \pm 0.06$) cells by flow cytometry (**Fig. 1B**) and we confirmed the presence of HLA⁺ cells within the lesion by immunohistochemistry 21 days post grafting.

- 5 The effect of LT grafting on OPC recruitment and differentiation was evaluated using the combination of Olig2 (expressed throughout the oligodendroglial lineage) and adenomatous polyposis coli/clone CC1 (expressed in mature stages of the lineage) immunolabelling in the lesion border underlined by a strong staining of astrocytic marker GFAP. While no difference in the total number of Olig2⁺ cells was observed, the
- 10 proportion of Olig2⁺CC1⁺ cells, representing OPC engaged in differentiation, was significantly reduced in multiple sclerosis LT grafted mice compared to HD LT grafted mice (**Fig. 1C**). These results demonstrate that multiple sclerosis LT did not interfere with OPC recruitment but impeded OPC differentiation.

- Our data revealed that some patients' LT have a deleterious effect while LT from other
- 15 patients did not disturb OPC differentiation. Therefore, we defined two subgroups of multiple sclerosis patients based on their LT effect on OPC differentiation (**Fig. 1D**): multiple sclerosis patient with a High Olig2⁺CC1⁺ (patient 1, 2, 3; **Fig. 1D**) and Low Olig2⁺CC1⁺ profile (patients 4, 5, 6; **Fig. 1D**). To investigate this heterogeneity, we performed electron microscopy on a second set of mice grafted with LT of HD, of the
- 20 Low subgroup and the High subgroup. The lesion area was calculated on semithin sections. Ultrathin sections revealed axons remyelinated by OL, Schwann cells or non remyelinated. The percentage of remyelinated area revealed a decrease of OL remyelination in mice grafted with Low subgroup LT that was significantly different both from the mice grafted with LT from HD or the High subgroup (**Fig. 1D**).

- 25 Overall, multiple sclerosis LT presence within the lesion is sufficient to disturb OL remyelination and different patterns of remyelination were observed among the mice grafted with multiple sclerosis LT.

In vitro assessment of LT effect on MIG activation and OPC proliferation/differentiation

We explored *in vitro* the underlying cellular mechanisms of the decreased remyelination *in vivo*. Since applying LT supernatant directly on OPC did not have a significant effect on OPC differentiation (data not shown), we investigated whether LT affected OPC
5 behavior through their influence on MIG.

LT supernatant from multiple sclerosis patient, HD or fresh culture media (control condition) were added to MIG (**Fig. 2A**). After 24h, cells were fixed and stained for two markers of MIG activation: inducible Nitric Oxide Synthase (iNOS) which labels the M1 state, and insulin growth factor 1 (IGF-1), a marker of the M2 state. We evaluated the
10 ratio of iNOS⁺ cells to IGF-1⁺ (**Fig. 2B**) and showed that HD LT supernatant did not change the M1/M2 ratio compared to control conditions while multiple sclerosis LT supernatant provoked a significant shift towards an M1 phenotype leading to an increase of the M1/M2 ratio.

The consequences of MIG differential activation on OPC behavior were next assessed.
15 The conditioned media of MIG pre-exposed to culture media (control condition), HD, or multiple sclerosis LT supernatants were placed in a primary culture of mouse OPCs (**Fig. 3A**). After 24h, OPCs were fixed and labeled for Olig2 combined with Ki67, a marker of proliferation (**Fig. 3B-D**). The proportion of proliferating Olig2⁺Ki67⁺ cells was evaluated (**Fig. 3E**). We observed, both in HD and multiple sclerosis conditions, an
20 increase of OPC proliferation compared to the control condition. Furthermore, MIG conditioned media pre-exposed to multiple sclerosis patient LT supernatant induced a significant increase of OPC proliferation compared to HD conditions. To analyze OPC differentiation, OPCs were fixed 72h after exposure to the different MIG conditioned media (**Fig. 3A**) and stained for 3 markers expressed at different stages of OPC
25 differentiation: O4 for immature OL, Galactocerebroside (GalC) for pre-OL, and 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase) for mature OL. The proportion of positive cells for each marker was evaluated (**Fig. 3F, G, H**). MIG activated by multiple sclerosis patient LT supernatants appear to disturb OPC differentiation, as demonstrated by a significant reduction of the proportion of O4⁺ cells (**Fig. 3F**), GalC⁺

pre-OL (**Fig. 3G**) and CNPase⁺ mature OL (**Fig. 3H**) in multiple sclerosis conditions compared to control or HD conditions.

Thus, the preferential polarization of MIG to the pro-inflammatory M1 phenotype induced by multiple sclerosis patients' LT supernatants results in an increase of OPC proliferation and impairment of OPC maturation.

Example 2: Identification of cellular and molecular cues leading to a successful remyelination

To assess potential differences between HD and multiple sclerosis patients, we characterized immune cells composition by flow cytometry and evaluated their secretory profiles by a Luminex-based multiplex assay. While no substantial difference in the subtypes of T helper or B cells between multiple sclerosis patients and HD was observed (**Table 2**), we found that, among the 72 molecules analyzed, 4 were statistically differentially expressed between multiple sclerosis patients and HD: IL-7 and IL-20 where increased while B cell-attracting chemokine 1 (BCA-1) and CCL19 were downregulated in multiple sclerosis patients versus HD conditions (**Fig. 4**). Thus, even if LT subtypes proportions do not differ between multiple sclerosis patients and HD, their LT have different intrinsic capacity to respond to stimulation.

	Healthy Donors	MS patients
Total CD4⁺ cells (%)	50.6 ± 7.2	58.5 ± 6.9
Th1 cells (%)	32.0 ± 13.1	33.2 ± 14
Th17 cells (%)	5.8 ± 4.7	6.8 ± 3.4
Treg cells (%)	20.9 ± 11.5	25.7 ± 9.9
B cells (%)	5.6 ± 2.1	7.2 ± 2.8

Table 2. MS patients and HD have the same LT composition. After *in vitro* activation of MS patient (n=26) or HD LT (n=8) with anti CD2/CD3/CD28 antibodies during 72h, the proportion of different subtypes of T helper (Th1, Treg and Th17) and B cells was assessed by flow cytometry.

Our *in vivo* and *in vitro* results reveal heterogeneity among multiple sclerosis patients (**Fig. 8A-C**): some patient exhibit a remyelination pattern close to HD whereas some other patients exhibit a low remyelination profile. In order to test whether individual features

of the patients or HD might reflect this heterogeneity, we evaluated the effect of age (**Fig. 5 A-B**) and sex (**Fig. 5C, D**) but no correlation was found with MIG activation (**Fig. 5A, C**) or OPC differentiation (**Fig. 5B, D**). Patients were next clustered according to their disease form (**Fig. 5C, G**), disease duration (**Fig. 5D, H**), treatment status (**Fig. 5**) and sex (**Fig. 5F, J**) but none of these clinical features could explain the heterogeneous pattern observed. To further exclude an effect of a disease modifying treatment on the heterogeneous remyelination pattern observed, we replicated our results in a second independent treatment-free cohort (characteristics in **table 1; Fig. 9A and B**). To characterize the cellular and molecular actors inducing OPC differentiation heterogeneity, multiple sclerosis patients were clustered into two subgroups for further analysis: those inducing a high percentage of CNPase⁺ cells *in vitro* (HIGH) and those inducing a low percentage of CNPase⁺ cells (LOW). To detect the influence of combinations of variables (Schaefer and Strimmer, Stat Appl Genet Mol Biol. 2005; 4:Article32.) on OPC differentiation, we performed a multivariate analysis on our *in vitro* data using barycentric discriminant analysis (BADA) with ranked data on the three subgroups (HIGH, LOW and HD) based on LT secretion profile. As expected, the HD profile is best discriminated from the two multiple sclerosis groups as indicated by their respective position on the first dimension (**Fig. 6A**). The second dimension highlighted the difference between the HIGH and LOW groups. Focusing on the differences among multiple sclerosis patients, we performed a partial least squares regression analysis (Vinzi VE, *et al.*, Handbook of partial least squares: Concepts, methods and applications. Springer H. Berlin, Germany, Springer-Verlag: 2010.) (PLS) to find the best combination of LT composition, LT cytokine secretion, and MIG activation that could predict high levels of CNPase (**Fig. 6B**). We found that both MIG activation ($p < 0.001$) and LT cytokine secretion ($p < 0.05$) significantly predicted the percentage of CNPase (**Fig. 6C**). Bootstrap ratio analysis identified the cytokines/chemokines that strongly correlated with the percentage of CNPase⁺ cells. Leukemia inhibitory factor (LIF), TNF-related apoptosis-inducing ligand (TRAIL), and IL-15 were positively correlated with the percentage of CNPase, whereas Stromal cell derived factor 1 (SDF-1/CXCL12), Epithelial neutrophil-activating protein 78 (ENA-78/CXCL5) and CCL19 were negatively correlated with the percentage of CNPase (**Fig. 6D**).

To validate molecular candidates, we added CCL19 to LT supernatants and studied its effect on MIG activation and OPC differentiation. The addition of CCL19 in the LT supernatant of LOW patients did not have any significant effect on M1/M2 ratio or OPC differentiation. However, when considering the HIGH group, adding CCL19 was enough
5 to increase the M1/M2 ratio (**Fig. 6E**) and, as a consequence, decrease the percentage of CNPase⁺ (**Fig. 6F**) cells to values close to the LOW group. This deleterious action of CCL19 was replicated in an independent cohort of treatment-free patients (**Fig. 9C and D**). Conversely, according to preliminary data, administering an anti-CCL19 antibody to LT supernatants of LOW patients allows increasing the percentage of
10 differentiated OPC (i.e. CNPase⁺). This result supports the therapeutic use of inhibitors of CCL19 signaling to improve remyelination in said patients.

In a comprehensive investigation, we demonstrated for the first time that multiple sclerosis patient LT control the local inflammatory environment and influence the remyelination process (**Fig. 7**). Our findings show that multiple sclerosis patient LT have
15 a specific molecular signature that directs and/or maintains MIG in a pro-inflammatory state, resulting in a remyelination defect. As expected from what is known about the natural history of multiple sclerosis, the effects of multiple sclerosis patients' LT were very heterogeneous.

In conclusion, while LT from some of the patients induced a pro-regenerative MIG
20 activation that led to proper OPC differentiation and a successful remyelination process *in vivo* (HIGH group), other patients' LT induced a hostile environment for the remyelination (LOW group), a pattern suggesting that the intrinsic capacity to induce remyelination is specific to each patient.

In addition, CCL19 was found to be negatively correlated with MIG M2 phenotype and
25 OPC maturation. Intriguingly, CCL19 is one of the chemokines that discriminate HD from MS patients since it is downregulated in MS LT supernatant compared to HD but is also negatively correlated to M2 MIG and CNPase⁺ OPC when comparing "LOW" versus "HIGH" subgroups. The BADA analysis indicates that HD profile and MS patient "HIGH" versus "LOW" profile do not seem to involve the same molecular players. Thus,
30 while we could have expected CCL19 to have a positive effect when considering its high

expression in HD conditions, the addition of CCL19 to the “HIGH” subgroup LT supernatant was sufficient to elicit an M1/M2 ratio and CNPase levels close to those observed with the “LOW” subgroup. This suggests that the molecular players in remyelination are really specific in pathological conditions compared to HD conditions.

- 5 We are therefore considering using different approaches on the basis on CCL19 antagonism to correct the negative effect observed in the “LOW” group.

Materials and Methods

Study design

- The aim of the study was to define the molecular and cellular elements leading to a proper remyelination in a humanized context. All patients fulfilled diagnostic criteria for MS, and individuals (MS patients and HD) with any other inflammatory or neurological disorders were excluded from the study. The number of individual patients or independent replicate is indicated in all Fig. legend. Data were analyzed in a blinded fashion (third party concealment). All statistics were performed under the supervision of the biostatistics platform. Alpha < 0.05 was considered as statistically significant.
- 10
15

Multiple sclerosis patients and HD recruitment

- Collection of blood and cells for the study was approved by the French Ethics committee and the French ministry of research (DC-2012-1535 and AC-2012-1536). Written informed consent was obtained from all study participants. All patients fulfilled diagnostic criteria for MS, and individuals (MS patients and HD) with any other inflammatory or neurological disorders were excluded from the study.
- 20

Mice

- Nude (RjOrl:NMRI-Foxn1nu/Foxn1nu) and wild type (C57BL/6JR) mice were purchased from Janvier (France). All animal protocols were performed in accordance with the guidelines published in the National Institute of Health Guide for the Care and Use of Laboratory Animals, EU regulations (agreement n° A75-1319) and the local “Charles Darwin” ethics committee.
- 25

Collection of patients and healthy donors PBMCs

Thirty mL of blood was collected from 26 multiple sclerosis patients (from the BRC-REFGENSEP cohort) and 12 HD (from the French Blood Organization EFS) in ACD tubes. Peripheral blood mononuclear cells (PBMCs) were purified through centrifugation
5 (2200 rpm, 20min) on a Ficoll gradient and several washing in 10% FCS RPMI. Cells were resuspended at 2×10^6 cells/mL in RPMI 10% FCS and activated using anti-CD2/anti-CD3/anti-CD28 antibodies conjugated to beads (Miltenyi, France) at a ratio of 1 bead per 2 cells in 24-well plates (TPP). After 72h, cells were collected for grafting and supernatants were harvested and used for MIG culture experiments and
10 cytokines/chemokines analysis by Luminex®.

Peripheral Blood Mononuclear Cells (PBMCs) culture

PBMCs (2×10^6 /ml) were activated in RPMI 10% FCS using anti-CD2/anti-CD3/anti-CD28 antibodies conjugated to beads (Miltenyi, France) at a ratio of 1 bead per 2 cells in 24-well plates (TPP). After 72h, cells were collected and the concentration adjusted to
15 10^8 cells per mL for the graft experiment. LT supernatants were harvested and used for MIG culture experiments and cytokines/chemokines analysis by Luminex®.

Flow cytometry

To test human LT survival after grafting, 50-200 μ L of blood were sampled retro-orbitally 8-12 days after surgery. Cells were fixed with 1-step Fix/Lyse solution (Affymetrix) for
20 15min, washed, resuspended in FACS buffer and stained with antibodies (Supplementary table 1). The percentages of hCD4+ and HLA+ cells were identified by forward and side scatter and calculated on total leucocytes.

For LT characterization, PBMCs were activated with 50 ng/ml Phorbol 12-myristate 13-acetate (Sigma-Aldrich), 500 ng/ml ionomycin (Sigma-Aldrich), and GolgiPlug
25 (1μ g/ 1×10^6 cells; BD Biosciences) during 4h. Cells were then washed in staining buffer (PBS 1% FCS and 0.1% sodium azide) and stained with antibodies (supplementary table 1).

Data were acquired on a FACSVerse (BD Biosciences) and analyzed using FlowJo software (Tree Star).

Secretion profile measurement

The Luminex®-based multiplexed immunoassays with fluorescent microspheres
5 Milliplex Map assays (HCYTMAG-60K-PX41, HCYP3MAG-63K-11 and HCP2MAG-62K-PX23, Merck Millipore) were used to measure 72 cytokines/chemokines (5 were not detectable).

Lysophosphatidylcholine injection and PBMC graft

Eight weeks old nude mice were anesthetized with a solution of Ketamine (100 mg/mL)
10 and Xylazine (10 mg/mL). Demyelination was performed by stereotaxic injection of 1 µL of lysophosphatidylcholine (1%; LPC, Sigma) in the dorsal horn of the spinal cord. After 48h, PBMCs from MS patients or HD (10^5 cells in 1 µL of XVIVO 15 medium (Lonza) were engrafted within the lesion site previously marked by charcoal.

Perfusion and tissue processing

15 Grafted mice were euthanized by over-anesthesia of Ketamine/Xylazine and perfused intracardially with a solution of PBS 4% PFA for immunohistochemistry (IHC) and 4% PFA, 5% glutaraldehyde for electron microscopy (EM). Spinal cords were removed and post-fixed 1 hour. For IHC, tissues were cryoprotected overnight in a 20% sucrose solution; frozen in cooled isopentane and cryosected longitudinally (12 µm slices). For
20 EM, spinal cords were cut into 1mm sections and contrasted first with 2% osmium tetroxide and with 5% uranyl acetate before several steps of dehydration. Spinal cord sections were embedded in Epon medium before being cut into semithin and ultrathin sections for analysis.

MIG and OPC culture

25 MIG and OPCs were obtained using mixed cultures of glial cells. Hemispheres from C57BL6 P0/P1 newborn mice were isolated, mechanically dissociated and put in culture in Poly Ornithine (Sigma) coated flasks (TPP) in DMEM supplemented with 10% of

horse serum and 10% FCS. After 12-14 days, MIG were collected in the floating fraction of the culture after 2h of shaking at 100 rpm. OPCs were collected following a shaking period of 18h at 250 rpm. OPCs were purified after 2 differential adhesions on low adhesion petri dishes (TPP).

5 *MIG pre-treatment, OPCs proliferation and differentiation Test*

MIG were plated on 4 well dishes (Gibco) at 10^5 cells per well or in 96 well plates (TPP) at 4×10^4 cells per well in DMEM/F12 media with N2 (1%), B27 (0.5%), insulin (25 μ g/mL), glucose (6 mg/mL), and supplemented with 2.5% FCS (differentiation test) or not (proliferation test). After adhesion, MIG were pre-treated during 24h with LT
10 supernatant from multiple sclerosis patients or HD. The MIG conditioned media was harvested and cells fixed using 4% PFA. OPCs were plated in 4 well dishes or 96 well plates (respectively 10^5 and 4×10^4 cells per well) coated with poly-lysine. After adhesion, MIG conditioned media was added to OPCs culture. After 24h (proliferation test) or 72h (differentiation test), cells were fixed using 4% PFA. To test the influence of
15 CCL19, recombinant human CCL19 (1 ng/mL, R&D systems) was added to LT supernatant.

Immunocytochemistry and immunohistochemistry

Cells and tissues were permeabilized, saturated and stained with antibodies described in **table 3**. All primary antibodies were incubated overnight at 4°C, secondary antibodies
20 45min at room temperature. Nuclei were counterstained with Hoechst (Sigma, 33342).

	Antibody	Fluoro- chrome	Target	Isotype	BPS	Source (reference or clone)
Flow cytometry: multiple sclerosis and HD LT characterization	hCD4	Cy7	CD4 T cells	Mouse IgG1	(1)	Biolegend (RPA-T4)
	CD19	APC	B cells	Mouse IgG1	(1)	BD Biosciences (HIB19)
	CD27	PE	mature T cells, activated B cells and NK cell	Mouse IgG1	(1)	BD Biosciences (M-T271)
	CD25	FITC	Activated T and B cells	Mouse IgG1	(1)	BD Biosciences (M-A251)
	HLA-DR	PE	B cells, activated T cells, Antigen presenting cells	Mouse IgG2b	(1)	BD Biosciences (TU36)
	CCR6	PercpCy5 .5	B cells, memory T cells, immature DC	Mouse IgG1	(1)	BD Biosciences (11A9)
	CCR4	FITC	Th2 cells and DC	Mouse IgG2b	(1)	R&D systems (FAB1567)
	CXCR3	Pecy7	Activate T cells	Mouse IgG1	(1)	BD Biosciences (1C6)
	CD127	PercpCy5 .5	T and B-cells progenitors, mature T cells	Mouse IgG1	(1)	BD Biosciences (HIL7RM21)
	IL-17A	PE	Th17 cells	Mouse IgG1	(2)	Biolegend (BL168)
	IFN γ	APC	Th1 cells	Mouse IgG1	(2)	Biolegend (B27)
	FoxP3	Alexa fluor 647	Treg cells	Mouse IgG1	(2)	BD Biosciences (259D/C7)
Flow cytometry: Human LT survival after graft	hCD4	APCcy7	CD4 T cells	Rat IgG2b	(1)	Biolegend (GK1.5)
	HLA	V500	HLA cells	Mouse IgG2a	(1)	Biolegend (W6/32)
Immunocytoche mistry and Immunohistoche mistry	Iba-1	None	Microglia	Rabbit IgG	(3)	Wako (019- 19741)
	IGF-1	None	M2 Microglia	Goat	(3)	Santa Cruz Biotech (sc- 18355)
	iNOS	None	M1 Microglia	IgG2a	(3)	BD biosciences (610330)
	O4	None	Immature Oligodendrocyt es	IgM	(3)	Homemade
	GalC	None	Pre- Oligodendrocyt es	IgG3	(3)	Homemade
	CNPase	None	Mature Oligodendrocyt es	IgG1	(3)	Sigma (C5922)
	APC/CC1	None	Mature Oligodendrocyt es	IgG1	(3)	Millipore (OP80)

	Olig2	None	Oligodendrocyte lineage	Rabbit IgG	(3)	Millipore (AB9610)
	Ki67	None	Proliferating cells	IgG1	(3)	BD biosciences (556003)
	GFAP	None	Astrocytes	Rabbit IgG	(3)	DAKO (Z0334)

Table 3. Blocking / Permeabilisation solution (BPS): (1) Fc Block, (2) Fc Block + Cytofix/Cytoperm (BD Biosciences), (3) PBS BSA 4% Triton X-100 0.1% (Sigma).

Electron microscopy

- 5 Mice were perfused intracardially with a solution of 4% PFA, 5% glutaraldehyde (Electron Microscopy Science) in 0.1M phosphate buffer. Spinal cord were extracted and post fixed 1h before being cut into 1mm sections. Tissues were contrasted with 2% osmium tetroxide then 5% uranyl acetate before several steps of dehydration. Spinal cord sections were embedded in epon before being cut into semithin and ultrathin sections for analysis. Ultrathin sections representative of the different layers of the lesion were viewed using a Philips CM 120-Bio-tween electron microscope.

Image acquisition

- Pictures were taken using fluoromicroscopes DMRB (Leica) and ApoTome.2 (Zeiss), plate scanner CellInsight CX5 (Thermoscientific) or slide scanners Axioscan.Z1 (Zeiss) and NanoZoomer-XR Digital slide scanner C12000 (Hamamatsu) and converted to TIFF for quantification when needed.

Photographic images quantifications

- In vitro:* To assess MIG polarization, the proportion of Arg-1⁺, iNOS⁺ and IGF-1⁺ cells on the total number of cells was evaluated using image J software. The same quantification method was used for the evaluation of O4⁺, GalC⁺ and CNPase⁺ cells on the total number of cells. Proliferative Olig-2⁺/Ki67⁺ oligodendroglial cells were quantified automatically using the cellinsight system (Thermoscientific).

In vivo: the remyelination efficacy was assessed by evaluating P0⁺ area within the center of the lesion using morphostrider. The number of Olig-2⁺ and Olig-2⁺/CC1⁺ *in vivo* was

quantified using image J software.

Statistical analysis

Errors bars on graphs represent mean $\pm$ SEM. Statistical tests were run by Prism GraphPad software (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). For analysis of 2 groups, an unpaired 2-tailed student *t*-test was performed. For more than two groups analysis, a one-way ANOVA test was performed. These parametric tests were used after checking the normal distribution of the data (Kolmogorov-Smirnov test).

Multivariate analysis

10 All the coding necessary for the multivariate analysis has been run with R software.

BADA

BADA is a robust form of discriminant analysis which explicitly looks for (linear) combination of variables called factors or dimensions that best discriminate between a-priori defined groups of observations. These factors can be used to draw maps that show the optimum separation of the groups of observations represented by points on these maps (and the observations). The variance of each dimension corresponds to a portion of the original between group variance and therefore the quality of a component is often expressed by the proportion of the variance it extracts from the total between group variance. BADA evaluates the quality of the group separations by using a non-parametric Bootstrap resampling scheme to derive confidence intervals that can draw around the points representing the groups on the factor maps. Before applying BADA, a ranking procedure was applied on the cytokine data block in order to take into account the fact (due to technical limitation) that there were in the data some 0s corresponding to undetectable levels of cytokine secretion.

25 *Partial least squares (PLS)*

To assess the contributions of each block of data to the remyelination process, we applied a Hierarchical PLS with a design specified on Figure 5. Hierarchical PLS consists in

applying PLS regression simultaneously to several blocks of data in order to evaluate their relative link to a given outcome (here, the % of CNPase⁺ cells). Therefore, it benefits from the properties of PLS itself, which is able to cope with high dimensional and highly correlated data.

- 5 Network inference was performed by computing partial correlations and assessing their significance with GeneNet, which is particularly adapted to high dimensional data.

CLAIMS

1. An *in vitro* method for the prognostic of a subject affected by a demyelinating disease comprising detecting and quantifying the level of at least one of CCL19,
5 IL-15, ENA78 and/or TRAIL in a biological fluid from the subject.
2. The *in vitro* method according to claim 1, further comprising detecting and quantifying the level of at least one of LIF and SDF-1.
3. The *in vitro* method according to claim 1 or 2, wherein the method is for classifying the subject in a group having a low remyelination profile or in a group having a
10 high remyelination profile.
4. The *in vitro* method according to any one of claims 1 to 3, wherein the measured levels of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL is/are compared to reference values, preferably said reference values correspond respectively to the median levels of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL in a group of
15 patients with a demyelinating disease.
5. A composition for use in the treatment of a demyelinating disease comprising at least one modulator of CCL19, IL-15, ENA78 and/or TRAIL.
6. The composition for use according to claim 5, further comprising at least one modulator of LIF and/or SDF-1.
- 20 7. The composition for use according to any one of claims 5 to 6, wherein said modulator is an antibody or fragment or mimetic thereof, an aptamer, a small molecule, a peptide mimetic, a siRNA, an asRNA, an antagonist, an agonist or an inverse agonist.
8. The composition for use according to any one of claims 5 to 7, comprising at least
25 one inhibitor of CCL19, ENA78 and/or SDF-1 and/or at least one activator of IL-15, LIF and/or TRAIL.

9. The composition for use according to any one of claims 5 to 7, comprising at least one antagonist or at least one inverse agonist of CCR7, CXCR2 and/or CXCR4 and/or at least one agonist of IL-15R, LIFR, TRAIL-RI and/or TRAIL-RII.
- 5 10. The composition for use according to any one of claims 5 to 9, wherein the demyelinating disease is selected from the group comprising multiple sclerosis, inflammatory demyelinating disorders, acute disseminated encephalomyelitis, transverse myelitis, acute transverse myelitis, Guillain-Barre syndrome, brainstem encephalitis, optic neuritis, neuromyelitis optica, leukodystrophy, adrenoleukodystrophy, adrenomyeloma neuropathy, idiopathic inflammatory
- 10 demyelinating disease, central pontine myelinolysis, optic neuritis, aquaporin 4 antibody-negative neuromyelitis optica, progressive multifocal leukoencephalopathy, periventricular leukomalacia, Vitamin B12 deficiency, Wernicke's encephalopathy, osmotic demyelination syndrome, Leigh's disease.
- 15 11. The composition for use according to any one of claims 5 to 10, wherein said modulator is to be administered in a subject having a low remyelination profile.

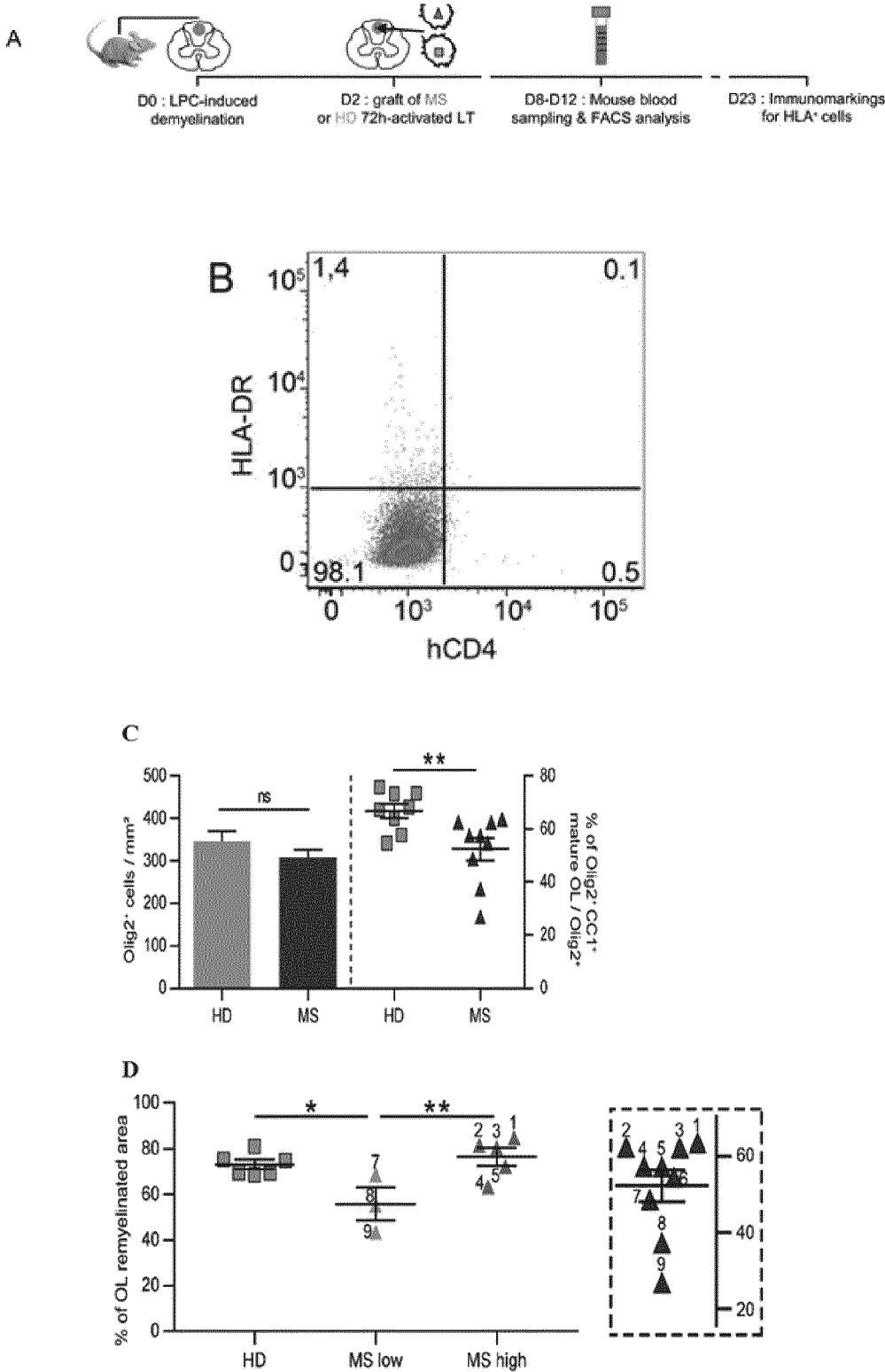


FIG. 1

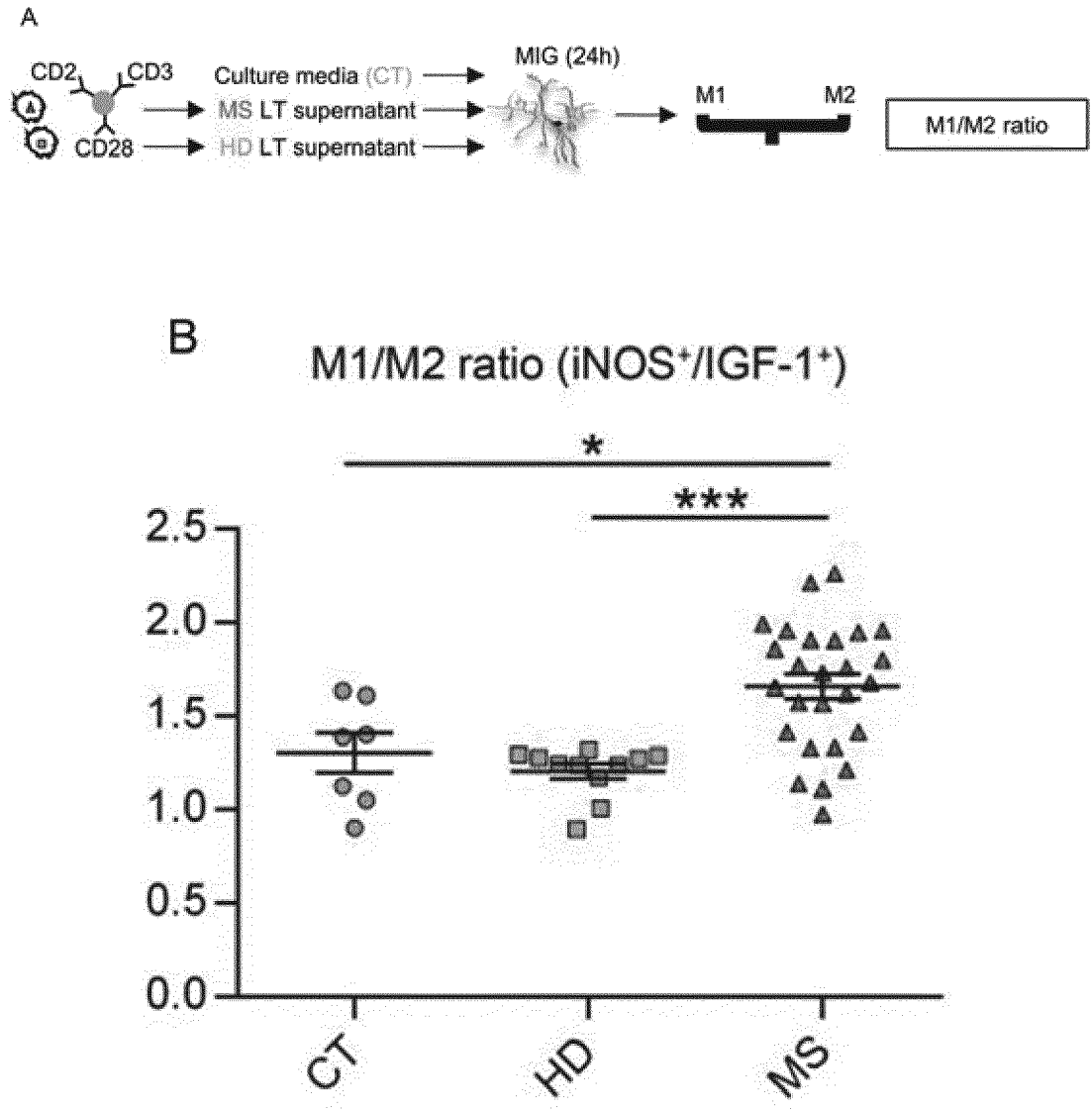


FIG. 2

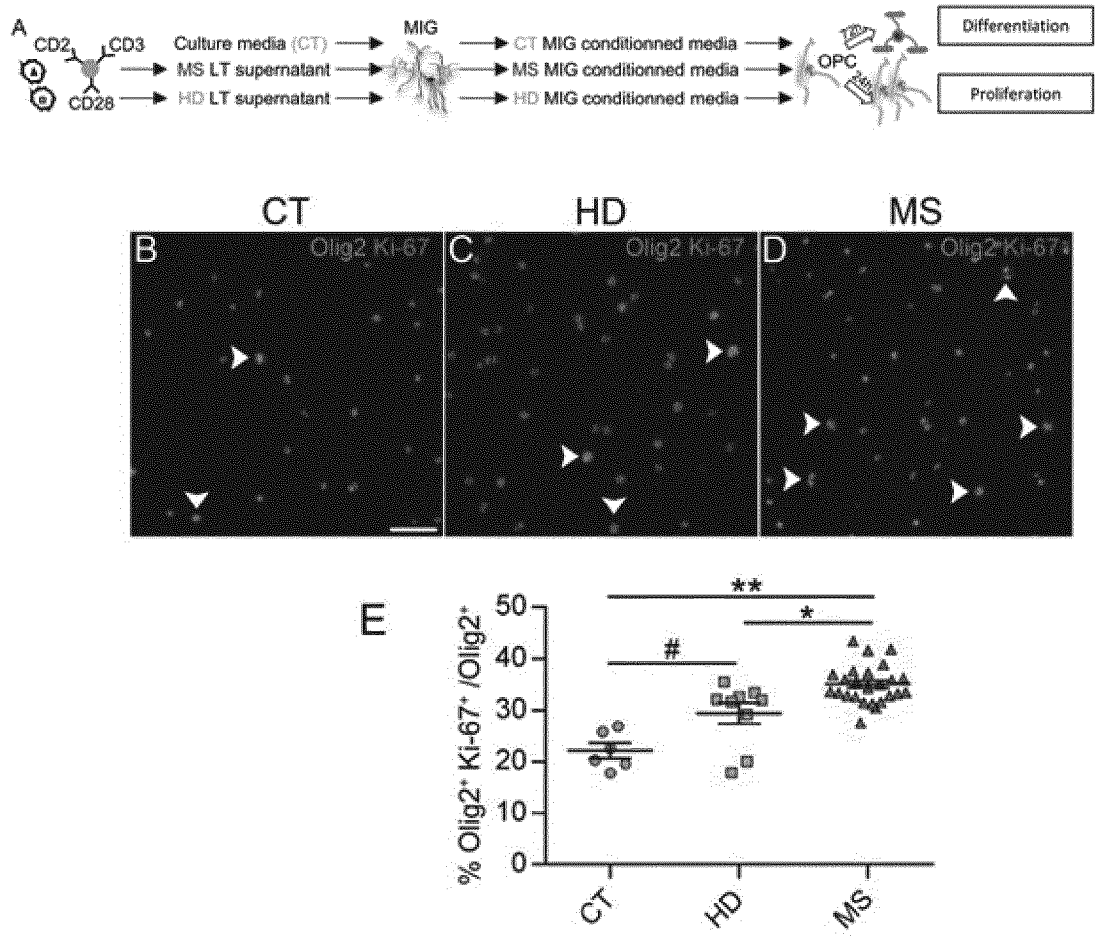


FIG. 3 (A-E)

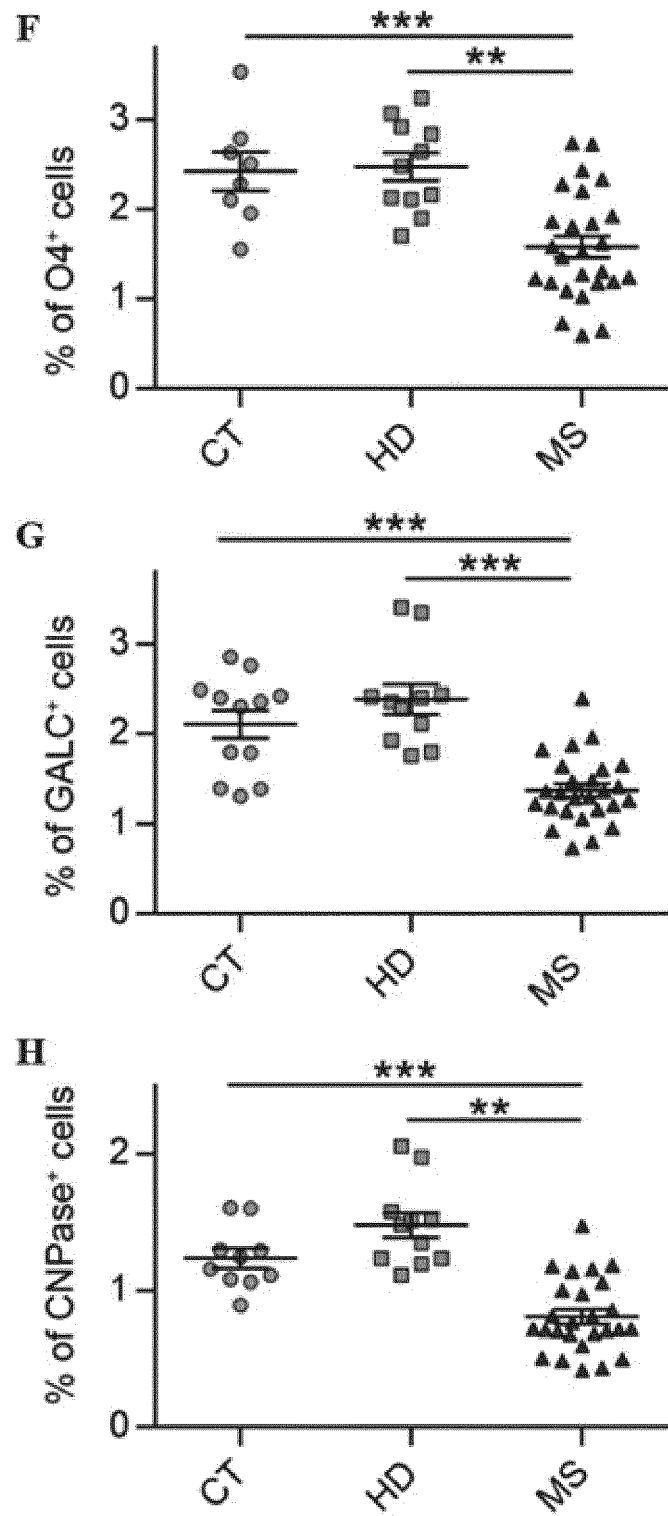


FIG. 3 (F-H)

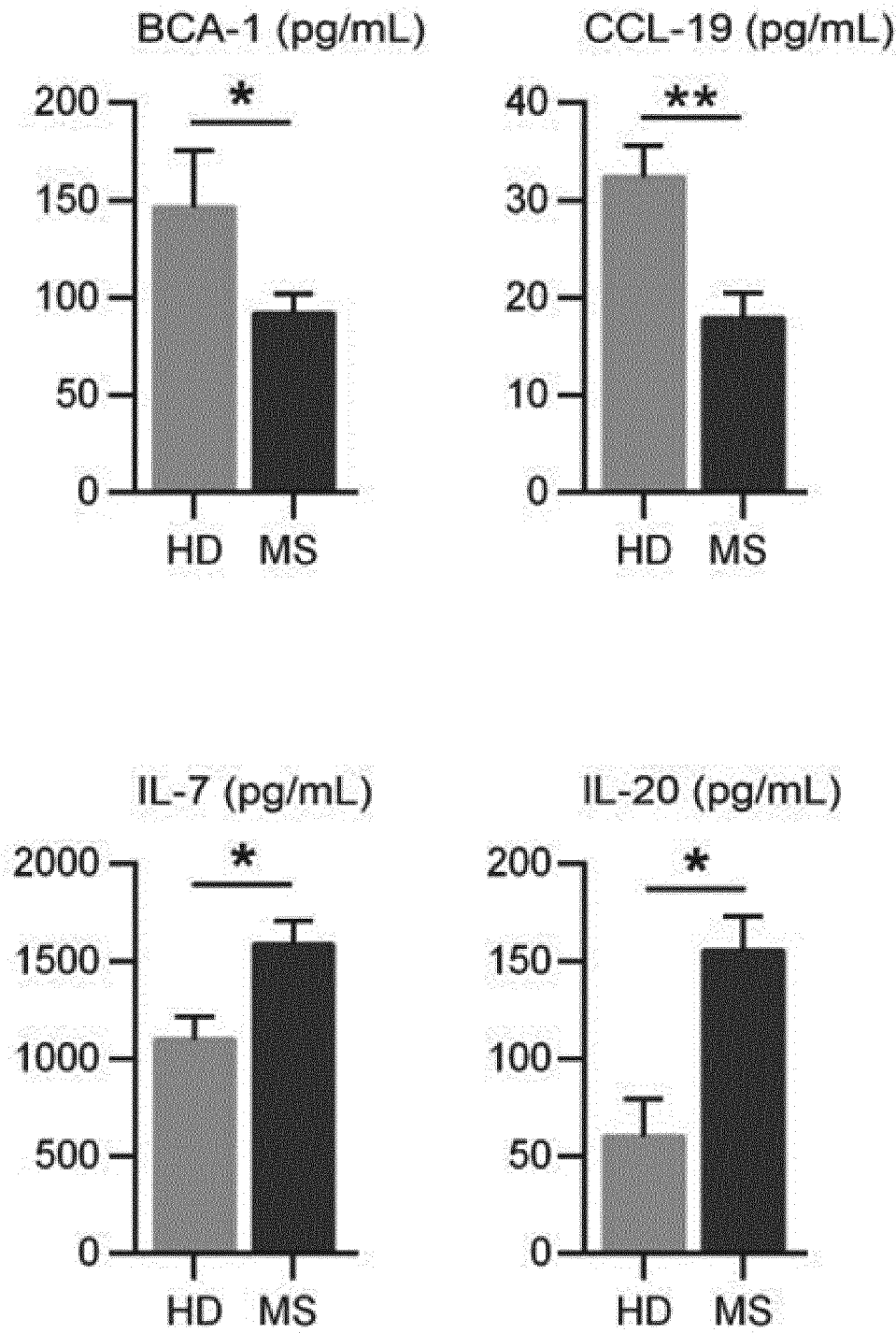


FIG. 4

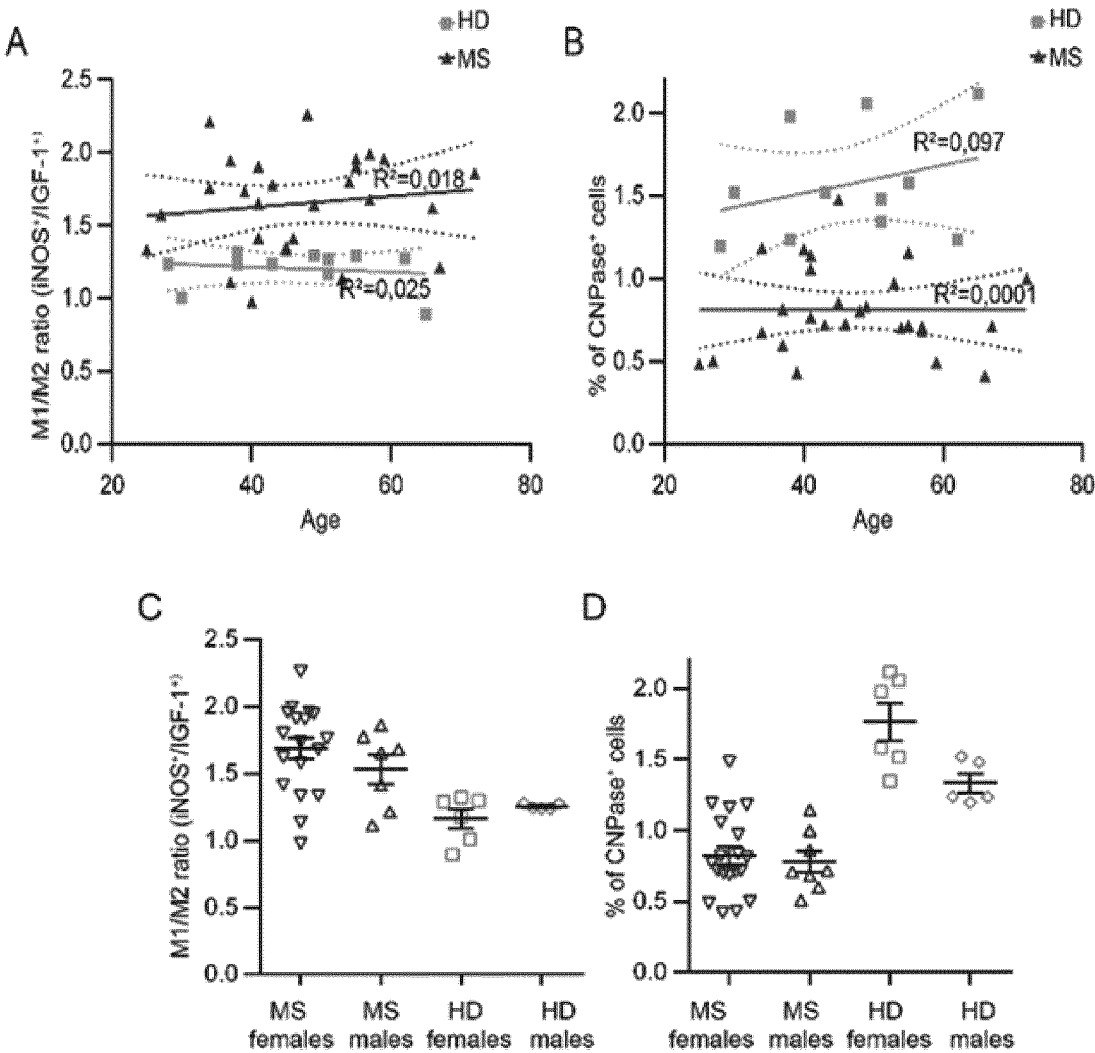


FIG. 5 (A-D)

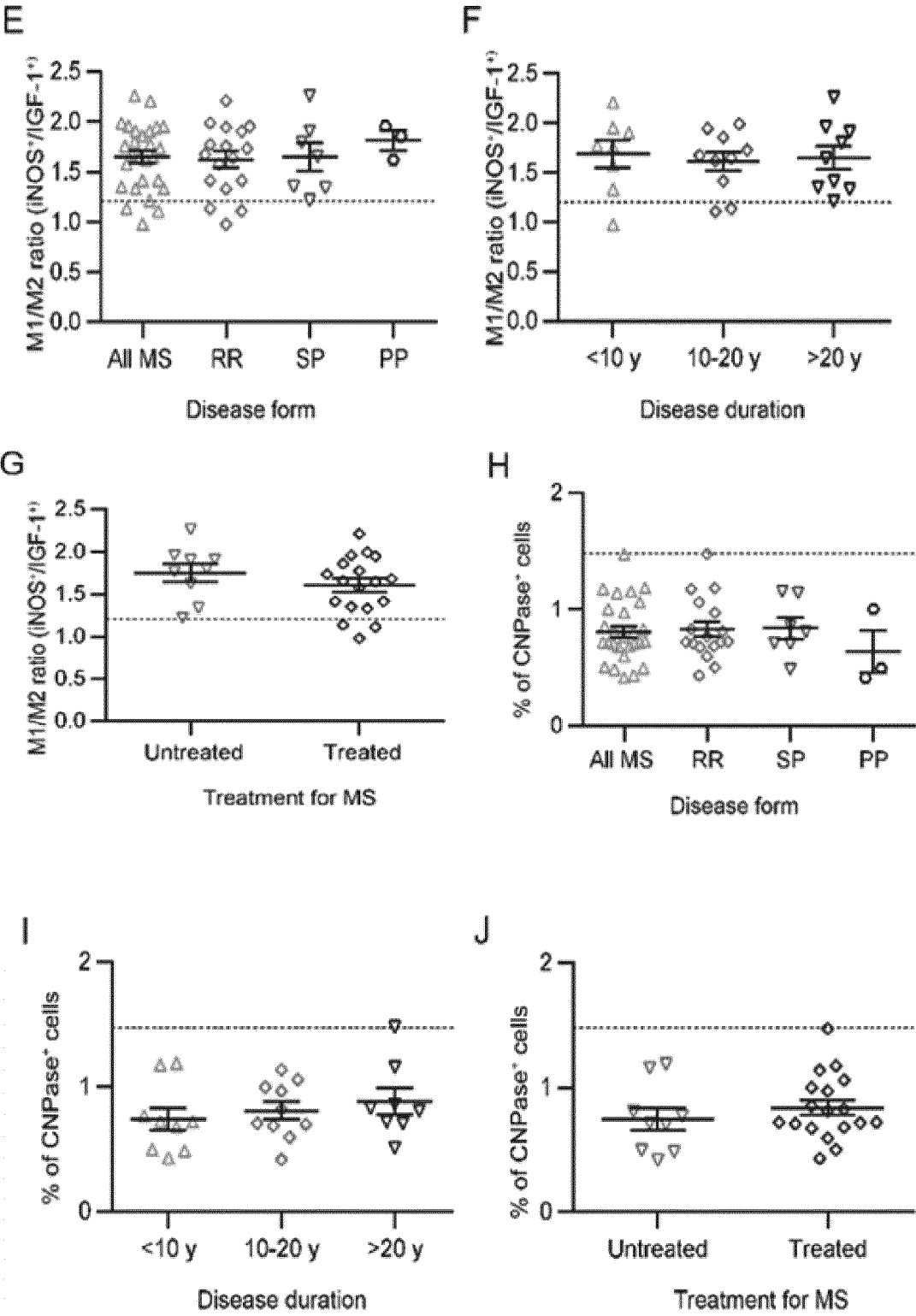


FIG. 5 (E-J)

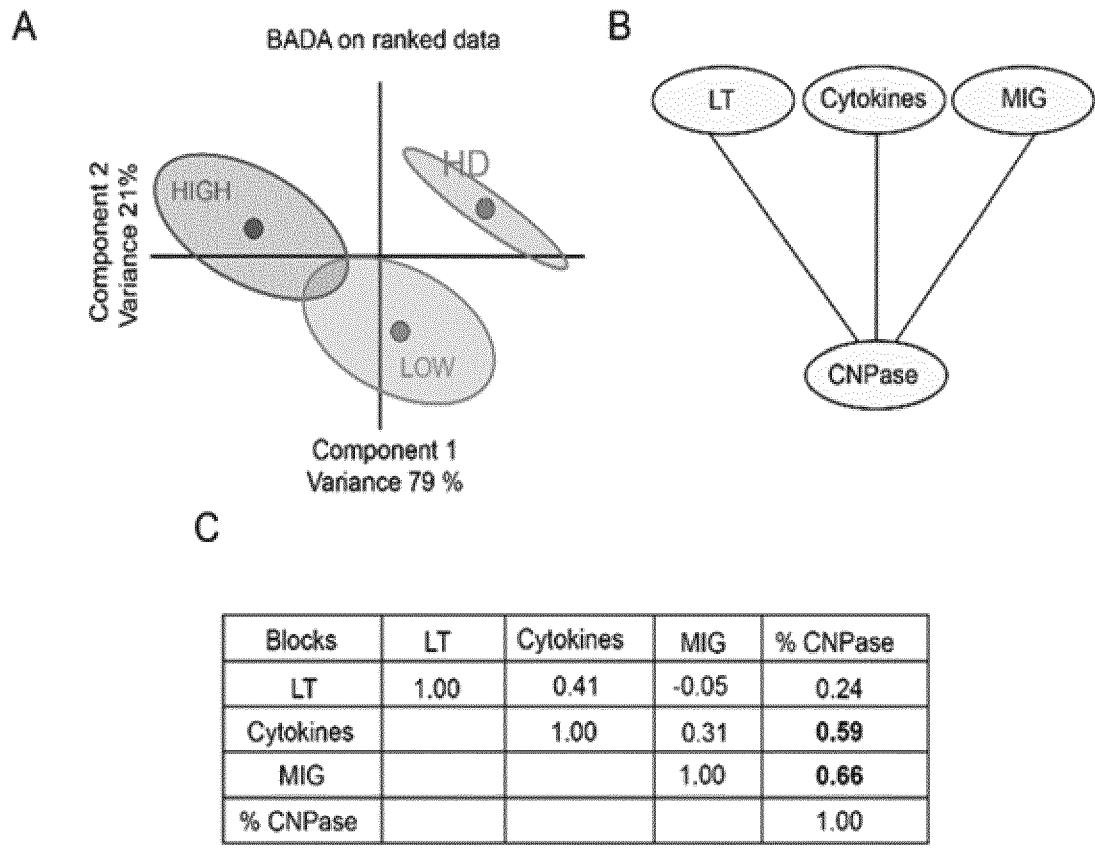


FIG. 6 (A-C)

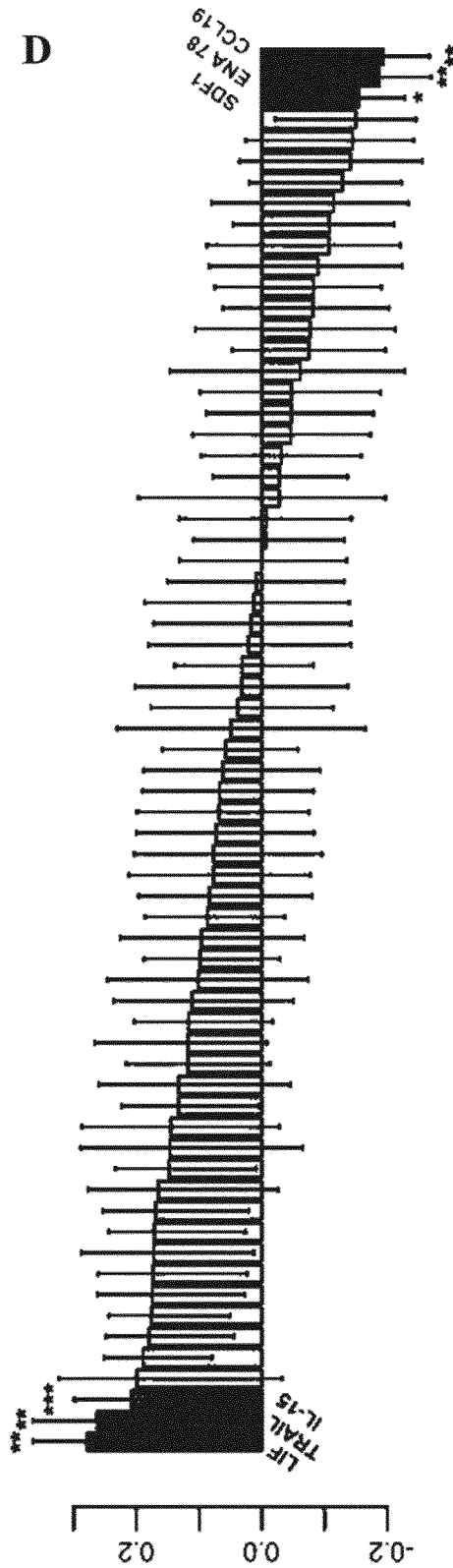


FIG. 6 (D)

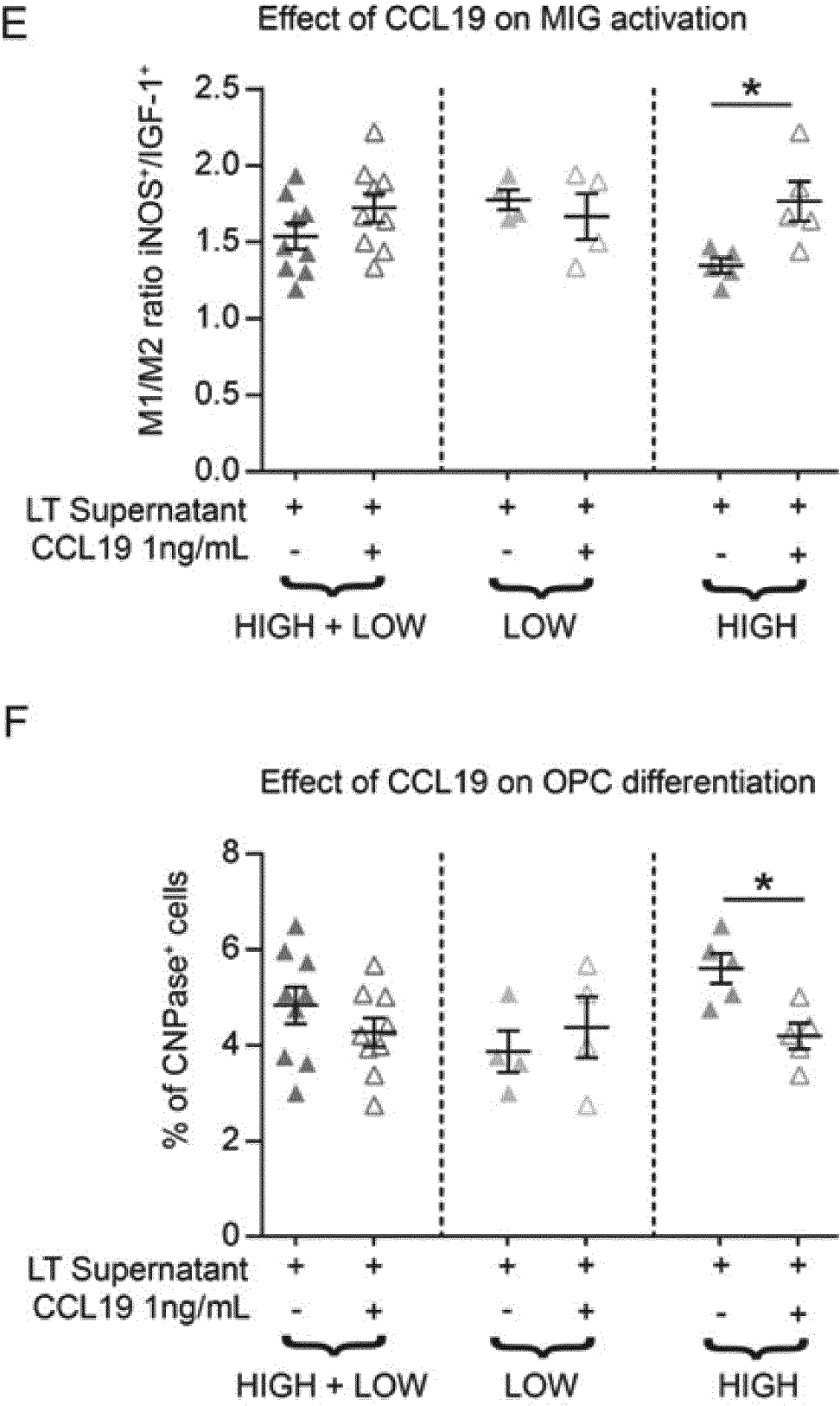


FIG. 6 (E-F)

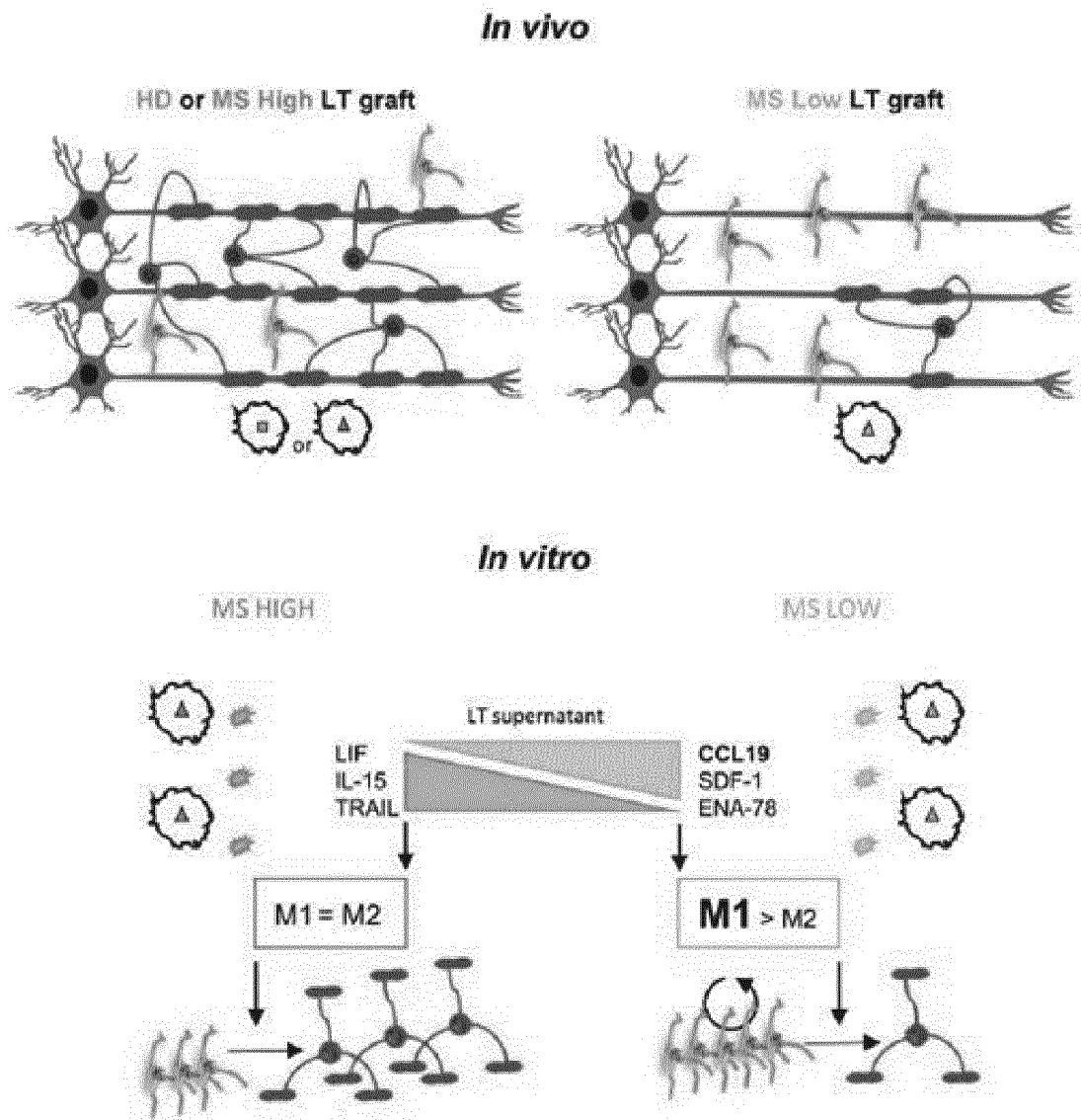


FIG. 7

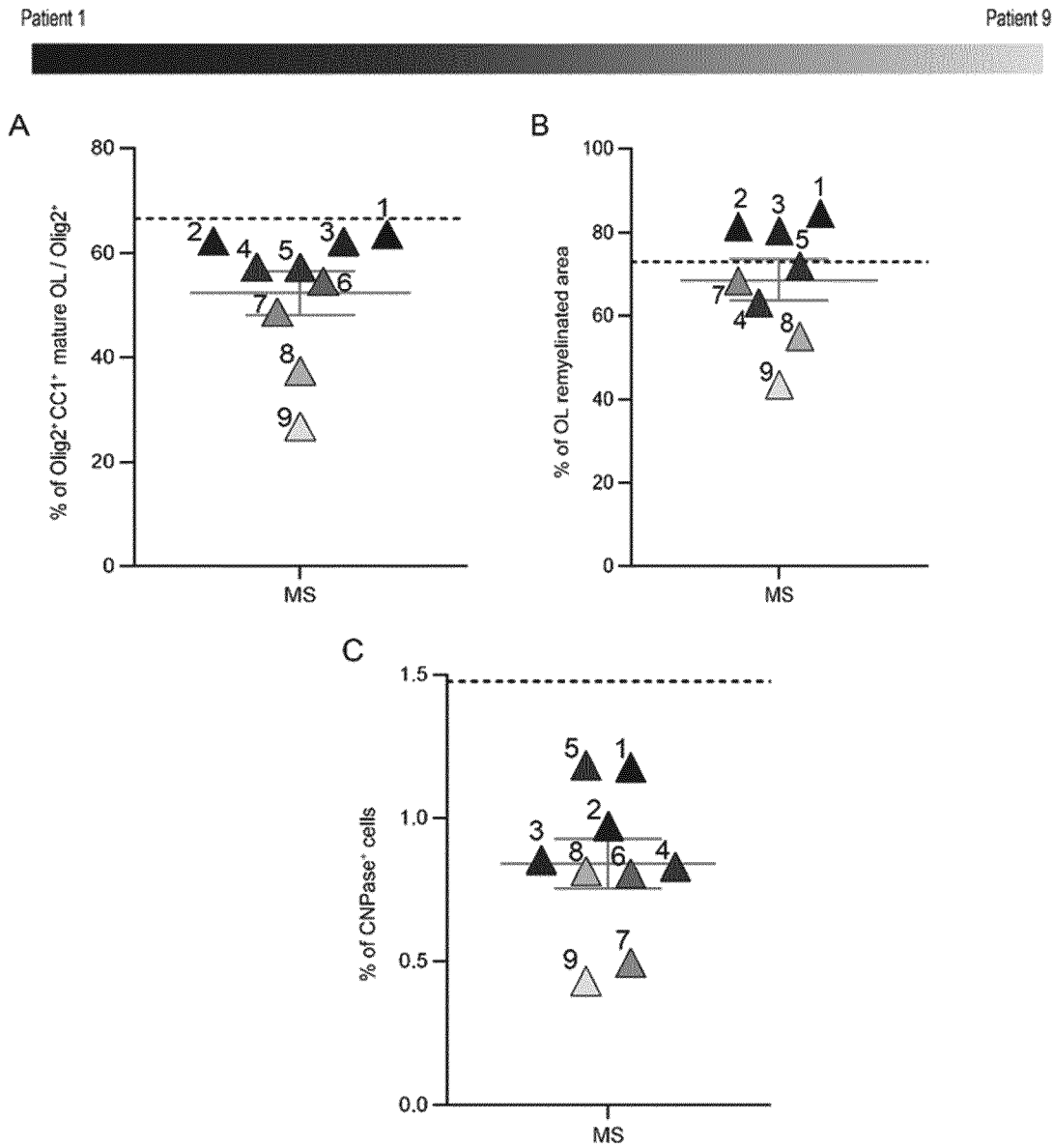
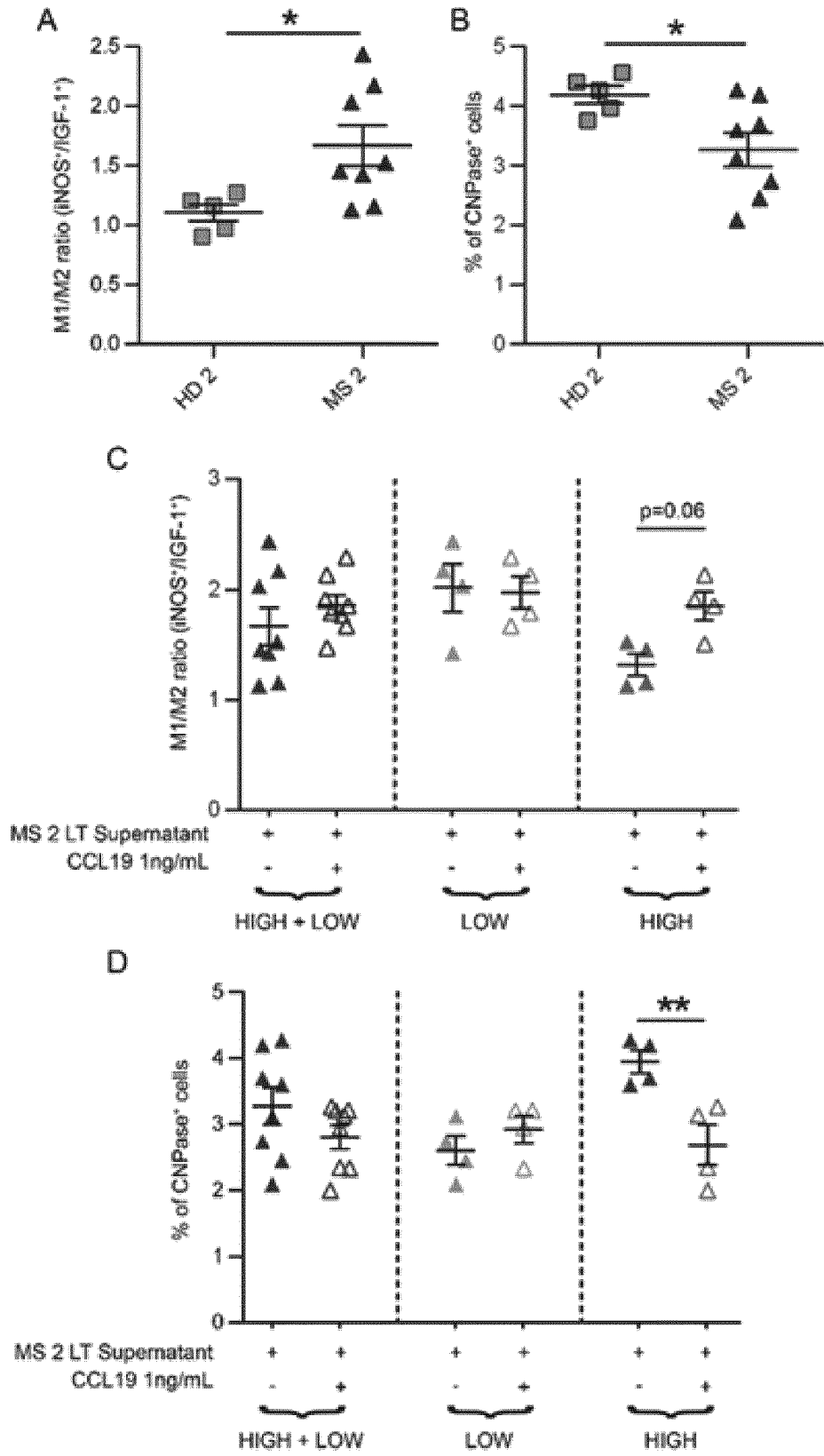


FIG. 8



INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/076474

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/68 A61K39/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU 2013 276 991 A1 (BAXTER INTERNATIONAL INC.) 23 January 2014 (2014-01-23)	1,3-5,
Y	abstract paragraphs [0008] - [0016], [0091] - [0092], [0164] claims 1-12,22-23	7-11 2,6
Y	----- US 2003/162700 A1 (SHIPHAM K.A.-M. [AU] ET AL.) 28 August 2003 (2003-08-28) abstract paragraphs [0053], [0054], [0068], [0069] example 12 claims 1-7,33-38 ----- -/-	6



Further documents are listed in the continuation of Box C.



See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search

8 December 2017

Date of mailing of the international search report

21/12/2017

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Authorized officer

Giry, Murielle

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/076474

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEN T. ET AL.: "Changes of CXCL12, CXCL14 and PDGF levels in the brain of patients with idiopathic demyelinating optic neuritis and neuromyelitis optica", J. NEUROIMMUNOL., vol. 279, 2015, pages 1-6, XP029138231, abstract page 2, column 1 page 4, column 2 page 5, column 2, last paragraph	2,6
Y	PATEL J.R. ET AL.: "CXCR4 promotes differentiation of oligodendrocyte progenitors and remyelination", PROC. NATL. ACAD. SCI. USA, vol. 107, no. 24, 15 June 2010 (2010-06-15), pages 11062-11067, XP055344626, abstract	6
A	HARRIS V.K. ET AL.: "Biomarkers of therapeutic response in multiple sclerosis: Current status", MOL. DIAGN. THER., vol. 18, 28 August 2014 (2014-08-28), pages 605-617, XP055153122, abstract paragraph [02.4]	1-11
A	OLSEN J.A. ET AL.: "Remyelination in multiple sclerosis: Cellular mechanisms and novel therapeutic approaches", J. NEUROSCI. RES., vol. 93, no. 5, 6 October 2014 (2014-10-06), pages 687-696, XP055344323, the whole document	1-11
X,P	EL BEHI M. ET AL.: "Adaptive human immunity drives remyelination in a mouse model of demyelination", BRAIN, vol. 140, no. 4, 22 February 2017 (2017-02-22), pages 967-980, XP55433649, the whole document	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/076474

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
AU 2013276991	A1	23-01-2014	
		AU 2013276991 A1	23-01-2014
		AU 2013276992 A1	23-01-2014
		AU 2013276993 A1	23-01-2014
		AU 2013276994 A1	23-01-2014

US 2003162700	A1	28-08-2003	
		CA 2401989 A1	07-09-2001
		EP 1267911 A1	02-01-2003
		US 2003162700 A1	28-08-2003
		US 2007212372 A1	13-09-2007
		WO 0164239 A1	07-09-2001
