

ANTI-CHEMOKINE LIKE RECEPTOR 1 HUMANIZED ANTIBODIES AND THEIR THERAPEUTIC APPLICATIONS

Field of the invention

5 [1] The invention pertains to the field of immunotherapy. The present invention provides new humanized anti-chemerin receptor antibodies which have a Resolvin E1-like agonist activity on chemokine like receptor-1 (CMKLR1). The present invention also provides uses of such antibody in therapy, in particular for treating autoimmune diseases and chronic inflammatory diseases, infectious diseases, cancers, and any condition wherein the
10 resolution phase of inflammation is disrupted or delayed.

Background of the invention

[2] The critical role of inflammatory processes in health and diseases has long been recognized. The detailed molecular mechanisms and biological events that regulate the
15 progression and the resolution of inflammation remain of critical interest. Recent investigations have provided strong evidence that the resolution of inflammation is not a passive process, as believed earlier. Resolution of the inflammation is instead a biosynthetically active process, regulated by biochemical mediators and receptors-signaling pathways. Resolution is therefore driven by specialized pro-resolving mediators.
20 Inflammation is a spontaneous mechanism that occurs during an infection, an injury or a traumatism. Inflammation is inevitable and usually salutary, and its response is orchestrated by a delicate balance between positive and negative feedback loops. The inflammation is usually divided in 3 steps: initiation, amplification and resolution.

[3] The resolution process which allows the ending of the inflammatory response is a complex
25 process involving the sequential and chronological engagement of cellular (e.g. granulocytes or macrophages) and chemical (e.g. cytokines or specialized pro-solving mediators or factors) effectors.

[4] Chemokine-like receptor 1 (CMKLR1), also known as ChemR23, and chemokine receptor-like 2 (CCRL2) are 7-transmembrane receptors identified by their homology to known G-protein-coupled receptors (AJ Kennedy and AP Davenport, 2018). The Chemokine-like
30 Receptor 1 (CMKLR1; also named Dez in Murine animals), is an orphan G protein-coupled receptor related to GPR-1 (38% overall amino acid identity), C3a receptor (38%), C5a anaphylatoxin receptor (36%) and formyl Met-Leu-Phe receptors (35%). ChemR23 is more distantly related to the chemokine receptors subfamily (Samson et al., 1998). CMKLR1 is
35 expressed on monocytes, on macrophages, on dendritic cells, and on NK cells, as well as on adipocytes and endothelial cells. Recent studies identified ligands for these receptors

[10] Further, as will be explained later in the present application, several very advantageous biological effects have been reached, associated in particular to the apoptosis of neutrophils and to the reduction of migration and/or transmigration of neutrophils and/or macrophages notably, leading to strong benefic action in the resolution of inflammation.

5 The new compounds allow thus to combine the production capacity and the biological activity.

[11] Accordingly, in a first example of the disclosure, it is disclosed an antibody or antigen-binding fragment thereof, which binds to Chemokine-like receptor 1 (CMKLR1), in particular human CMKLR1, comprising:

- 10 a) an antibody heavy chain variable (VH) domain comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3, wherein:
- VHCDR1 is selected from the group consisting of SEQ ID No.3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7;
 - VHCDR2 is selected from the group consisting of SEQ ID No. 9, SEQ ID No. 10, 15 SEQ ID No. 11, SEQ ID No. 12 and SEQ ID No. 61;
 - VHCDR3 is selected from the group consisting of SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15 and SEQ ID No. 16;
- b) an antibody light chain variable (VL) domain comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3, wherein:
- 20 - VLCDR1 is selected from the group consisting of SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 20, SEQ ID No. 21, SEQ ID No. 22 and SEQ ID No. 23;
- VLCDR2 is selected from the group consisting of SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, 25 SEQ ID No. 31, SEQ ID No. 32 and SEQ ID No. 33;
- VLCDR3 is selected from the group consisting of SEQ ID No. 34, SEQ ID No. 35 and SEQ ID No. 36.

In a first aspect of the disclosure, it is disclosed an anti- Chemerin Like Receptor 1 (CMKLR1) antibody or antigen-binding fragment thereof which binds to CMKLR1, in particular human CMKLR1, said antibody or antigen-binding fragment thereof comprising:

30 a. a heavy chain variable (VH) domain comprising SEQ ID NO: 91 and a light chain variable (VL) domain comprising SEQ ID NO: 93; or

b. a VH domain comprising SEQ ID NO: 91 and a VL domain comprising SEQ ID NO: 35 55.

In another aspect of the disclosure, it is disclosed the anti-CMKLR1 antibody or antigen-binding fragment thereof according to the disclosure for use in therapy.

5 In another aspect of the disclosure, it is disclosed a use of the anti-CMKLR1 antibody or antigen-binding fragment thereof according to the disclosure in the manufacture of a medicament for preventing or treating:

- 10 - an inflammatory disease, in particular acute inflammatory diseases, chronic inflammatory diseases such as chronic inflammatory pulmonary diseases, keratoconjunctivitis, periodontal disease, eczema, inflammatory bowel disease, in particular Crohn's disease or colitis, in particular ulcerative colitis or spontaneous colitis, cystic fibrosis, NASH (Nonalcoholic steatohepatitis), scleroderma, anti-neutrophil cytoplasm antibodies-related disease ANCA associated disease, in particular wherein, as a result of the administration of the treatment, the resolution of inflammation is enhanced;
- 15 - an autoimmune disease such as diabetes, in particular type I diabetes, psoriasis, lupus, rheumatoid arthritis, multiple sclerosis, Sjögren's syndrome, celiac disease, vasculitis, myasthenia gravis, or an infection disease such as sepsis, peritonitis, degenerative diseases, wound healing disorders or dry eye syndrome, in particular wherein, as a result of the administration of the treatment, the resolution of inflammation is enhanced;
- 20 - a cancer, in particular metastatic cancers, solid or liquid cancers such as carcinoma, more particularly hepatocarcinoma, in particular mammary carcinoma or colon carcinoma, colorectal cancer or lung cancer or mesothelioma or myeloid cancer such as leukemia, in particular a cancer wherein cancer cells express CMKLR1 or where the microenvironment of the tumor is invaded by cells expressing or overexpressing CMKLR1, in particular wherein, as a result of the administration of the treatment, the resolution of inflammation is enhanced.
- 25

30 In another aspect of the disclosure, it is disclosed a use of the anti-CMKLR1 antibody or antigen-binding fragment thereof according to the disclosure in the manufacture of a medicament for preventing or treating NASH (Nonalcoholic steatohepatitis), scleroderma, cystic fibrosis or anti-neutrophil cytoplasm antibodies-related disease (ANCA).

35 [12] In a particular example, said antibody or antigen-binding fragment thereof, which binds to Chemokine-like receptor 1 (CMKLR1), in particular human CMKLR1, comprises:

4b

- a) an antibody heavy chain variable (VH) domain comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3, wherein:
- VHCDR1 is selected from the group consisting of SEQ ID No.3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7;
 - 5 - VHCDR2 is selected from the group consisting of SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11 and SEQ 61; or VHCDR2 corresponds to the amino acid sequence of SEQ ID No. 12 or SEQ ID No. 63 with the proviso that VHCDR1 is not SEQ ID No. 3 or SEQ ID No. 4;

- VHCDR3 is selected from the group consisting of SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15 and SEQ ID No. 16;
- b) an antibody light chain variable (VL) domain comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3, wherein:
 - 5 - VLCDR1 is selected from the group consisting of SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 20, SEQ ID No. 21, SEQ ID No. 22 and SEQ ID No. 23;
 - VLCDR2 is selected from the group consisting of SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 31, SEQ ID No. 32 and SEQ ID No. 33;
 - 10 - VLCDR3 is selected from the group consisting of SEQ ID No. 34, SEQ ID No. 35 and SEQ ID No. 36.
- [13] In another particular example, said antibody or antigen-binding fragment thereof, which binds to Chemokine-like receptor 1 (CMKLR1), in particular human CMKLR1, comprises:
 - 15 a) an antibody heavy chain variable (VH) domain comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3, wherein:
 - VHCDR1 is selected from the group consisting of SEQ ID No.3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7;
 - VHCDR2 is selected from the group consisting of SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11 and SEQ No. 61,
 - 20 ○ when VHCDR1 is SEQ ID No. 3 or SEQ ID No. 4 and VHCDR2 corresponds to the amino sequence of SEQ ID NO: 12 said heavy chain variable (VH) domain does not comprise a framework VHFR3 of SEQ ID No. 70, preferably with the proviso that said heavy chain variable (VH) domain comprises a framework FR3 of SEQ ID NO: 69,
 - 25 - VHCDR3 is selected from the group consisting of SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15 and SEQ ID No. 16;
 - b) an antibody light chain variable (VL) domain comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3, wherein:
 - 30 - VLCDR1 is selected from the group consisting of SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 20, SEQ ID No. 21, SEQ ID No. 22 and SEQ ID No. 23;
 - VLCDR2 is selected from the group consisting of SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 31, SEQ ID No. 32 and SEQ ID No. 33;
 - 35 - VLCDR3 is selected from the group consisting of SEQ ID No. 34, SEQ ID No. 35 and SEQ ID No. 36.

- VLCDR3 is selected from the group consisting of SEQ ID No. 34, SEQ ID No. 35 and SEQ ID No. 36.

In particular advantageously, the antibody or antigen-binding fragment thereof binds specifically to the third extra-cellular loop (EL3) of CMKLR1, in particular an epitope located within the third extra-cellular loop (EL3) of CMKLR1; more particularly the antibody or antigen-binding fragment thereof binds specifically to a polypeptide comprising amino acid sequence SEQ ID No: 2 or SEQ ID No. 59 or to an epitope located within amino acid sequence SEQ ID No. 60.

[14] An antibody or an antigen-binding fragment thereof according to this example is suitable for production in different cell lines, including but not limited to mammalian cell lines, with a yield of production suitable for purposes of development of a drug candidate, while the specific binding capability to the extra cellular third loop of CMKLR1 and its Resolvin E1-like agonist capability on CMKLR1 are retained. The humanized antibodies disclosed herein may therefore be efficiently produced and share the functional capabilities of their parental antibody.

[15] In a particular example of the disclosure, which may be distinguished from the definition of the antibodies of the disclosure solely by their CDR domains, but which may be combined with such a definition in particular examples, it is disclosed an antibody or antigen-binding fragment thereof, which binds to Chemokine-like receptor 1 (CMKLR1), in particular human CMKLR1, suitable for the production in mammalian cells such as COS or CHO or HEK cells, in particular with a yield over 0.1mg/ml, in particular with a yield over 1 mg/ml, more particularly with a yield of at least 10 mg/ml, and again more particularly with a yield over 100 mg/ml wherein:

- a) the Variable Heavy (VH) domain comprises the amino acid sequence of the frameworks (FR1, FR2, FR3 and FR4) of a Heavy Chain variable domain wherein each framework harbors a sequence identity respectively with the framework of the same rank in the sequence of SEQ ID No. 41 that is 100 % for FR1, at least 60 % for FR2, at least 78 % for FR3 and at least 80 % for FR4; more particularly 100 % for FR1, at least 80 % for FR2, at least 85 % for FR3 and at least 90 % for FR4;
- b) the Variable Light (VL) domain comprises the amino acid sequence of the frameworks (FR1, FR2, FR3 and FR4) of a Light Chain Variable domain wherein each framework harbors a sequence identity respectively with the framework of the same rank in the sequence of SEQ ID No.50 that is at least 60 % for FR1, at least 70 % for FR2, at least 75 % for FR3 and at least 80 % for FR4, and more particularly of 100% for FR1, at least 90 % for FR2, at least 90% for FR3 and 100 % for FR4.

[22] Such antibody presents the same advantageous features as the antibodies defined according to the first example and the second example of the disclosure; *i.e.* high yield production, specific binding to an epitope located within the third extra loop of the chemerin-like Receptor 1, a Resolvin E1-like agonist capability.

5 [23] The disclosed antibodies are suitable for being used in the treatment of a condition wherein the resolution of the inflammation is delayed or disrupted. All antibodies described herein are suitable for inducing the resolution of the inflammation, and/or enhancing resolution of the inflammation, and/or initiating resolution of the inflammation.

[24] In the following disclosure, a humanized anti-CMKLR1 compound is considered as
10 being either a humanized anti-CMKLR1 antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic or modified antibody. In a particular example of the disclosure, said compound is defined by the sequences of its CDR. In a more particular example of the disclosure, the anti-CMKLR1 compound is an antibody defined by the sequences of its CDRs and its framework regions (FR). An anti-CMKLR1 compound is a
15 compound which binds specifically to the Chemokine-like receptor 1 (CMKLR1). In the following disclosure, the terms Chemokine-like receptor 1, CMKLR1 and ChemR23 are used interchangeably, and all designate the receptor encoded by gene *CMKLR1* in human or *cmklr1* in non-human animals. In a particular example of the disclosure, the anti-CMKLR1 compound binds specifically to human CMKLR1 or, in other words, the disclosure
20 relates to an anti-human CMKLR1 compound. As used herein, the term "CMKLR1" refers to a Chemokine-Like Receptor 1 protein (also designated as chemR23), a member of the G-protein coupled receptor family from a mammal species, preferably a human CMKLR1. A reference sequence of the human CMKLR1 protein, used in the examples of the present application, corresponds to the sequence associated to the Uniprot Accession number
25 Q99788 (SEQ ID No: 1).

[25] In a particular example, the disclosure relates to an anti-CMKLR1 antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic or modified antibody defined by at least one functional feature. In a preferred example, said anti-CMKLR1 compound is defined by its capability to inhibit secretion of pro-inflammatory cytokines, in particular IL12,
30 and/or its capability to enhance secretion of anti-inflammatory cytokines, in particular IL10. In a more particular example, the anti-CMKLR1 compound inhibits or enhances cytokine secretion by macrophages, in particular by pro-inflammatory macrophages and/or by pro-resolution of the inflammation macrophages. In a particular example, the anti-CMKLR1 compound of the disclosure enhances the polarization of macrophages into anti-inflammatory macrophages, in particular pro-resolution macrophages. In a particular
35 example, the anti-CMKLR1 compound of the disclosure enhances the apoptosis of neutrophils as compared to a control antibody.

[56] In a particular aspect of the invention, it is disclosed anti-Chemokine Like Receptor 1 (CMKLR1) antibody or an antigen-binding fragment thereof which binds to CMKLR1, in particular human CMKLR1, said antibody of antigen-binding fragment thereof comprising:

- 5 **a.** an antibody heavy chain variable (VH) domain comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3, wherein:
 - VHCDR1 is selected from the group consisting of SEQ ID No.3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7;
 - VHCDR2 is selected from the group consisting of SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12 and SEQ ID No. 61;
 - 10 - VHCDR3 is selected from the group consisting of SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15 and SEQ ID No. 16;
- b.** an antibody light chain variable (VL) domain comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3, wherein:
 - VLCDR1 is selected from the group consisting of SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 20, SEQ ID No. 21, SEQ ID No. 22 and SEQ ID No. 23;
 - 15 - VLCDR2 is selected from the group consisting of SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 31, SEQ ID No. 32 and SEQ ID No. 33;
 - 20 - VLCDR3 is selected from the group consisting of SEQ ID No. 34, SEQ ID No. 35 and SEQ ID No. 36.

In particular, the antibody or antigen-binding fragment thereof binds specifically to an epitope located within the third extra-cellular loop (EL3) of CMKLR1, more particularly the antibody or antigen-binding fragment thereof binds specifically to a polypeptide comprising amino acid sequence SEQ ID No: 2 or SEQ ID No. 59 or to an epitope located within amino acid sequence SEQ ID No. 60.

[57] In another particular aspect of the invention, it is disclosed anti-Chemokine Like Receptor 1 (CMKLR1) antibody or an antigen-binding fragment thereof which binds to CMKLR1, in particular human CMKLR1, said antibody of antigen-binding fragment thereof comprising:

- 30 **a.** an antibody heavy chain variable (VH) domain comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3, wherein:
 - VHCDR1 is selected from the group consisting of SEQ ID No.3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7;
 - 35 - VHCDR2 is selected from the group consisting of SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11 and SEQ ID No. 61; or VHCDR2 corresponds to the amino acid

residues of SEQ ID No. 12 or SEQ ID No. 63 with the proviso that VHCDR1 is not SEQ ID No. 3 or SEQ ID No. 4;

- VHCDR3 is selected from the group consisting of SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15 and SEQ ID No. 16;

5 **b.** an antibody light chain variable (VL) domain comprising the three CDRs VLCDR1, VLCDR2 and VLCDR3, wherein:

- VLCDR1 is selected from the group consisting of SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 20, SEQ ID No. 21, SEQ ID No. 22 and SEQ ID No. 23;

10 - VLCDR2 is selected from the group consisting of SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 31, SEQ ID No. 32 and SEQ ID No. 33;

- VLCDR3 is selected from the group consisting of SEQ ID No. 34, SEQ ID No. 35 and SEQ ID No. 36.

15 In particular, the antibody or antigen-binding fragment thereof binds specifically to an epitope located within the third extra-cellular loop (EL3) of CMKLR1, more particularly the antibody or antigen-binding fragment thereof binds specifically to a polypeptide comprising amino acid sequence SEQ ID No: 2 or SEQ ID No. 59 or to an epitope located within amino acid sequence SEQ ID No. 60.

20 [58] In another particular aspect of the invention, it is disclosed anti-Chemokine Like Receptor 1 (CMKLR1) antibody or an antigen-binding fragment thereof which binds to CMKLR1, in particular human CMKLR1, said antibody of antigen-binding fragment thereof comprising:

25 **a.** an antibody heavy chain variable (VH) domain comprising the three CDRs VHCDR1, VHCDR2 and VHCDR3, wherein:

- VHCDR1 is selected from the group consisting of SEQ ID No.3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6 and SEQ ID No. 7;

VHCDR2 is selected from the group consisting of SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11 and SEQ ID No. 61;

30 when VHCDR1 is SEQ ID No. 3 or SEQ ID No. 4 and VHCDR2 corresponds to the amino sequence of SEQ ID NO: 12 said heavy chain variable (VH) domain does not comprise a framework VHFR3 of SEQ ID No. 70, preferably with the proviso that said heavy chain variable (VH) domain comprises a framework FR3 of SEQ ID NO: 69,
; and

35 - VHCDR3 is selected from the group consisting of SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15 and SEQ ID No. 16;

Germline	Amino acid Sequence	SEQ ID No.
VL_IGKV1-13*02	AIQLTQSPSSLSASVGDRTITCSASSVSFMHWYQQKPGKAPKRWIY DTTKLTSGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQWNSKPPLTF GGGTKVEIK	50
VL_IGKV6-21*01	EIVLTQSPDFQSVTPKEKVTITCSASSVSFMHWYQQKPDQSPKRWIY DTTKLTSGVPSRFSGSGSGTDYTLTINSLEAEDAATYYCQQWNSKPPLTF GGGTKVEIK	87
VL_IGKV3-11*01	EIVLTQSPATLSLSPGERATLSCASSVSFMHWYQQKPGQAPRRWIY DTTKLTSGIPARFSGSGSGTDYTLTISLEPEDFAVYYCQQWNSKPPLTF GGGTKVEIK	88

Table 2. Light chain variable domain germlines

Examples of therapeutic efficacy of anti-CMKLR1 antibody treatment on preclinical models of autoimmune and inflammatory diseases

5 EXAMPLE 1. Induction of colitis by DSS

Colitis was induced in 8-10 weeks-old-C57Bl/6 male mice by adding 2% (wt/vol) of DSS to the sterile drinking ad libitum water for 6 days. Treatments were injected intra-peritoneally: Isotype control hlgG1 (10µg per mouse), RvE1 (1µg per mouse) daily, or 2G1 antibody (10µg per mouse) three times for 5 days. Colitis follow-up consisting of body weight and stool score (0: normal stool; 4: blood in stool) parameters were performed daily. When mice were euthanized, colon length representing the pathology severity was measured. Resolution index was determined in the different conditions as described in Bannenberg et al., 2005.

RESULTS: The DSS animal model presented on **Figure 2** is an acute inflammatory model. Figure 2 shows a better overall state of the animals treated with the anti-CMKLR1 antibody than states of animals receiving the control antibody or the resolvin RvE1. The anti-CMKLR1 treated mice lost significantly less weight (**Fig. 2A**) and the score stool (**Fig. 2B**) was significantly better. Regarding the colon length and resolution index (**Figure 2C and 2D**), the animals receiving the anti-CMKLR1 or RvE1 presented similar results.

EXAMPLE 2. Induction of colitis by TNBS

Colitis was induced in 8-10 weeks-old-C57Bl/6 male mice by intrarectal injection of 200 µL of the haptening agent TNBS at 5% in 50% ethanol on day 0. Treatments were injected intra-peritoneally; RvE1 (1µg per mouse) daily for three days, or 2G1 antibody (10µg per mouse) twice for 3 days. Colitis follow-up consisting of body weight and stool score (0: normal stool; 4: blood in stool) parameters were performed daily (data not represented). When mice are euthanized, colon length representing the pathology severity was measured.

RESULTS: Colitis induced by TNBS is another model of acute inflammation. **Figure 3** shows that animals treated with anti-CMKLR1 or RvE1 have a colon length which is the same as

mucosa biopsies performed before anti-TNF treatment (within a week) in patients refractory to corticosteroids and/or immunosuppression. In these three cohorts, anti-TNF response was defined as histological healing analyzed 4-6 weeks after their first anti-TNF infusion (altogether: n=18 non-IBD controls, n=41 UC non-responders and n=28 UC responders).

5 RESULTS: The analysis showed that CMKLR1 transcript expression is significantly increased in colon biopsies of primary UC non-responder patients before and after treatment with anti-TNF therapy as compared to non-IBD controls or patients with UC before anti-TNF and who will respond to anti-TNF therapy (**Figure 10A**). Mucosal CMKLR1 expression is also significantly increased in colon or ileal biopsies of Crohn's Disease patients (n=24 non-IBD
10 controls, n=17 CD non-responders and n=20 CD responders; GSE16879(Arijs et al., 2009a)) before and after anti-TNF therapy in patients who will not respond to anti-TNF as compared to non-IBD controls or future responders (**Figure 10B**). Finally, colon mucosa gene expression analysis in a cohort of UC patients (GSE7366146) treated with anti- α 4 β 7 (vedolizumab) therapy confirmed also that CMKLR1 expression is also significantly increased in non-
15 responders before and after treatment with Vedolizumab (**Figure 11**).

Altogether, the meta-analysis shows that CMKLR1 is over-expressed in inflamed tissues of IBD patients, in particular in patients non-responding to current immunosuppressive or immunotherapies even before initiation of the treatment. Our meta-analysis provide evidence that the CMKLR1 expression in the colon, or rather ileon for CD, from UC or CD patients who
20 are treatment refractory may in contrast qualify these patients as being responsive to an agonist anti-CMKLR1 antibody treatment such as an antibody of the invention.

EXAMPLE 9. CMKLR1 expression and antibody binding study

- ELISA binding CMKLR1 (Figure 12)

CMKLR1 peptide (273NH₂-PYHTLNLELHHTAMPGSVFSLGLPLATALAIA-COOH305) (SEQ
25 ID No: 60) (5 μ g/ml) was coated in borate buffer overnight. Saturation was performed with PBS-Tween 0,1%-Gelatin 0,25% for 2 hours at 37°C. Then, 2G1 or hlgG1 antibodies were added at different concentrations for 2 hours at 37°C. Then peroxidase-conjugated secondary antibody (0,8 μ g/ml) was added for 1 hour at 37°C and revealed by a TMB substrate. The colorimetric reaction was read with TECAN.

30 - CMKLR1 expression by FACS (Figure 13A)

Cells were resuspended in PBS-FBS-EDTA and incubated with Fc block (1/50) for 30 minutes on ice. Staining on T cell lines (Thp-1 and U937) was performed using A488-labeled 2G1 (5 μ g) or A488-labeled hlgG1 (5 μ g).

- Western blot analysis CMKLR1 (Figure 13B)

35 After protein migration and transfer as described previously, 2G1 antibody (10 μ g/membrane) was incubated overnight at 4°C and revealed with a peroxidase-conjugated secondary

monolayer especially under inflammatory condition when PMN and endothelial cells were activated with TNF α in healthy volunteers and in AIDs' patients (**Fig. 28B**).

The antibody of the invention has the capability to reduce the transmigration capability of neutrophils.

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EXAMPLE 19 - CD62L expression

MATERIAL AND METHODS. PMN from healthy volunteers were incubated in culture medium with coated Ab at 10 μ g/mL for different times and collected for CD62L staining analyzed by flow cytometry (**Fig. 29**, left panel). The cell surface expression of CD62L in cells incubated
10 with antibody of the invention is reduced compared to cells incubated in absence of the antibody. The soluble form of the CD62L released by shedding is detected by ELISA in the supernatant of PMN incubated with coated Ab. The treatment of PMN with the anti-ChemR23 antibody increases the concentration of soluble CD62L compared to the isotype control condition (**Fig. 29**, right panel).

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EXAMPLE 20 - Mesothelioma model Survival

Mice treated according to the method explained in the description of the figure with an agonist of CMKLR1 have a higher survival rate than mice treated with a control antibody, thereby illustrating the positive effect of a compound according to the invention for treating
20 mesothelioma (**Fig. 30**).

EXAMPLE 21– CRC model

As illustrated on **Fig. 31A**, the tumor volume is reduced in animals treated with the anti-CMKLR1 antibody of the invention, as compared to the control antibody. In several cases,
25 complete remission is also observed, thereby illustrating the positive effect of a compound according to the invention for treating CRC.

Moreover, as exemplified on **Fig. 31B**, the treatment with an anti-CMKLR1 monoclonal antibody (2G1) leads to a reduction of the stool score, and the reduction of tumor number.

30 EXAMPLE 22 - autoimmune encephalomyelitis experimental model

In this model, the curative administration of the antibody of the invention in EAE model does not lead to the reduction of the weight (**Fig. 32A**), as compared to animals treated with a control antibody. But the illness score is significantly reduced when the treatment is performed with the anti-CMKLR1 antibody (**Fig. 32B**). The anti-CMKLR1 antibody leads to a great
35 improvement of the illness score as soon as ten-day post treatment as compared to the control, since the score is approximately 30 % lower in animals treated with the agonist compound. It

CLAIMS

1. An anti- Chemerin Like Receptor 1 (CMKLR1) antibody or antigen-binding fragment thereof which binds to CMKLR1, in particular human CMKLR1, said antibody or antigen-binding fragment thereof comprising:
- 5
- a. a heavy chain variable (VH) domain comprising SEQ ID NO: 91 and a light chain variable (VL) domain comprising SEQ ID NO: 93; or
 - b. a VH domain comprising SEQ ID NO: 91 and a VL domain comprising SEQ ID NO: 55.
- 10
2. The anti-CMKLR1 antibody or antigen-binding fragment thereof according to claim 1 wherein the antibody or antigen-binding fragment thereof binds specifically to the third extra-cellular loop (EL3) of CMKLR1, in particular to an epitope located within third extra-cellular loop (EL3) of CMKLR1, or within the polypeptide comprising amino acid
- 15
- sequence SEQ ID No: 2 or SEQ ID No. 59 or to an epitope located within amino acid sequence SEQ ID No. 60.
3. The anti-CMKLR1 antibody or antigen-binding fragment thereof according to claim 1 or 2, in particular wherein the anti-CMKLR1 antibody or antigen-binding fragment thereof
- 20
- has an effect of a pro-resolution factor of inflammation, in particular has such an effect by interacting with myeloid cell.
4. The anti-CMKLR1 antibody or antigen-binding fragment thereof according to any one of claims 1 to 3, which comprises a heavy chain variable domain comprising of the
- 25
- amino acid sequence of SEQ ID No. 91 and a light chain variable domain comprising of the amino acid sequence of SEQ ID No. 93.
5. The anti-CMKLR1 antibody or antigen-binding fragment thereof according to any one of claims 1 to 4, which does not activate the beta-arrestin signaling pathway in
- 30
- CMKLR1-positive cells *in vitro* and/or *in vivo*.
6. The anti-CMKLR1 antibody or antigen-binding fragment thereof according to any one of claims 1 to 5, which does not exhibit a significant depletion of CMKLR1-positive cells
- 35
- in vitro* and/or *in vivo*

7. The anti-CMKLR1 antibody or antigen-binding fragment thereof according to any one of claims 1 to 6, which does not compete with Chemerin for the binding to CMKLR1, and/or which does not interfere with the binding of Chemerin to CMKLR1.
- 5 8. The anti-CMKLR1 antibody or antigen-binding fragment according to any one of claims 1 to 7, which is a humanized monoclonal antibody or antigen-binding fragment thereof, in particular wherein the antibody light chain constant domain is derived from a human kappa light chain constant domain, in particular wherein the light chain constant domain comprises or consists of the sequence of SEQ ID No: 79, and wherein the antibody
10 heavy chain constant domain is derived from a human IgG1, IgG2, IgG3, or IgG4 heavy chain constant domain, in particular wherein the antibody heavy chain constant domain comprises or consists of the amino acid sequence of SEQ ID 80, SEQ ID No. 81, SEQ ID No. 82, SEQ ID No. 83 or SEQ ID No. 84, in particular from a human IgG1 heavy chain constant domain, in particular wherein the antibody heavy chain constant domain
15 comprises or consists of the amino acid sequence of SEQ ID No:80 or SEQ ID No. 83.
9. The anti-CMKLR1 antibody or antigen-binding fragment thereof according to any one of claims 1 to 8 for use in therapy.
- 20 10. Use of the anti-CMKLR1 antibody or antigen-binding fragment thereof according to any one of claims 1 to 8 in the manufacture of a medicament for preventing or treating:
 - an inflammatory disease, in particular acute inflammatory diseases, chronic inflammatory diseases such as chronic inflammatory pulmonary diseases, keratoconjunctivitis, periodontal disease, eczema, inflammatory bowel disease, in
25 particular Crohn's disease or colitis, in particular ulcerative colitis or spontaneous colitis, cystic fibrosis, NASH (Nonalcoholic steatohepatitis), scleroderma, anti-neutrophil cytoplasm antibodies-related disease ANCA associated disease, in particular wherein, as a result of the administration of the treatment, the resolution of inflammation is enhanced;
 - 30 - an autoimmune disease such as diabetes, in particular type I diabetes, psoriasis, lupus, rheumatoid arthritis, multiple sclerosis, Sjögren's syndrome, celiac disease, vasculitis, myasthenia gravis, or an infection disease such as sepsis, peritonitis, degenerative diseases, wound healing disorders or dry eye syndrome, in particular wherein, as a result of the administration of the treatment, the resolution of
35 inflammation is enhanced;
 - a cancer, in particular metastatic cancers, solid or liquid cancers such as carcinoma, more particularly hepatocarcinoma, in particular mammary carcinoma

or colon carcinoma, colorectal cancer or lung cancer or mesothelioma or myeloid cancer such as leukemia, in particular a cancer wherein cancer cells express CMKLR1 or where the microenvironment of the tumor is invaded by cells expressing or overexpressing CMKLR1, in particular wherein, as a result of the administration of the treatment, the resolution of inflammation is enhanced.

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- 11.** Use of the anti-CMKLR1 antibody or antigen-binding fragment thereof according to any one of claims 1 to 8 in the manufacture of a medicament for preventing or treating NASH (Nonalcoholic steatohepatitis), scleroderma, cystic fibrosis or anti-neutrophil cytoplasm antibodies-related disease (ANCA).

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