ADVANCE E-MAIL

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION, OBTENTION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

GEVERS & ORES
Association N° 92
41, avenue de Friedland
75008 PARIS
FRANCE

Date of mailing *(day/month/year)* 09 May 2017 (09.05.2017)	
Applicant's or agent's file reference ERBIsafl-F2173/8/WO	**IMPORTANT NOTIFICATION**
International application No. PCT/EP2017/059071	International filing date *(day/month/year)* 14 April 2017 (14.04.2017)
International publication date *(day/month/year)* **Not yet published**	Priority date *(day/month/year)* 14 April 2016 (14.04.2016)
Applicant OSE IMMUNOTHERAPEUTICS	

The applicant is hereby notified of the date of receipt (or of obtaining by the International Bureau) of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to the date of receipt, **the priority document concerned was submitted or transmitted to or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-*bis*)**. This Form replaces any previously issued notification concerning submission, transmittal or obtaining of priority documents.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
14 April 2016 (14.04.2016)	62/322,707	US	NR
17 February 2017 (17.02.2017)	17305182.2	EP	05 May 2017 (05.05.2017)

The letters "NR" denote a priority document which, on the date of mailing of this Form, had not yet been received or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-*bis*). Where the applicant has failed to either submit, request to prepare and transmit, or to request the International Bureau to obtain the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

An asterisk "*" next to a date of receipt, denotes a priority document submitted or transmitted to or obtained by the International Bureau but not in compliance with Rule 17.1(a), (b) or (b-*bis*) (the priority document was received after the time limit prescribed in Rule 17.1(a); the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b) or the request to the International Bureau to obtain the priority document was made after the applicable time limit under Rule 17.1(b-*bis*)). Even though the priority document was not furnished in compliance with Rule 17.1(a), (b) or (b-*bis*), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
05 July 2018 (05.07.2018)

(54) Title: NEW ANTI-SIRPa ANTIBODIES AND THEIR THERAPEUTIC APPLICATIONS

(57) Abstract: The present invention provides new anti-SIRPa antibodies able to specifically antagonize the interaction between SIRPa and CD47, without affecting the interaction between SIRPg and CD47, and their uses.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/059071

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
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)
C07K 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, Sequence Search, 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	D. HATHERLEY ET AL: "The Structure of the Macrophage Signal Regulatory Protein (SIRP) Inhibitory Receptor Reveals a Binding Face Reminiscent of That Used by T Cell Receptors", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 282, no. 19, 16 March 2007 (2007-03-16), pages 14567-14575, XP055081473, ISSN: 0021-9258, DOI: 10.1074/jbc.M611511200 figure 2	21

X	WO 2015/138600 A2 (UNIV LELAND STANFORD JUNIOR [US]) 17 September 2015 (2015-09-17) claim 20	17

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 2018	05/06/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Scheffzyk, Irmgard

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIU ET AL: "Functional Elements on SIRP@a IgV Domain Mediate Cell Surface Binding to CD47", JOURNAL OF MOLECULAR BIO, ACADEMIC PRESS, UNITED KINGDOM, vol. 365, no. 3, 23 December 2006 (2006-12-23), pages 680-693, XP005733360, ISSN: 0022-2836, DOI: 10.1016/J.JMB.2006.09.079 figure 1	1-16, 18-22
X	Y. LIU ET AL: "Signal Regulatory Protein (SIRPalpha), a Cellular Ligand for CD47, Regulates Neutrophil Transmigration", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 12, 15 March 2002 (2002-03-15), pages 10028-10036, XP055030073, ISSN: 0021-9258, DOI: 10.1074/jbc.M109720200 the whole document	1-16, 18-22
X	LEE ET AL: "Novel structural determinants on SIRP alpha that mediate binding to CD47.", JOURNAL OF IMMUNOLOGY, 1 January 2007 (2007-01-01), pages 7741-7750, XP55081468, figure 2	1-16, 18-22
X	US 2010/215640 A1 (CLEMMONS DAVID R [US] ET AL) 26 August 2010 (2010-08-26) paragraph [0060]; sequence 12	1-27
Y	US 2012/039896 A1 (CLEMMONS DAVID R [US] ET AL) 16 February 2012 (2012-02-16) sequence 12	1-27
Y	US 2014/141002 A1 (CLEMMONS DAVID R [US] ET AL) 22 May 2014 (2014-05-22) sequence 13	1-27

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/059071

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2015138600 A2	17-09-2015	AU 2015229448 A1	01-09-2016
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		CN 106456749 A	22-02-2017
		EP 3116544 A2	18-01-2017
		JP 2017510251 A	13-04-2017
		SG 11201607143U A	29-09-2016
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		US 2016297866 A1	13-10-2016
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		WO 2013032948 A1	07-03-2013
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		KR 20140090975 A	18-07-2014
		NZ 621515 A	31-03-2016
		RU 2014111472 A	27-11-2015
		US 2012039896 A1	16-02-2012
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		US 2016297866 A1	13-10-2016
		US 2017114134 A1	27-04-2017
		WO 2013032948 A1	07-03-2013
		ZA 201401450 B	26-11-2014

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NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

DESAIX, Anne
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FRANCE

Date of mailing *(day/month/year)*
24 August 2018 (24.08.2018)

Applicant's or agent's file reference
B12986B - AD/HLQ/DL

IMPORTANT NOTIFICATION

International application No.
PCT/EP2017/059071

International filing date *(day/month/year)*
14 April 2017 (14.04.2017)

1. The following indications appeared on record concerning:

☐ the applicant ☒ the inventor ☐ the agent ☐ the common representative

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☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

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☐ Notifications by e-mail authorized

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
☒ the International Searching Authority
☐ the Authority(ies) specified for supplementary search
☐ the International Preliminary Examining Authority
☒ the designated Offices concerned
☐ the elected Offices concerned
☐ other:

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WIPO | PCT

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(30) Priority Data:
62/322,707 14 April 2016 (14.04.2016) US
17305182.2 17 February 2017 (17.02.2017) EP

(71) Applicant: **OSE IMMUNOTHERAPEUTICS** [FR/FR]; 22 Bd Benoni Goullin, 44200 NANTES (FR).

(72) Inventors: **POIRIER, Nicolas**; 1 Chemin du Passe-Temps, 44119 TREILLIÈRES (FR). **MARY, Caroline**; 7 rue du Buisson, 44680 SAINTE PAZANNE (FR). **VANHOVE, Bernard**; 72 bis rue Henri Barbusse, 44400 REZÉ (FR). **GAUTTIER, Vanessa**; 2, Rue Félicien Thomazeau, 44400 REZÉ (FR). **THEPENIER, Virginie**; 9 Rue des Frênes, 44710 PORT-SAINT-PÈRE (FR). **PENGAM, Sabrina**; 15 Rue Guillaume Grootaers, Bâtiment 5, 44300 NANTES (FR).

(74) Agent: **GEVERS & ORES**; Association N° 92, 41, avenue de Friedland, 75008 PARIS (FR).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Published:

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— *with sequence listing part of description (Rule 5.2(a))*

WO 2017/178653 A2

(54) Title: NEW ANTI-SIRPa ANTIBODIES AND THEIR THERAPEUTIC APPLICATIONS

(57) Abstract: The present invention provides new anti-SIRPa antibodies able to specifically antagonize the interaction between SIRPa and CD47, without affecting the interaction between SIRPg and CD47, and their uses.

NEW ANTI-SIRPa ANTIBODIES AND THEIR THERAPEUTIC APPLICATIONS

The invention pertains to the field of immunotherapy. The present invention provides new anti-SIRPa antibodies able to specifically decreases the interaction between SIRPa and CD47 without affecting the interaction between SIRPg and CD47.

Targeting immune checkpoints of the adaptive immunity has shown great therapeutic efficacy to fight numerous cancers, but in a limited proportion of patients. Immune checkpoint on myeloid cells (macrophages, dendritic cells, MDSC, PMN) remain poorly studied while these cells represent the most abundant immune cell type in many solid tumors and are often associated with a poor outcome.

Signal regulatory protein alpha or SIRPa (also designated as SIRPα, CD172a or SHPS-1), is expressed on monocytes, most subpopulations of tissue macrophages, granulocytes, subsets of dendritic cells in lymphoid tissues, some bone marrow progenitor cells, and to varying levels on neurons, with a notably high expression in synapse-rich areas of the brain, such as the granular layer of the cerebellum and the hippocampus. SIRPa is the prototypic member of the SIRP paired receptor family of closely related SIRP proteins. The gene coding for human SIRPa is a polymorphic gene and several variants were described in human population. The most common protein variants are SIRPa v1 and v2 (accession numbers NP_542970 (P78324) and CAA71403). The polymorphisms in human SIRP lead to changes in surface-exposed amino acids, but this does not affect binding to CD47.

Interaction of SIRPa, expressed by myeloid cells, with the ubiquitous receptor CD47 is an important immune checkpoint of the innate response, involved in the regulation of myeloid functions. The SIRPa interaction with CD47 is largely described and provides a downregulatory signal that inhibits host cell phagocytosis. CD47 is widely expressed at lower levels by most healthy cells but it is also overexpressed in some cancer cells. Therefore, CD47 functions as a "don't-eat-me" signal. Both CD47 and SIRPa also engage in many other interactions. One of the best characterized physiological functions of the CD47-SIRPa interaction is its role in the homeostasis of hematopoietic cells, in particular red blood cells and platelets. Because CD47 serves as a "don't-eat-me" signal and, as such, is an important determinant of host cell phagocytosis by macrophages, the potential contribution of CD47-SIRPa interaction in cancer cell clearance has been intensely investigated in recent years. It was shown that abundance of CD47 receptors in tumors is inversely correlated with patient overall survival and constitute an adverse prognostic factor for several cancer types.

The SIRPa/CD47 pathway is nowadays subject to different pharmaceutical developments to enhance macrophages phagocytosis. In fact, like infected cells, cancer cells carry aberrant cargo such as unfamiliar proteins or normal proteins at abnormal levels, yet these cells frequently subvert innate immune control mechanisms by concurrently over-expressing immunoregulatory molecules. It is

becoming clear that one such mechanism involves CD47, a protein of "self" expressed by normal cells. CD47 interacts with SIRPa and leads to the transmission of a "don't eat me" signal to phagocytic macrophages, which then leave target cells unaffected. Over-expression of CD47 by cancer cells renders them resistant to macrophages, even when the cancer cells are coated with therapeutic antibodies, and correlates with poor clinical outcomes in numerous solid and hematological cancers. In experimental models, in particular human tumor-xenograft models in immunodeficient mice, blockade of the CD47/SIRPa pathway via agents targeting CD47 was very effective to promote tumor elimination by macrophages and to decrease cancer cell dissemination and metastasis formation. Blockade of the CD47/SIRPa pathway via agents targeting CD47, by enhancing antibody-dependent phagocytosis by macrophages, has been described to synergize with depleting therapeutic anticancer antibodies such as Trastuzumab (anti-Her2), Cetuximab (anti-EGFR), Rituximab (anti-CD20) and Alemtuzumab (anti-CD52).

However, it has recently been shown that agents targeting CD47 (anti-CD47 or SIRPa-Fc) present hematological toxicity (anemia or thrombocytopenia) related to CD47 physiological role.

Besides, CD47 also engages with another member of the SIRP family, SIRP-gamma (also designated as SIRPg, SIRPγ, CD172g or SIRP beta 2) that is present at the surface of human T cells and not on human myeloid cells. SIRPg is the result of a duplication of SIRPb gene in old-world primates nearly 35 million years ago and it is expressed in a restricted manner on T lymphocytes as opposed to SIRPa expression on myeloid cells. SIRPg is absent in mice. It has been shown that the SIRPg-CD47 interaction mediates cell-cell adhesion, enhances superantigen-dependent T-cell-mediated proliferation and co-stimulates T-cell activation (Piccio et al., Blood, 105:6, 2005).

Due to the high similarity of sequences between SIRPa and SIRPg, in particular in the region that interacts with CD47, the anti-SIRPa antibodies disclosed in the prior art also bind SIRPg and have undesirable effects in humans such as an inhibition of the proliferation of T-cells and a decrease of the immune response. Such side effects of anti-CD47 or non-selective anti-SIRPa antibodies could not be predicted since the tests of the known antibodies were performed in mice models, which do not possess the SIRPg gene, and thus such side effects were absent.

There remains therefore a significant need in the art for new and improved agents, in particular antibodies, for safe immunotherapy, notably against cancer, targeting innate immune cells without deleterious impact on T cell immune responses. In particular, there is a need for inhibiting the SIRPa-CD47 interaction without affecting the SIRPg-CD47 interaction. The present inventors have made a significant step forward with the invention disclosed herein.

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

Here, the Inventors provide new anti-SIRPa antibodies, in particular humanized antibodies, that antagonize the SIRPa-CD47 interaction but do not specifically bind SIRPg and, thus, do not affect the SIRPg-CD47 interaction.

The antibodies of the invention have in particular the following advantages:

- They avoid hematological toxicity due to restricted expression of SIRPa (no binding to human Red Blood Cells (RBC) and platelets);
- They reduce tumor growth and modify tumor microenvironment in monotherapy;
- They present synergistic effect with checkpoint inhibitors and costimulatory agents;
- They induce durable and robust anti-tumor memory T lymphocytes responses;
- They enable human T cell immune responses, being selective antagonist of SIRPa-CD47 interaction, not disturbing the CD47/SIRPg interaction. Unexpectedly, the inventors provide such selective antibodies despite the high sequence identity between SIRPa and SIRPg sequences.

These new antibodies selected on the basis of these characteristics are particularly promising for numerous therapeutic applications, in particular for the treatment of cancer including inflammatory cancers and cancers with infiltrated myeloid cells (in particular with infiltrated MDSCs and/or TAM cells).

SIRPa ANTIBODIES (WITH EPITOPES)

In an aspect, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least one peptide comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one skilled in the relevant art.

For convenience, the meaning of certain terms and phrases employed in the specification, examples, and claims are provided.

As used herein, the term "antibody" comprises polyclonal antibodies, monoclonal antibodies or recombinant antibodies.

As used herein, a "monoclonal antibody" is intended to refer to a preparation of antibody molecules, antibodies which share a common heavy chain and common light chain amino acid sequence, in contrast with "polyclonal" antibody preparations which contain a mixture of antibodies of different amino acid sequence. Monoclonal antibodies can be generated by several known technologies like phage, bacteria, yeast or ribosomal display, as well as by classical methods exemplified by

hybridoma-derived antibodies. Thus, the term "monoclonal" is used to refer to all antibodies derived from one nucleic acid clone.

The antibodies of the present invention include recombinant antibodies. As used herein, the term "recombinant antibody" refers to antibodies which are produced, expressed, generated or isolated by recombinant means, such as antibodies which are expressed using a recombinant expression vector transfected into a host cell; antibodies isolated from a recombinant combinatorial antibody library; antibodies isolated from an animal (*e.g.* a mouse) which is transgenic due to human immunoglobulin genes; or antibodies which are produced, expressed, generated or isolated in any other way in which particular immunoglobulin gene sequences (such as human immunoglobulin gene sequences) are assembled with other DNA sequences. Recombinant antibodies include, for example, chimeric and humanized antibodies.

As used herein, a "chimeric antibody" refers to an antibody in which the sequence of the variable domain derived from the germline of a mammalian species, such as a mouse, have been grafted onto the sequence of the constant domain derived from the germline of another mammalian species, such as a human.

As used herein, a "humanized antibody" refers to an antibody in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

As used herein, an "antigen-binding fragment of an antibody" means a part of an antibody, *i.e.* a molecule corresponding to a portion of the structure of the antibody of the invention, that exhibits antigen-binding capacity for SIRPa, possibly in its native form; such fragment especially exhibits the same or substantially the same antigen-binding specificity for said antigen compared to the antigen-binding specificity of the corresponding four-chain antibody. Advantageously, the antigen-binding fragments have a similar binding affinity as the corresponding 4-chain antibodies. However, antigen-binding fragment that have a reduced antigen-binding affinity with respect to corresponding 4-chain antibodies are also encompassed within the invention. The antigen-binding capacity can be determined by measuring the affinity between the antibody and the target fragment. These antigen-binding fragments may also be designated as "functional fragments" of antibodies.

Antigen-binding fragments of antibodies are fragments which comprise their hypervariable domains designated CDRs (Complementary Determining Regions) or part(s) thereof encompassing the recognition site for the antigen, *i.e.* the extracellular domain of SIRPa, thereby defining antigen recognition specificity.

Each Light and Heavy chain variable domains (respectively VL and VH) of a four-chain immunoglobulin has three CDRs, designated VL-CDR1 (or LCDR1), VL-CDR2 (or LCDR2), VL-CDR3 (or LCDR3) and VH-CDR1 (or HCDR1), VH-CDR2 (or HCDR2), VH-CDR3 (or HCDR3), respectively.

The skilled person is able to determine the location of the various regions/domains of antibodies by reference to the standard definitions in this respect set forth, including a reference numbering system, a reference to the numbering system of KABAT or by application of the IMGT "collier de perle" algorithm. In this respect, for the definition of the sequences of the invention, it is noted that the delimitation of the regions/domains may vary from one reference system to another. Accordingly, the regions/domains as defined in the present invention encompass sequences showing variations in length or localization of the concerned sequences within the full-length sequence of the variable domains of the antibodies, of approximately +/- 10%.

Based on the structure of four-chain immunoglobulins, antigen-binding fragments can thus be defined by comparison with sequences of antibodies in the available databases and prior art, and especially by comparison of the location of the functional domains in these sequences, noting that the positions of the framework and constant domains are well defined for various classes of antibodies, especially for IgGs, in particular for mammalian IgGs. Such comparison also involves data relating to 3-dimensional structures of antibodies.

For illustration purpose of specific embodiments of the invention, antigen binding fragments of an antibody that contain the variable domains comprising the CDRs of said antibody encompass Fv, dsFv, scFv, Fab, Fab', F(ab')2. Fv fragments consist of the VL and VH domains of an antibody associated together by hydrophobic interactions; in dsFv fragments, the VH:VL heterodimer is stabilised by a disulphide bond; in scFv fragments, the VL and VH domains are connected to one another via a flexible peptide linker thus forming a single-chain protein. Fab fragments are monomeric fragments obtainable by papain digestion of an antibody; they comprise the entire L chain, and a VH-CH1 fragment of the H chain, bound together through a disulfide bond. The F(ab')2 fragment can be produced by pepsin digestion of an antibody below the hinge disulfide; it comprises two Fab' fragments, and additionally a portion of the hinge region of the immunoglobulin molecule. The Fab' fragments are obtainable from F(ab')2 fragments by cutting a disulfide bond in the hinge region. F(ab')2 fragments are divalent, *i.e.* they comprise two antigen binding sites, like the native immunoglobulin molecule; on the other hand, Fv (a VHVL dimmer constituting the variable part of Fab), dsFv, scFv, Fab, and Fab' fragments are monovalent, *i.e.* they comprise a single antigen-binding site. These basic antigen-binding fragments of the invention can be combined together to obtain multivalent antigen-binding fragments, such as diabodies, tribodies or tetrabodies. These multivalent antigen-binding fragments are also part of the present invention.

As used herein, the term "bispecific" antibodies refers to antibodies that recognize two different antigens by virtue of possessing at least one region (*e.g.* derived from a variable region of a first antibody) that is specific for a first antigen, and at least a second region (*e.g.* derived from a variable region of a second antibody) that is specific for a second antigen. A bispecific antibody specifically

binds to two target antigens and is thus one type of multispecific antibody. Multispecific antibodies, which recognize two or more different antigens, can be produced by recombinant DNA methods or include, but are not limited to, antibodies produced chemically by any convenient method. Bispecific antibodies include all antibodies or conjugates of antibodies, or polymeric forms of antibodies which are capable of recognizing two different antigens. Bispecific antibodies include antibodies that have been reduced and reformed so as to retain their bivalent characteristics and to antibodies that have been chemically coupled so that they can have several antigen recognition sites for each antigen such as BiME (Bispecific Macrophage Enhancing antibodies), BiTE (bispecific T cell engager), DART (Dual affinity retargeting); DNL (dock-and-lock), DVD-Ig (dual variable domain immunoglobulins), HAS (human serum albumin), kih (knobs into holes).

Accordingly, bispecific antibodies of the invention are directed against SIRPa and a second antigen.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that is bispecific.

Several researches to develop therapeutic antibodies had led to engineer the Fc regions to optimize antibody properties allowing the generation of molecules that are better suited to the pharmacology activity required of them. The Fc region of an antibody mediates its serum half-life and effector functions, such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP). Several mutations located at the interface between the CH2 and CH3 domains, such as T250Q/M428L and M252Y/S254T/T256E + H433K/N434F, have been shown to increase the binding affinity to FcRn and the half-life of IgG1 *in vivo.* However, there is not always a direct relationship between increased FcRn binding and improved half-life. One approach to improve the efficacy of a therapeutic antibody is to increase its serum persistence, thereby allowing higher circulating levels, less frequent administration and reduced doses. Engineering Fc regions may be desired to either reduce or increase the effector function of the antibody. For antibodies that target cell-surface molecules, especially those on immune cells, abrogating effector functions is required. Conversely, for antibodies intended for oncology use, increasing effector functions may improve the therapeutic activity. The four human IgG isotypes bind the activating Fcγ receptors (FcγRI, FcγRIIa, FcγRIIIa), the inhibitory FcγRIIb receptor, and the first component of complement (C1q) with different affinities, yielding very different effector functions. Binding of IgG to the FcγRs or C1q depends on residues located in the hinge region and the CH2 domain. Two regions of the CH2 domain are critical for FcγRs and C1q binding, and have unique sequences in IgG2 and IgG4.

As used herein, a "modified antibody" corresponds to a molecule comprising an antibody or an antigen-binding fragment thereof, wherein said monoclonal antibody or functional fragment thereof is associated with a functionally different molecule. A modified antibody of the invention may be

either a fusion chimeric protein or a conjugate resulting from any suitable form of attachment including covalent attachment, grafting, chemical bonding with a chemical or biological group or with a molecule, such as a PEG polymer or another protective group or molecule suitable for protection against proteases cleavage *in vivo*, for improvement of stability and/or half-life of the antibody or functional fragment. With similar techniques, especially by chemical coupling or grafting, a modified antibody can be prepared with a biologically active molecule, said active molecule being for example chosen among toxins, in particular Pseudomonas exotoxin A, the A-chain of plant toxin ricin or saporin toxin, especially a therapeutic active ingredient, a vector (including especially a protein vector) suitable for targeting the antibody or functional fragment to specific cells or tissues of the human body, or it may be associated with a label or with a linker, especially when fragments of the antibody are used. PEGylation of the antibody or functional fragments thereof is a particular interesting embodiment as it improves the delivery conditions of the active substance to the host, especially for a therapeutic application. PEGylation can be site specific to prevent interference with the recognition sites of the antibodies or functional fragments, and can be performed with high molecular weight PEG. PEGylation can be achieved through free cysteine residues present in the sequence of the antibody or functional fragment or through added free Cysteine residues in the amino sequence of the antibody or functional fragment.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that is modified.

The macromolecules of the invention comprise antibodies and fragments thereof but also comprise artificial proteins, peptides and any chemical compounds with the capacity to bind antigens mimicking that of antibodies, also termed herein antigen-binding antibody mimetic. Such proteins comprise affitins and anticalins. Affitins are artificial proteins with the ability to selectively bind antigens. They are structurally derived from the DNA binding protein Sac7d, found in *Sulfolobus acidocaldarius*, a microorganism belonging to the archaeal domain. By randomizing the amino acids on the binding surface of Sac7d, *e.g.* by generating variants corresponding to random substitutions of 11 residues of the binding interface of Sac7d, an affitin library may be generated and subjecting the resulting protein library to rounds of ribosome display, the affinity can be directed towards various targets, such as peptides, proteins, viruses and bacteria. Affitins are antibody mimetics and are being developed as tools in biotechnology. They have also been used as specific inhibitors for various enzymes (Krehenbrink *et al.*, J. mol. Biol., 383:5, 2008). The skilled person may readily develop anticalins with the required binding properties using methods know in the art, in particular as disclosed in patent application WO2008068637 and the above-cited publication, in particular the generation of phage display and/or ribosome display libraries and their screening using an antigen as dislosed herein. Anticalins are artificial proteins that are able to bind to antigens, either to proteins

or to small molecules. They are antibody mimetic derived from human lipocalins which are a family of naturally binding proteins. Anticalins are about eight times smaller with a size of about 180 amino acids and a mass of about 20 kDa (Skerra, Febs J., 275:11, 2008). Anticalin phage display libraries have been generated which allow for the screening and selection, in particular of anticalins with specific binding properties. The skilled person may readily develop affitins with the required binding properties using methods know in the art, in particular as disclosed in EP patent EP1270725 B1 , US patent US8536307 B2, (Schlehuber and Skerra, Biophys. Chem., 96:2-3, 2002) and the above-cited publication, in particular the generation of phage display and/or ribosome display libraries and their screening using an antigen as dislosed herein. Anticalins and affitins may both be produced in a number of expression system comprising bacterial expressin systems. Thus, the invention provides affitins, anticalins and other similar antibody mimetics with the features of the antibodies described herein, in particular with regard to the binding to SIRPa, the inhibition of the interaction between SIRPa and CD47, the non-binding to SIRPg, the non binding to T cells, the non inhibition of the proliferation of T cells, the non inhibition of the interaction between SIRPg and CD47 all of which are contemplated as macromolecules of the invention.

All the embodiments disclosed herein for antibodies or fragments thereof are transposed *mutatis mutandis* to the macromolecules of the invention, in particular to antigen-binding antibody mimetics.

As used herein, the term "epitope" means the part of an antigen to which the antibody binds. The epitopes of protein antigens can be divided into two categories, conformational epitope and linear epitope. A conformational epitope corresponds to discontinuous sections of the antigen's amino acid sequence. A linear epitope corresponds to a continuous sequence of amino acids from the antigen.

In the invention, the peptides that are present within SIRPa and that are bound by the anti-SIRPa antibodies are constitutive of the epitope specifically recognized by these antibodies.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least two, three, four or five peptides comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to the peptide comprising or consisting of amino acid sequence SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E) within SIRPa and to at least one peptide comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 4

(QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to the peptides comprising or consisting of amino acid sequence SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least one peptide comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 7 (GRELIYNQKEGH), SEQ ID NO: 8 (KFRKGSPDDVE), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to the peptides of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 7 (GRELIYNQKEGH), SEQ ID NO: 8 (KFRKGSPDDVE), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least one peptide comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 9 (ARELIYNQKEGH), SEQ ID NO: 10 (KFRKGSPDTE), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE).

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to the peptides of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 9 (ARELIYNQKEGH), SEQ ID NO: 10 (KFRKGSPDTE), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least one peptide comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 11 (GRELIYN), DVE, SEQ ID NO: 12 (HTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to the peptides

of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 11 (GRELIYN), DVE, SEQ ID NO: 12 (HTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least one peptide comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 13 (ARELIYN), SEQ ID NO: 12 (HTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to the peptides of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 13 (ARELIYN), SEQ ID NO: 12 (HTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

The peptides of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13 correspond to linear epitopes.

These linear epitopes have been identified by the inventors by array-based oligopeptide scanning (sometimes called overlapping peptide scan or pepscan analysis). This technique uses a library of oligo-peptide sequences from overlapping and non-overlapping segments of a target protein and tests for their ability to bind the antibody of interest. By combining non-adjacent peptide sequences from different parts of the target protein and enforcing conformational rigidity onto this combined peptide (such as by using CLIPS scaffolds) (Timmerman et al., 2007, J Mol Recognit., Sep-Oct;20(5):283-99), discontinuous epitopes can be mapped with very high reliability and precision (Gaseitsiwe et al., 2010 - Clin Vaccine Immunol. Jan; 17(1): 168–175). All of the tested antibodies of the invention, including HEFLB, specifically bind to said epitopes.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to a conformational epitope comprising at least one peptide selected from the group consisting of SEQ ID NO: 70 (ELIYNQKEGHFPR), SEQ ID NO: 71 (RNNMDFSIRIGN) and SEQ ID NO: 72 (SPRDITLKW) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to a conformational epitope comprising or consisting of the peptides of SEQ ID NO: 70 (ELIYNQKEGHFPR), SEQ ID NO: 71 (RNNMDFSIRIGN) and SEQ ID NO: 72 (SPRDITLKW) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to a

conformational epitope comprising at least one peptide selected from the group consisting of SEQ ID NO: 70 (ELIYNQKEGHFPR) and SEQ ID NO: 71 (RNNMDFSIRIGN).

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to a conformational epitope comprising or consisting of the peptides of SEQ ID NO: 70 (ELIYNQKEGHFPR) and SEQ ID NO: 71 (RNNMDFSIRIGN) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to a conformational epitope comprising at least one peptide selected from the group consisting of SEQ ID NO: 73 (YNQK) and "SIR" within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to a conformational epitope comprising or consisting of the peptide of amino acid sequence set forth in SEQ ID NO: 73 (YNQK) and the peptide of SIR amino acid sequence within SIRPa.

The peptides of SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73 and SIRP correspond to conformational epitopes. These conformational epitopes have been determined by the inventors using proteolysis protection procedures (enzymatic digestion: chymotrypsin, trypsin of the antibody-antigen complex immobilized on affinity chromatography) following by mass spectrometry analyses (MALDI-TOF/TOF) to detect and sequence such peptides of interest, as well known by one skilled in the art (Van de Water et al., Clinical Immunology and Immunopathology, 1997, vol. 85). The antigen used was the human SIRPa (accession numbers NP_542970) and one of the antibodies of the invention used was the HEFLB variant.

The anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to the invention specifically bind said conformational epitopes comprising or consisting of said peptides in their conformational arrangement within the native SIRPa.

The peptide of amino acid sequence set forth in SEQ ID NO: 73 (YNQK) corresponds to the peptide consisting of amino acids at position 80 to 83 in the human SIRPa amino acid sequence referenced by the NP_542970 accession number.

The peptide of SIR amino acid sequence, the SIR peptide, corresponds to the peptide consisting of amino acids at position 105 to 107 in the human SIRPa amino acid sequence referenced by the NP_542970 accession number.

As used herein, the term "SIRPa" refers to a SIRPa protein from a mammal species, preferably a human SIRPa (e.g. accession numbers NP_542970 (P78324) and CAA71403).

As used herein, the term "anti-SIRPa antibody" refers to an antibody which specifically binds to SIRPa, in particular to a human SIRPa.

The specific binding between the antibody or antigen-binding fragment thereof of the invention and the epitope (or the region comprising the epitope) implies that the antibody exhibits appreciable affinity for the epitope (the region comprising the epitope) on a particular protein or antigen (here SIRPa). "Appreciable affinity" includes binding with an affinity of about 10^{-9} M (KD) or stronger. Preferably, binding is considered specific when the binding affinity is between 10^{-9} M and 10^{-12} M, optionally between 10^{-9} M and 10^{-10} M, in particular 10^{-10} M. Whether a binding domain specifically reacts with or binds to a target can be tested readily by, inter alia, comparing the reaction of said binding domain with a target protein or antigen with the reaction of said binding domain with proteins or antigens other than the target protein.

The affinity can be determined by various methods well known from the one skilled in the art. These methods include, but are not limited to, Biacore Analysis, Blitz analysis and Scatchard plot.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that has a KD value inferior to 10^{-9} M, preferably inferior to 10^{-10} M for SIRPa, more preferably inferior to 1.10^{-11} M, particularly by Biacore Analysis.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that decreases the interaction between SIRPa and CD47.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that partially or fully, in particular fully, inhibits the binding of CD47 to SIRPa, in particular of human CD47 to human SIRPa.

Such an antibody of the invention specifically binds SIRPa and antagonizes the interaction between SIRPa and CD47.

In particular, the anti-SIRPa antagonist antibody of the invention is capable of reducing or inhibiting the binding of CD47 to SIRPa by at least 50%, 60%, 70%, preferably 80%, more preferably 90% or most preferably 100%, as compared to a negative control molecule, in a binding assay.

In particular, the anti-SIRPa antagonist antibody of the invention is capable of reducing or inhibiting the binding of CD47 to SIRPa by from 50% to 100%, preferably from 60% to 90%, more preferably from 70% to 80%, as compared to a negative control molecule, in a binding assay.

Methods for determining antibody specificity and affinity by competitive inhibition are known in the art (see, e.g., Harlow *et al.*, Antibodies:A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1998); Colligan *et al.*, Current Protocols in Immunology, Green Publishing Assoc., NY (1992; 1993); Muller, Meth. Enzym., 92:589-601 (1983)) and described in the examples below.

These methods include, but are not limited to, Biacore Analysis, Blitz analysis, flow cytometry and ELISA assay.

In an embodiment, the invention relates to an anti-SIRPa antibody as defined above that has an IC50 lower than 500 ng/ml, in particular lower than 400 ng/ml, 300 ng/ml, more particularly lower than 200 ng/ml, as determined in a competitive SIRPa binding assay between CD47 and the anti-SIRPa antibody by ELISA.

In an embodiment, the invention relates to an anti-SIRPa antibody as defined above that has an IC50 lower than 500 ng/ml, in particular lower than 400 ng/ml, 300 ng/ml, more particularly lower than 200 ng/ml, lower than 150 ng/ml and even more particularly lower than 100 ng/ml as determined by competition cytometry assay on human monocytes between CD47 and the anti-SIRPa antibody.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not specifically bind to SIRPg, preferably to human SIRPg.

Such an antibody of the invention does not affect or does not prevent the interaction between SIRPg and CD47.

As used herein, the term "SIRPg" relates to a signal regulatory protein gamma (also designated SIRP gamma, CD172g or SIRP beta 2), from a mammal species, preferably a human SIRPg.

A reference sequence of the human SIRPg protein, used in the examples of the present application, corresponds to the sequence associated to the Accession number Q9P1W8.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that has a KD value superior to 10^{-9} M, preferably superior to 10^{-8} M, more preferably superior to 10^{-7} M for SIRPg, in particular by Blitz analysis.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not significantly inhibit, antagonize, the binding of CD47 to SIRP-g, that does not significantly compete with the binding of CD47 to SIRPg.

This antagonist effect can be determined using the methods as defined the examples of the present application.

In the invention, it can be considered that an antibody (or antigen-binding fragment thereof or antigen-binding antibody mimetic) does not antagonize the binding of CD47 to SIRPg if said antibody (or antigen-binding fragment thereof or antigen-binding antibody mimetic) induces no increase, or induces an increase inferior to 1 log, of the KD value of CD47 in a SIRPg binding competitive assay by Blitz.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not specifically bind to T-cells, in particular to CD3+ T-cells.

In particular, the anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention does not bind to T-cells from mammal species, in particular to human T-cells.

In particular, it is considered that an anti-SIRPa antibody (or antigen-binding fragment thereof or antigen-binding antibody mimetic) does not specifically bind to human T cells if, in a population of human T cells isolated from PBMC from a healthy donor, less than 10%, preferably less than 5%, more preferably less than 2%, most preferably less than 1% of the population of human T cells are recognized by said anti-SIRPa antibody (or antigen-binding fragment thereof or antigen-binding antibody mimetic).

This effect can be measured by the methods as described in the examples of the present application.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not significantly inhibit the proliferation of T-cells, in particular CD3+ T-cells, preferably from mammal species and more preferably of human T cells.

In particular, it is considered that an anti-SIRPa antibody does not significantly inhibit the proliferation of T-cells if the proliferation of T-cells is reduced by less than 30%, preferably less than 20%, more preferably less than 10%, most preferably less than 5% as compared with a negative control.

The proliferation of T-cells can be determined by various methods. For example, the proliferation of T-cells can be measured by incorporation of H^3-thymidine as described in the examples of the present application.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not significantly inhibit, antagonize, the binding of the surfactant proteins to SIRP-a, that does not significantly compete with the binding of the surfactant proteins to SIRPa.

As used herein, the "surfactant proteins" are collagen-containing C-type (calcium dependent) lectins, which contribute significantly to surfactant homeostasis and pulmonary immunity (for review, see Kishore *et al.*, Surfactant proteins SP-A and SP-D: structure, function and receptors, Mol Immunol, 43(9), 1293-315, 2006).

As used herein, the term "surfactant protein" refers to a surfactant protein from a mammal species, preferably a human surfactant protein.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not inhibit the binding of the human surfactant protein D (SP-D) to SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not inhibit the binding of the human surfactant protein A (SP-A) to SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not antagonize the interaction between the surfactant proteins and SIRPa.

The competition between SIRPa and surfactant proteins can be determined by competitive assay using methods well known from the one skilled in the art. These methods include, but are not limited to, Biacore Analysis, Blitz analysis and ELISA assay.

In the invention, it can be considered that an antibody (or antigen-binding fragment thereof or antigen-binding antibody mimetic) does not antagonize the binding of a surfactant protein to SIRPa if said antibody (or antigen-binding fragment thereof or antigen-binding antibody mimetic) induces no increase, or induces an increase inferior to 1 log, of the KD value of the surfactant protein in a SIRPa binding competitive assay by Blitz.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that weakly binds, or does not specifically bind to SIRPb.

As used herein, the term "SIRPb" refers to a SIRPb protein (also designated as SIRPβ, signal-regulatory protein beta-1, SIRP-beta-1, CD172 antigen-like family member B or CD172b) from a mammal species, preferably a human SIRPb.

A reference sequence of the human SIRPb protein, used in the examples of the present application, corresponds to the sequence associated to the Accession number Q5TFQ8-1.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that has KD value superior to 10^{-9} M, preferably superior to 10^{-8} M for SIRPb, in particular by Blitz analysis.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 15 (NIDPSDSDTHYNQKFKD) or SEQ ID NO: 16 (NIDPSDSDTHYSPSFQG),

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 17 (GGTGTMAWFAY) SEQ ID NO: 18 (GGTGTLAWFAY), SEQ ID NO: 19 (GGTGTMAYFAY) or SEQ ID NO: 20 (GGTGTLAYFAY),

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23 (FQGTHVPYT).

18D5 / Variant A /Variant B

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 15 (NIDPSDSDTHYNQKFKD),

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 17 (GGTGTMAWFAY),

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23 (FQGTHVPYT).

Variant C

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 16 (NIDPSDSDTHYSPSFQG),

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 17 (GGTGTMAWFAY),

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23 (FQGTHVPYT).

Variant E

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 16 (NIDPSDSDTHYSPSFQG),

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 18 (GGTGTLAWFAY),

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23 (FQGTHVPYT).

Variant F

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 16 (NIDPSDSDTHYSPSFQG),

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 19 (GGTGTMAYFAY),

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23 (FQGTHVPYT).

Variant EF

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 16 (NIDPSDSDTHYSPSFQG),

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 20 (GGTGTLAYFAY),

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23 (FQGTHVPYT).

The anti-SIRPa antibodies of the invention may have a heavy chain variable region comprising the amino acid sequence of HCDR1 and/or HCDR2 and/or HCDR3 of the human antibodies as provided herein; and/or a light chain variable region comprising the amino acid sequence of LCDR1 and/or LCDR2 and/or LCDR3 of the human antibodies as provided herein.

In an embodiment, the antibody comprises an amino acid sequence variant of one or more of the CDRs of the provided human antibodies, which variant comprises one or more amino acid insertion(s) within or adjacent to a CDR residue and/or deletion(s) within or adjacent to a CDR residue and/or substitution(s) of CDR residue(s) (with substitution(s) being the preferred type of amino acid alteration for generating such variants).

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

- a heavy chain variable domain comprising or consisting of the amino acid sequence selected from SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, and/or

- a light chain variable domain comprising or consisting of the amino acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32 and SEQ ID NO: 33.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

- a heavy chain variable domain comprising or consisting of the amino acid sequence 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 and SEQ ID NO: 30, and

- a light chain variable domain comprising or consisting of the amino acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32 and SEQ ID NO: 33.

The sequences of the specific variable domains are given in Table 1 below.

Heavy chain variable domain of the wild-type antibody **(chimeric and mouse 18D5)**	QVQLQQPGAELVRPGSSVKLSCKASGYTFTSYWVHWVKQ RPIQGLEWIGNIDPSDSDTHYNQKFKDKASLTVDKSSSTAY MQLSSLTFEDSAVYYCVRGGTGTMAWFAYWGQGTLVTVS A	SEQ ID NO: 24
Heavy chain variable domain of humanized variant **(HA)**	EVQLVQSGAEVKKPGESLRISCKASGYTFTSYWVHWVRQM PGKGLEWIGNIDPSDSDTHYNQKFKDHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLVTVSS	SEQ ID NO: 25
Heavy chain variable domain of humanized variant **(HB)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYNQKFKDHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLVTVSS	SEQ ID NO: 26
Heavy chain variable domain of humanized	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLVTVSS	SEQ ID NO: 27

variant **(HC)**		
Heavy chain variable domain of humanized variant **(HE)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTLAWFAYWGQGTLVTVSS	SEQ ID NO: 28
Heavy chain variable domain of humanized variant **(HF)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAYFAYWGQGTLVTVSS	SEQ ID NO: 29
Heavy chain variable domain of humanized variant **(HEF)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTLAYFAYWGQGTLVTVSS	SEQ ID NO: 30
Light chain of the wild-type antibody **(chimeric and mouse 18D5)**	DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSYGNTYLYWY LQKPGQSPKLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRVE AEDLGVYFCFQGTHVPYTFGSGTKLEIK	SEQ ID NO: 31
Light chain variable domain of humanized variant A **(LA)**	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSYGNTYLYWY QQRPGQSPRLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYFCFQGTHVPYTFGGGTKVEIK	SEQ ID NO: 32
Light chain variable domain of humanized variant **(LB)**	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSYGNTYLYWF QQRPGQSPRLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYYCFQGTHVPYTFGGGTKVEIK	SEQ ID NO: 33

Table 1. Examples of heavy chain variable domains and light chain variable domains of antibodies according to the invention.

The sequences of the variable domains of the antibodies exemplified in the present invention can be deduced from the combinations of the sequences shown in Table 2.

Antibody	Heavy chain variable domain	Lightchain variable domain
18D5 (chimeric and mouse)	SEQ ID NO: 24	SEQ ID NO: 31
HALA	SEQ ID NO: 25	SEQ ID NO: 32
HALB	SEQ ID NO: 25	SEQ ID NO: 33
HBLA	SEQ ID NO: 26	SEQ ID NO: 32

HBLB	SEQ ID NO: 26	SEQ ID NO: 33
HCLA	SEQ ID NO: 27	SEQ ID NO: 32
HCLB	SEQ ID NO: 27	SEQ ID NO: 33
HELA	SEQ ID NO: 28	SEQ ID NO: 32
HELB	SEQ ID NO: 28	SEQ ID NO: 33
HFLA	SEQ ID NO: 29	SEQ ID NO: 32
HFLB	SEQ ID NO: 29	SEQ ID NO: 33
HEFLA	SEQ ID NO: 30	SEQ ID NO: 32
HEFLB	SEQ ID NO: 30	SEQ ID NO: 33

Table 2. Heavy chain variable domain and light chain variable domain of specific antibodies according to the invention.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises a light chain variable domain comprising or consisting of the amino acid sequence SEQ ID NO: 33.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

- a light chain variable domain comprising or consisting of the amino acid sequence SEQ ID NO: 33, and

- a heavy chain variable domain comprising or consisting of the amino acid sequence 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 and SEQ ID NO: 30,

preferably

- a heavy chain variable domain comprising or consisting of the amino acid sequence selected from the group consisting of SEQ ID NO: 29 and SEQ ID NO: 30,

more preferably

- a heavy chain variable domain comprising or consisting of the amino acid sequence selected from the group consisting of SEQ ID NO: 30,

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 24, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 31,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 25, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 25, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 26, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 26, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 27, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 27, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 28, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 28, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 29, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 29, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 30, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 30, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33.

In an embodiment, the antibody or antigen-binding fragment or antigen-binding antibody mimetic has no substitution of the amino acid W at position 33 (W33) in the heavy chain variable domain, said position being identified with respect to SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29 or SEQ ID NO: 30, and/or no substitution of the amino acids Y at position 39 (Y39), R at position 55 (R55) and/or F at position 60 (F60) in the light chain variable domain, said positions being identified with respect to SEQ ID NO: 31, SEQ ID NO: 32 or SEQ ID: 33, in particular has no substitution at position W33 in the heavy chain variable domain and no substitution at positions Y39, R55 and F60 in the light chain variable domain.

In the invention, the antibodies can be produced with any heavy chain and light chain constant domains.

In one embodiment, the anti-human SIRPa antibody of the invention is a humanized monoclonal antibody, in particular wherein the antibody light chain constant domain is derived from a human kappa light chain constant domain, more particularly wherein the light chain constant domain consists of the sequence of SEQ ID NO: 35, and wherein the antibody heavy chain constant domain is derived from a human IgG1, IgG2, IgG3, or IgG4 (wild type or mutated) heavy chain constant domain, in particular from a human IgG4 heavy chain constant domain, more particularly wherein the antibody heavy chain constant domain consists of the sequence with SEQ ID NO: 34.

As well known by one skilled in the art, the choice of IgG isotypes of the heavy chain constant domain centers on whether specific functions are required and the need for a suitable *in vivo* half-life. For example, antibodies designed for selective eradication of cancer cells typically require an active isotype that permits complement activation and effector-mediated cell killing by antibody-dependent cell-mediated cytotoxicity. Both human IgG1 and IgG3 (shorter half-life) isotypes meet these criteria, particularly human IgG1 isotype (wild type and variants). In particular, depending of the IgG isotype of the heavy chain constant domain (particularly human wild type and variants IgG1 isotype), the anti-human SIRPa antibody of the invention can be cytotoxic towards cells expressing SIRPa via a CDC, ADCC and/or ADCP mechanism (Salfeld, nature biotechnology, vol. 25, N°12, 2007; Irani et al. Molecular Immunology, vol. 67, issue 2, part A, 2015). In fact, the fragment crystallisable (Fc) region interacts with a variety of accessory molecules to mediate indirect effector functions such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC).

Heavy chain constant domain (IgG4m-S228P) SEQ ID NO: 34	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPGK
Light chain constant domain (CLkappa) SEQ ID NO: 35	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Table 3. Examples of a heavy chain constant domain and a light chain constant domain suitable for the antibodies according to the invention.

In an embodiment, the invention relates to an anti-SIRPa antibody as defined above that comprises:

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 56, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 57,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 36, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 43,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 37, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 44,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 37, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 45,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 38, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 44,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 38, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 45,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 39, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 44,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 39, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 45,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 40, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 44,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 40, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 45,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 41, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 44,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 41, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 45,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 42, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 44,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 42, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 45.

Heavy chain of the wild-type antibody (**mouse 18D5**)	QVQLQQPGAELVRPGSSVKLSCKASGYTFTSYWVHWVKQ RPIQGLEWIGNIDPSDSDTHYNQKFKDKASLTVDKSSSTAY MQLSSLTFEDSAVYYCVRGGTGTMAWFAYWGQGTLVTVS AAKTTPPSVYPLAPGCGDTTGSSVTLGCLVKGYFPESVTVT WNSGSLSSSVHTFPALLQSGLYTMSSSVTVPSSTWPSQTVT CSVAHPASSTTVDKKLEPSGPISTINPCPPCKECHKCPA PNLEGGPSVFIFPPNIKDVLMISLTPKVTCVVVDVSEDDPDV QISWFVNNVEVHTAQTQTHREDYNSTIRVVSTLPIQHQDW MSGKEFKCKVNNKDLPSPIERTISKIKGLVRAPQVYILPPPAE QLSRKDVSLTCLVVGFNPGDISVEWTSNGHTEENYKDTAPV LDSDGSYFIYSKLNMKTSKWEKTDSFSCNVRHEGLKNYYLKK TISRSPGK	SEQ ID NO: 56
Heavy chain of the wild-type antibody **(chimeric 18D5)**	QVQLQQPGAELVRPGSSVKLSCKASGYTFTSYWVHWVKQ RPIQGLEWIGNIDPSDSDTHYNQKFKDKASLTVDKSSSTAY MQLSSLTFEDSAVYYCVRGGTGTMAWFAYWGQGTLVTVS AASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPP CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGL PSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSPGK	SEQ ID NO: 36
Heavy chain of humanized variant **(HA)**	EVQLVQSGAEVKKPGESLRISCKASGYTFTSYWVHWVRQM PGKGLEWIGNIDPSDSDTHYNQKFKDHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD	SEQ ID NO: 37

	GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSPGK	
Heavy chain of humanized variant **(HB)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYNQKFKDHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSPGK	SEQ ID NO: 38
Heavy chain of humanized variant **(HC)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSPGK	SEQ ID NO: 39
Heavy chain of humanized variant **(HE)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTLAWFAYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSPGK	SEQ ID NO: 40
Heavy chain of humanized variant **(HF)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAYFAYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV	SEQ ID NO: 41

	VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSPGK	
Heavy chain of humanized variant **(HEF)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTLAYFAYWGQGTLVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCP PCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY TQKSLSLSPGK	SEQ ID NO: 42
Light chain of the wild-type antibody **(mouse 18D5)**	DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSYGNTYLYWY LQKPGQSPKLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRVE AEDLGVYFCFQGTHVPYTFGSGTKLEIKRADAAPTVSIFPPSS EQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNS WTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTS PIVKSFNRNEC	SEQ ID NO: 57
Light chain of the wild-type antibody **(chimeric 18D5)**	DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSYGNTYLYWY LQKPGQSPKLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRVE AEDLGVYFCFQGTHVPYTFGSGTKLEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 43
Light chain variable domain of humanized variant A **(LA)**	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSYGNTYLYWY QQRPGQSPRLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYFCFQGTHVPYTFGGGTKVEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 44
Light chain variable domain of humanized variant **(LB)**	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSYGNTYLYWF QQRPGQSPRLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYYCFQGTHVPYTFGGGTKVEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 45

Table 4. Heavy chain and light chain sequences of specific antibodies according to the invention.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that is able to induce the differentiation of monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, wherein said non suppressive cells secrete pro-inflammatory cytokines such as IL6, IL12 and TNF, and no or low level of anti-inflammatory cytokines such as IL10 and TGFβ.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, wherein said non suppressive cells express iNOS.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, wherein said non suppressive cells do not express the MHC Class II markers and express the markers CD80-CD86.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, wherein said non suppressive cells express at least one marker of the natural killer (NK) cells.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, wherein said antibody or antigen-binding fragment thereof is able to inhibit M2 polarization of macrophages and/or favors pro-inflammatory M1-type macrophages.

The antibodies of the invention can modify the macrophage polarization in order to induce a pro-inflammatory environment, *i.e.* they can inhibit the anti-inflammatory signal provided by M2-type macrophages and/or favor the pro-inflammatory signal provided by M1-type macrophages This approach allows to reestablish an inflammatory environment favorable to the action of the T effector cells, in particular in eliminating the cancer cells.

SIRPa ANTIBODIES

The embodiments recited for the antibodies as defined above are repeated *mutadis mutandis* to the antibodies recited in the other aspects of the invention.

In another aspect, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to a conformational epitope comprising at least one peptide selected from the group consisting of SEQ ID NO: 70 (ELIYNQKEGHFPR), SEQ ID NO: 71 (RNNMDFSIRIGN) and SEQ ID NO: 72 (SPRDITLKW) within SIRPa.

In another aspect, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to a conformational epitope

comprising or consisting of the peptides of SEQ ID NO: 70 (ELIYNQKEGHFPR), SEQ ID NO: 71 (RNNMDFSIRIGN) and SEQ ID NO: 72 (SPRDITLKW) within SIRPa.

In another aspect, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to a conformational epitope comprising at least one peptide selected from the group consisting of SEQ ID NO: 70 (ELIYNQKEGHFPR) and SEQ ID NO: 71 (RNNMDFSIRIGN).

In another aspect, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to a conformational epitope comprising or consisting of the peptides of SEQ ID NO: 70 (ELIYNQKEGHFPR) and SEQ ID NO: 71 (RNNMDFSIRIGN) within SIRPa.

In another aspect, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to a conformational epitope comprising at least one peptide selected from the group consisting of SEQ ID NO: 73 (YNQK) and SIR.

In another aspect, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to a conformational epitope comprising or consisting of the peptides of SEQ ID NO: 73 (YNQK) and SIR within SIRPa.

In another aspect, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of a peptide of the amino acid sequence SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of a peptide of the amino acid sequence SEQ ID NO: 15 (NIDPSDSDTHYNQKFKD) or SEQ ID NO: 16 (NIDPSDSDTHYSPSFQG),

- HCDR3 comprising or consisting of a peptide of the amino acid sequence SEQ ID NO: 17 (GGTGTMAWFAY), SEQ ID NO: 18 (GGTGTLAWFAY), SEQ ID NO: 19 (GGTGTMAYFAY) or SEQ ID NO: 20 (GGTGTLAYFAY),

- LCDR1 comprising or consisting of a peptide of the amino acid sequence SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of a peptide of the amino acid sequence SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of a peptide of the amino acid sequence SEQ ID NO: 23 (FQGTHVPYT).

The invention also relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg, in particular which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

APPLICATIONS

In another aspect, the invention relates to:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

for use as a medicament.

The present invention also relates to a method of treatment in a subject in need thereof comprising administering to said subject an effective amount of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

The present invention also relates to the use of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or

which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

in the manufacture of a medicament.

In another aspect, the invention relates to:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

for use in the treatment of any condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

As defined herein, "a condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells" corresponds to a cancer including inflammatory cancers and cancers with infiltrated myeloid cells (in particular with infiltrated MDSCs and/or TAM cells), an infectious disease, a trauma, an auto-immune disease (such as rheumatoid arthritis, type 1 diabetes, lupus, psoriasis), a vaccination, a chronic inflammatory diseases (such as Inflammatory bowel diseases including Crohn disease and Ulcerative colitis), a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis or a transplant dysfunctions.

The present invention also relates to a method of treatment of any condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells in a subject in need thereof comprising administering to said subject an effective amount of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or

which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

The present invention also relates to the use of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

in the manufacture of a medicament for the treatment of any condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

In an embodiment, the invention relates to:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

for use in the treatment of any condition susceptible of being improved or prevented by modifying macrophage polarization to pro-inflammatory macrophages.

Indeed, SIRPa acts as a checkpoint inhibitor and participates to macrophage polarization. In particular, blocking SIRPa induces a pro-inflammatory function of macrophages associated to type 1 macrophages (M1 pro-inflammatory = M (IFNg)) and inhibits the suppressive activity of macrophages in the tumor, since the pro-inflammatory profile of macrophages is obtained at the expense of type 2 macrophages (M2 type high phagocytic activity = M (IL4)). Thus, an antagonist of SIRPa is able to

inhibit M2 phenotypic polarization of macrophages and/or favors pro-inflammatory M1-type macrophage function and can be used in therapeutic.

As defined herein, "a condition susceptible of being improved or prevented by modifying macrophage polarization to favor pro-inflammatory macrophages" corresponds for example to a solid cancer, a liquid cancer, an infectious disease, a trauma, an auto-immune disease, a vaccination, a brain injury, a nerve injury, a polycythemia, a hemochromatosis or a chronic inflammatory disease.

The present invention also relates to a method of treatment of any condition susceptible of being improved or prevented by modifying macrophage polarization to pro-inflammatory macrophages in a subject in need thereof comprising administering to said subject an effective amount of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

In an embodiment, the invention also relates to the use of :

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

in the manufacture of a medicament for the treatment of any condition susceptible of being improved or prevented by modifying macrophage polarization to pro-inflammatory macrophages.

Modifying the polarization of macrophages to favor pro-inflammatory cells can be useful in a number of pathologies or situations. As described above, this modification is particularly useful in the context of cancers, to restore an anti-tumor activity of macrophages and/or prevent the pro-tumoral activity of M2-type macrophages. Inappropriate immune responses due to an excess of M2-type

macrophage polarization also occur in infectious diseases, fibrosis, vaccination, trauma and chronic inflammatory diseases.

Thus, according to a particular embodiment, anti-SIRPa antibody can be used to treat an individual who has a cancer selected from the group consisting of lung cancers, mesothelioma cancers, ovary cancers, liver cancers, bladder cancers, brain cancers, breast cancers, colon cancers, sarcomas, pancreas cancers, head and neck cancers, kidney cancers, thymomas, gliomas, melanomas and hematologic cancers such as lymphomas (Hodgkin's lymphoma and non-Hodgkin's lymphoma), leukemias such as T and B Acute or Chronic Lymphoblastic Leukemia (ALL or CLL) or Acute or Chronic myeloid leukemia (AML or CML) and Myelomas.

In an embodiment, the invention relates to:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

for use in the treatment of a pathology selected from the group consisting of a cancer (in particular inflammatory cancers and cancers with infiltrated myeloid cells particularly with infiltrated MDSCs and/or TAM cells), an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis, obesity, type II diabetes and a transplant dysfunction.

In an embodiment, the invention relates to a method of treatment of a pathology selected from the group consisting of a cancer (in particular inflammatory cancers and cancers with infiltrated myeloid cells particularly with infiltrated MDSCs and/or TAM cells), an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis, obesity, type II diabetes and a transplant dysfunction in a subject in need thereof comprising administering to said subject an effective amount of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

In an embodiment, the invention relates to the use of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

in the manufacture of a medicament for the treatment of a pathology selected from the group consisting of a cancer (in particular inflammatory cancers and cancers with infiltrated myeloid cells particularly with infiltrated MDSCs and/or TAM cells), an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis, obesity, type II diabetes and a transplant dysfunction.

In an embodiment, the invention relates to an anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, for its uses as defined above, wherein said anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention is administered to a patient presenting a SIRPa-positive tumor.

In an embodiment, the invention relates to:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or

which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

for use in vaccination.

In an embodiment, the invention relates to a method of vaccination of a subject comprising administering to said subject an effective amount of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

In an embodiment, the invention relates to the use of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

for the manufacture of a vaccine.

Suppressive myeloid cells limit the effectiveness of vaccination, especially in young children. Thus, an anti-SIRPa /g would limit the benefit provided by an anti-SIRPa on the vaccine response, preventing T lymphocytes from responding to vaccination.

The antibody or antigen-binding fragment thereof of the invention can be administered in a variety of suitable routes, *e.g.*, intravenously (IV), subcutaneously (SC), or, intramuscularly (IM) to the subject.

The antibody or antigen-binding fragment thereof can be administered alone or in combination with another therapeutic agent, *e.g.*, a second human monoclonal antibody or antigen binding fragment thereof. In another example, the antibody is administered together with another agent, for example, an immunosuppressive agent, an erythropoiesis-stimulating agent (ESA), in combination with therapeutic cell compositions, and the like.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic for its use as defined above, wherein the anti-SIRPa antibody or antigen-binding fragment is combined with a second therapeutic agent.

In particular, anti-SIRPa antibodies of the present invention can be combined with some other potential strategies for overcoming tumor immune evasion mechanisms with agents in clinical development or already on the market (see table 1 from Antonia *et al.* Immuno-oncology combinations: a review of clinical experience and future prospects. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. *20*, 6258–6268, 2014):

1- Reversing the inhibition of adaptive immunity (blocking T-cell checkpoint pathways), for example by using an anti-CTLA4, an anti-PD1 or an anti-PD-L1 molecule;

2- Switching on adaptive immunity (promoting T-cell costimulatory receptor signaling using agonist molecules, in particular antibodies), for example by targeting CD137 (4-1BB) using agonist molecules including agonist anti-CD137 (anti-4-1BB) antibodies or CD137 (4-1BB) ligands ;

3- Improving the function of innate immune cells;

4- Activating the immune system (potentiating immune-cell effector function), for example through vaccine-based strategies.

The administration of the second therapeutic agent can be simultaneous or not with the administration of the anti-SIRPa antibody. Depending on the nature of the second agent, a co-administration can be prepared in the form of a combination drug (product), also known as a "combo". A combo is a fixed-dose combination that includes two or more active pharmaceutical ingredients combined in a single dosage form, which is manufactured and distributed in fixed doses. But the dose regimen and/or the administration route can also differ.

In a preferred embodiment, this second therapeutic agent is selected from the group consisting of chemotherapeutic agents, radiotherapy agents, immunotherapeutic agents, cell therapy agents (such as CAR-T cells), antibiotics and probiotics.

In particular, immunotherapeutic agents useful in the context of the invention are selected from the group consisting of therapeutic vaccines (DNA, RNA or peptide vaccins), immune checkpoint blockers or activators, in particular of adaptive immune cells (T or B lymphocytes) or immunoconjugates such as antibody-drug conjugates.

As used herein, the term "immunotherapeutic agents" refers in particular to agents that could take cancer vaccines from interesting biological phenomena to effective therapeutic agents including: T-cell growth factors to increase number and repertoire of naive T cells, growth factors to increase the number of dendritic cells (DCs), agonists to activate DCs and other antigen-pre senting cells (APCs), adjuvants to allow and augment cancer vaccines, agonists to activate and stimulate T cells, inhibitors of T-cell checkpoint blockade, T-cell growth factors to increase the growth and survival of immune T cells, agents to inhibit, block, or neutralize cancer cell and immune cell-derived immunosuppressive cytokine.

Numerous immune checkpoint blocker or activator are known in the art. In the context of the invention, examples of immune checkpoint blockers or activators of adaptive immune cells (B or T lymphocytes) that could be useful are anti-PDL1, anti-PD1, anti-CTLA4, anti-CD137, anti-CD2, anti-CD28, anti-CD40, anti-HVEM, anti-BTLA, anti-CD160, anti-TIGIT, anti-TIM-1/3, anti-LAG-3, anti-2B4, and anti-OX40, anti-CD40 agonist, CD40-L, TLR agonists, anti-ICOS, ICOS-L and B-cell receptor agonists, in particular anti-PDL1, anti-PD1, anti-CD137.

Said immunotherapeutic agent can also be an antibody targeting tumoral antigen, particularly selected from the group consisting of anti-Her2, anti-EGFR, anti-CD20, anti-CD19, anti-CD52.

The antibody may be provided at an effective dose from about 1 ng/kg body weight to about 30 mg/kg body weight, or more. In specific embodiments, the dosage may range from 1 µg/kg to about 20 mg/kg, optionally from 10 µg/kg up to 10 mg/kg or from 100 µg/kg up to 5 mg/kg.

The term "effective dose" or "effective dosage" or "effective amount" is defined as an amount sufficient to achieve or at least partially achieve the desired effect. The term "effective dose" is meant to encompass an amount sufficient to cure or at least partially arrest the disease and its complications in a patient already suffering from the disease. Amounts or doses effective for this use will depend on the condition to be treated, the delivered antibody construct, the therapeutic context and objectives, the severity of the disease, prior therapy, the patient's clinical history and response to the therapeutic agent, the route of administration, the size (body weight, body surface or organ size) and/or condition (the age and general health) of the patient, and the general state of the patient's own immune system. The proper dose can be adjusted such that it can be administered to the patient once or over a series of administrations, and in order to obtain the optimal therapeutic effect.

Dosing for such purposes may be repeated as required, *e.g.* daily, semi-weekly, weekly, semi-monthly, monthly, or as required during relapses.

In an aspect, the invention also relates to:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

for use in a diagnostic test, particularly in personalized medicine, more particularly in a companion diagnostic test.

In an embodiment, the invention relates to a method of diagnostic, particularly in personalized medicine, more particularly in a companion diagnostic test, using:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

In an embodiment, the invention relates to the use of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

in the manufacture of a medicament for a diagnostic test, particularly in personalized medicine, more particularly in a companion diagnostic test.

In an aspect, the invention also relates to an *in vitro* or *ex vivo* method of diagnosis, in particular a method of diagnostic suitable for use in personalized medicine, more particularly in a companion diagnosis, wherein an anti-SIRPa antibody or an antigen-binding fragment thereof or an antigen-

binding mimetic thereof of the invention is used for the detection of SIRPa+ cells in a sample previously obtained from a subject and optionally for the quantification of the expression of SIRPa.

In an aspect, the invention also relates to the use of an anti-SIRPa antibody or an antigen-binding fragment thereof or an antigen-binding mimetic of the invention, in the manufacture of a medicament suitable for use in a diagnostic test, in particular for use in personalized medicine, or in a companion diagnostic test.

In an aspect, the invention also relates to the use of at least one anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention, as a means for determination of the expression and/or level of expression of SIRPa in a biological sample previously obtained from an individual.

In an aspect, the invention also relates to an *in vitro* or *ex vivo* method to determine a SIRPa positive cells in a subject from a biological sample previously obtained from said subject, comprising:

i) determining *in vitro* the expression and/or the level of expression of SIRPa, in a biological sample previously obtained from said subject using the anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention.

In an aspect, the invention also relates to the use, in particular *in vitro* or *ex vivo,* of at least one anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention in a method wherein SIRPa is used as a biomarker that is predictive for the response to a treatment in a subject, in particular in a cancer subject.

In an aspect, the invention also relates to an *in vitro* or *ex vivo* method of predicting the response of a cancer subject to a treatment, in particular with anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention, comprising:

- determining the expression level of SIRPa in a tumour sample previously obtained from a subject, in particular with anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention, and
- comparing the expression level of SIRPa to a value representative of an expression level of SIRPa in a non-responding subject population,

wherein a higher expression level of SIRPa in the tumour sample of the subject is indicative for a subject who will respond to the treatment.

In an aspect, the invention also relates to a method of *in vitro* or *ex vivo* determining the presence of SIRPa+ cells in a sample previously obtained from a subject which comprises determining presence of SIRPa as a biomarker that is predictive for the response of a subject to a treatment, in particular a response of a subject diagnosed with a cancer, wherein said method comprises:

- determining the expression level of SIRPa in a tumor sample previously obtained from a subject, in particular with anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 13, and

- comparing the expression level of SIRPa to a value representative of an expression level of SIRPa in a non-responding subject population,

wherein a higher expression level of SIRPa in the tumor sample of the subject is indicative for a patient who will respond to the treatment.

COMPOSITIONS

In another aspect, the invention relates to a pharmaceutical composition comprising an antibody or antigen-binding fragment thereof as defined above and a pharmaceutically acceptable carrier.

As used herein, a "pharmaceutical composition" is meant to encompass a composition suitable for administration to a subject or patient, such as a mammal, especially a human. In general, a "pharmaceutical composition" is sterile and is usually free of contaminants that are capable of eliciting an undesirable response within the subject (*e.g.*, the compound(s) in the pharmaceutical composition is pharmaceutical grade). Pharmaceutical compositions can be designed for administration to subjects or patients in need thereof via a number of different routes of administration including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, intracheal and the like.

As used herein, a "pharmaceutically acceptable carrier" is meant to encompass an excipient, diluent, carrier, and adjuvant that are useful in preparing a pharmaceutical composition that are generally safe, non-toxic and neither biologically nor otherwise undesirable, and include an excipient, diluent, carrier, and adjuvant that are acceptable for veterinary use as well as human pharmaceutical use. "A pharmaceutically acceptable carrier" as used herein includes both one and more than one such excipient, diluent, carrier, and adjuvant.

In particular, the invention relates to a pharmaceutical composition which comprises as an active ingredient an antibody or antigen-binding fragment thereof as defined above and a pharmaceutically acceptable carrier.

COMBINATION PRODUCTS

In another aspect, the invention relates to a therapeutic means, in particular a combination product means, which comprises as active ingredients: an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above and a second therapeutic agent, wherein said active ingredients are formulated for separate, sequential or combined therapy, in particular for combined or sequential use.

In particular, the invention relates to a combination product comprising an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above and a second therapeutic agent for simultaneous, separate or sequential use a medicament.

In an embodiment, the invention relates to a combination product as defined above, wherein the second therapeutic agent is selected from the group consisting of chemotherapeutic agents, radiotherapy agents, cell therapy agents, immunotherapeutic agents, antibiotics and probiotics.

In an embodiment, the invention relates to a combination product as defined above, wherein said immunotherapeutic agent is selected from the group consisting of therapeutic vaccines, immune checkpoint blockers or activators, in particular of adaptive immune cells (T and B lymphocytes) and antibody-drug conjugates.

In an embodiment, the invention relates to a combination product as defined above, wherein said immune checkpoint blocker or activator of adaptive immune cells (T and B lymphocytes) is selected from the group consisting of anti-PDL1, anti-PD1, anti-CTLA4, anti-CD137, anti-CD2, anti-CD28, anti-CD40, anti-HVEM, anti-BTLA, anti-CD160, anti-TIGIT, anti-TIM-1/3, anti-LAG-3, anti-2B4, and anti-OX40, anti-CD40 agonist, CD40-L, TLR agonists, anti-ICOS, ICOS-L and B-cell receptor agonists, in particular selected from the group consisting of anti-PDL1, anti-PD1 and anti-CD137.

In one embodiment, said immunotherapeutic agent is an antibody targeting tumoral antigen, particularly selected from the group consisting of anti-Her2, anti-EGFR, anti-CD20, anti-CD19, anti-CD52.

In an aspect, the invention relates to a combination product as defined above, for simultaneous, separate or sequential use in the treatment of any condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

In an embodiment, the invention relates to a method of treatment of any condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells in a subject in need thereof comprising administering simultaneously, separately or sequentially to said subject an effective amount of a combination product as defined above.

In an embodiment, the invention relates to the use of a combination product as defined above in the manufacture of a medicament for the treatment any condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

In an aspect, the invention relates to a combination product as defined above, for simultaneous, separate or sequential use in the treatment of any condition susceptible of being improved or prevented by modifying macrophage polarization to pro-inflammatory macrophages.

In an embodiment, the invention relates to a method of treatment of any condition susceptible of being improved or prevented by modifying macrophage polarization to pro-inflammatory macrophages in a subject in need thereof comprising administering simultaneously, separately or sequentially to said subject an effective amount of a combination product as defined above.

In an embodiment, the invention relates to the use of a combination product as defined above in the manufacture of a medicament for the treatment any condition susceptible of being improved or prevented by modifying macrophage polarization to pro-inflammatory macrophages.

In an aspect, the invention relates to a combination product as defined above, for simultaneous, separate or sequential use in the treatment of a pathology selected from the group consisting of a cancer, an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis, obesity, type II diabetes and a transplant dysfunction or for use in vaccination.

In an embodiment, the invention relates to a method of treatment of a pathology selected from the group consisting of a cancer, an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis, obesity, type II diabetes and a transplant dysfunction of a subject in need thereof comprising administering simultaneously, separately or sequentially to said subject an effective amount of a combination product as defined above.

In an embodiment, the invention relates to the use of a combination product as defined above, in the manufacture of a medicament for the treatment of a pathology selected from the group consisting of a cancer, an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis, obesity, type II diabetes and a transplant dysfunction or for use in vaccination.

NUCLEIC ACIDS

In another aspect, the invention relates to an isolated nucleic acid molecule encoding an antibody or antigen-binding fragment thereof as defined above.

As used herein, a nucleic acid molecule can be double stranded and single stranded, linear and circular. It is preferably comprised in a vector which is preferably comprised in a host cell.

In an embodiment, the invention relates to an isolated nucleic acid molecule encoding an antibody or antigen-binding fragment thereof as defined above, said nucleic acid molecule comprising or consisting of at least one sequence selected from SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ

ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 and SEQ ID NO: 69.

In an embodiment, the invention relates to an isolated nucleic acid molecule encoding an antibody or antigen-binding fragment thereof as defined above, said nucleic acid molecule comprising:

- a sequence encoding the heavy chain variable domain of said antibody, preferably comprising:
  - SEQ ID NO: 58, SEQ ID NO: 59 or SEQ ID NO: 60,
  - SEQ ID NO: 61 or SEQ ID NO: 62, and
  - SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 66,

and/or

- a sequence encoding the light chain variable domain of said antibody, preferably comprising:
  - SEQ ID NO: 67,
  - SEQ ID NO: 68, and
  - SEQ ID NO: 69.

Nucleic acid sequences coding the HCDR1 (corresponding to the amino acid sequence SEQ ID NO: 14)	AGCTATTGGGTGCAC TCTTATTGGGTGCAC TCCTATTGGGTGCAC	SEQ ID NO: 58 SEQ ID NO: 59 SEQ ID NO: 60
Nucleic acid sequence coding the HCDR2 (corresponding to the amino acid sequence SEQ ID NO: 15)	AACATCGACCCCAGCGACTCTGATACC CATTACAATCAGAAGTTTAAGGAC	SEQ ID NO: 61
Nucleic acid sequence coding the HCDR2 (corresponding to the amino acid sequence SEQ ID NO: 16)	AACATCGACCCCAGCGACTCTGATACA CACTACTCCCCTAGCTTTCAGGGC	SEQ ID NO: 62
Nucleic acid sequence coding the HCDR3 (corresponding to the amino acid sequence SEQ ID NO: 17)	GGAGGAACCGGAACAATGGCTTGGTT TGCTTAC	SEQ ID NO: 63
Nucleic acid sequence coding the HCDR3 (corresponding to the amino acid sequence SEQ ID NO: 18)	GGAGGAACCGGCACACTGGCTTGGTT CGCTTAC	SEQ ID NO: 64
Nucleic acid sequence coding the HCDR3 (corresponding to the amino acid sequence SEQ ID NO: 19)	GGAGGAACCGGAACAATGGCTTACTT CGCTTAT	SEQ ID NO: 65
Nucleic acid sequence coding the HCDR3	GGAGGAACCGGCACACTGGCTTACTT	SEQ ID NO: 66

(corresponding to the amino acid sequence SEQ ID NO: 20)	CGCTTAT	
Nucleic acid sequence coding the LCDR1 (corresponding to the amino acid sequence SEQ ID NO: 21)	AGGTCCAGCCAGTCCCTGGTGCACAG CTATGGCAACACATACCTGTAT	SEQ ID NO: 67
Nucleic acid sequence coding the LCDR2 (corresponding to the amino acid sequence SEQ ID NO: 22)	AGGGTGTCTAATCGGTTCTCC	SEQ ID NO: 68
Nucleic acid sequence coding the LCDR3 (corresponding to the amino acid sequence SEQ ID NO: 23)	TTTCAGGGCACCCATGTGCCATACACA	SEQ ID NO: 69

Table 5. Sequences coding the CDRs of the heavy chain variable domains and the CDRs of the light chain variable domains of antibodies according to the invention.

In particular, the invention relates to a nucleic acid molecule comprising or consisting of a sequence selected from SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 and SEQ ID NO: 55.

Nucleic acid sequence coding the heavy chain variable domain of the wild-type antibody **(chimeric and mouse 18D5)**	CAGGTGCAGCTGCAGCAGCCAGGAGCTGAGCTGGTGAG GCCTGGCTCCAGCGTGAAGCTGTCCTGCAAGGCTAGCGG CTACACCTTCACAAGCTATTGGGTGCACTGGGTGAAGCA GCGGCCAATCCAGGGCCTGGAGTGGATCGGCAACATCG ACCCCAGCGACTCTGATACCCATTACAATCAGAAGTTTAA GGACAAGGCCTCTCTGACCGTGGATAAGTCTTCCAGCAC AGCTTATATGCAGCTGTCTTCCCTGACATTCGAGGATTCC GCCGTGTACTATTGCGTGAGGGGAGGAACCGGAACAAT GGCTTGGTTTGCTTACTGGGGCCAGGGCACCCTGGTGAC AGTGTCTGCT	SEQ ID NO: 46
Nucleic acid sequence coding the heavy chain variable domain of humanized variant **(HA)**	GAGGTGCAGCTGGTGCAGAGCGGAGCTGAGGTGAAGA AGCCAGGCGAGTCTCTGAGGATCTCCTGCAAGGCTAGCG GCTACACCTTCACATCTTATTGGGTGCACTGGGTGCGGC AGATGCCAGGCAAGGGCCTGGAGTGGATCGGCAACATC GACCCTAGCGACTCTGATACCCACTACAATCAGAAGTTTA AGGACCATGTGACCCTGTCTGTGGATAAGTCCATCAGCA CAGCCTATCTGCAGCTGTCCAGCCTGAAGGCCTCCGATAC AGCTATGTACTATTGCGTGAGGGGAGGAACCGGAACAA TGGCTTGGTTCGCTTACTGGGGCCAGGGCACCCTGGTGA CAGTGTCTTCC	SEQ ID NO: 47
Nucleic acid sequence coding the heavy chain	GAGGTGCAGCTGGTGCAGTCCGGAGCTGAGGTGAAGAA GCCAGGCGAGTCTCTGAGGATCTCCTGCAAGGCTTCTGG CTACTCCTTCACCAGCTATTGGGTGCACTGGGTGCGGCA	SEQ ID NO: 48

variable domain of humanized variant **(HB)**	GATGCCAGGCAAGGGCCTGGAGTGGATGGGCAACATCG ACCCTAGCGACTCTGATACACACTACAATCAGAAGTTTAA GGACCATGTGACCCTGAGCGTGGATAAGTCCATCAGCAC AGCCTATCTGCAGCTGTCCAGCCTGAAGGCCTCTGATACC GCTATGTACTATTGCGTGAGGGGAGGAACCGGAACAAT GGCTTGGTTCGCTTACTGGGGCCAGGGCACCCTGGTGAC AGTGTCTTCC	
Nucleic acid sequence coding the heavy chain variable domain of humanized variant **(HC)**	GAGGTGCAGCTGGTGCAGTCTGGCGCCGAGGTGAAGAA GCCAGGCGAGAGCCTGAGGATCTCTTGCAAGGCTAGCG GCTACTCTTTCACCTCCTATTGGGTGCACTGGGTGCGGCA GATGCCAGGCAAGGGCCTGGAGTGGATGGGCAACATCG ACCCCAGCGACTCTGATACACACTACTCCCCTAGCTTTCA GGGCCATGTGACCCTGTCCGTGGACAAGTCTATCTCCAC AGCCTATCTGCAGCTGTCCAGCCTGAAGGCCAGCGATAC CGCTATGTACTATTGCGTGAGGGGAGGAACCGGAACAAT GGCTTGGTTCGCTTACTGGGGCCAGGGCACCCTGGTGAC AGTGTCTTCC	SEQ ID NO: 49
Nucleic acid sequence coding the heavy chain variable domain of humanized variant **(HE)**	GAGGTGCAGCTGGTGCAGTCTGGCGCCGAGGTGAAGAA GCCAGGCGAGAGCCTGAGGATCTCTTGCAAGGCTAGCG GCTACTCTTTCACCTCCTATTGGGTGCACTGGGTGCGGCA GATGCCAGGCAAGGGCCTGGAGTGGATGGGCAACATCG ACCCCAGCGACTCTGATACACACTACTCCCCTAGCTTTCA GGGCCATGTGACCCTGTCCGTGGACAAGTCTATCTCCAC AGCCTATCTGCAGCTGTCCAGCCTGAAGGCCAGCGATAC CGCTATGTACTATTGCGTGAGGGGAGGAACCGGCACACT GGCTTGGTTCGCTTACTGGGGCCAGGGCACCCTGGTGAC AGTGTCTTCC	SEQ ID NO: 50
Nucleic acid sequence coding the heavy chain variable domain of humanized variant **(HF)**	GAGGTGCAGCTGGTGCAGTCTGGCGCCGAGGTGAAGAA GCCAGGCGAGAGCCTGAGGATCTCTTGCAAGGCTAGCG GCTACTCTTTCACCTCCTATTGGGTGCACTGGGTGCGGCA GATGCCAGGCAAGGGCCTGGAGTGGATGGGCAACATCG ACCCCAGCGACTCTGATACACACTACTCCCCTAGCTTTCA GGGCCATGTGACCCTGTCCGTGGACAAGTCTATCTCCAC AGCCTATCTGCAGCTGTCCAGCCTGAAGGCCAGCGATAC CGCTATGTACTATTGCGTGAGGGGAGGAACCGGAACAAT GGCTTACTTCGCTTATTGGGGCCAGGGCACCCTGGTGAC AGTGTCTTCC	SEQ ID NO: 51
Nucleic acid sequence coding the heavy chain variable domain of humanized variant **(HEF)**	GAGGTGCAGCTGGTGCAGTCTGGCGCCGAGGTGAAGAA GCCAGGCGAGAGCCTGAGGATCTCTTGCAAGGCTAGCG GCTACTCTTTCACCTCCTATTGGGTGCACTGGGTGCGGCA GATGCCAGGCAAGGGCCTGGAGTGGATGGGCAACATCG ACCCCAGCGACTCTGATACACACTACTCCCCTAGCTTTCA GGGCCATGTGACCCTGTCCGTGGACAAGTCTATCTCCAC AGCCTATCTGCAGCTGTCCAGCCTGAAGGCCAGCGATAC CGCTATGTACTATTGCGTGAGGGGAGGAACCGGCACACT GGCTTACTTCGCTTATTGGGGCCAGGGCACCCTGGTGAC AGTGTCTTCC	SEQ ID NO: 52
Nucleic acid sequence coding the light chain of the wild-type antibody	GACGTGGTCATGACCCAGACACCACTGAGCCTGCCCGTG TCCCTGGGCGATCAGGCCTCTATCTCCTGCAGGTCCAGCC AGTCCCTGGTGCACAGCTATGGCAACACATACCTGTATTG GTACCTGCAGAAGCCAGGCCAGTCCCCCAAGCTGCTGAT	SEQ ID NO: 53

(chimeric and mouse 18D5)	CTACAGGGTGTCTAATCGGTTCTCCGGCGTGCCTGACAG GTTCTCCGGCTCTGGCTCCGGCACCGATTTCACACTGAAG ATCAGCAGGGTGGAGGCTGAGGACCTGGGCGTGTATTT CTGTTTTCAGGGCACCCATGTGCCATACACATTTGGCTCT GGCACCAAGCTGGAGATCAAG	
Nucleic acid sequence coding the light chain variable domain of humanized variant A **(LA)**	GACGTGGTCATGACACAGAGCCCACTGTCTCTGCCTGTG ACCCTGGGACAGCCAGCCTCTATCTCCTGCAGGTCCAGCC AGTCCCTGGTGCACAGCTATGGCAACACATACCTGTATTG GTACCAGCAGAGGCCCGGACAGAGCCCAAGGCTGCTGA TCTACAGGGTGTCTAATCGGTTCTCCGGCGTGCCTGACA GGTTTAGCGGCTCTGGCTCCGGCACCGATTTCACACTGA AGATCTCTAGAGTGGAGGCTGAGGATGTGGGCGTGTAT TTCTGTTTTCAGGGCACCCATGTGCCATACACATTTGGCG GCGGCACCAAGGTGGAGATCAAG	SEQ ID NO: 54
Nucleic acid sequence coding the light chain variable domain of humanized variant **(LB)**	GACGTGGTCATGACACAGAGCCCACTGTCTCTGCCTGTG ACCCTGGGACAGCCAGCCTCTATCTCCTGCAGGTCCAGCC AGTCCCTGGTGCACAGCTACGGCAACACATACCTGTATT GGTTCCAGCAGAGGCCCGGACAGAGCCCAAGGCTGCTG ATCTATAGGGTGTCTAATCGGTTCTCCGGCGTGCCTGACA GGTTTAGCGGATCTGGATCCGGAACCGACTTCACCCTGA AGATCTCTAGAGTGGAGGCTGAGGATGTGGGCGTGTAC TATTGTTTCCAGGGCACCCATGTGCCATACACATTTGGCG GCGGCACCAAGGTGGAGATCAAG	SEQ ID NO: 55

Table 6. Sequences coding the heavy chain variable domains and the light chain variable domains of antibodies according to the invention.

VECTORS

In another aspect, the invention relates to a vector comprising the nucleic acid molecule as defined above.

As used herein, a "vector" is a nucleic acid molecule used as a vehicle to transfer genetic material into a cell. The term "vector" encompasses plasmids, viruses, cosmids and artificial chromosomes. In general, engineered vectors comprise an origin of replication, a multicloning site and a selectable marker. The vector itself is generally a nucleotide sequence, commonly a DNA sequence, that comprises an insert (transgene) and a larger sequence that serves as the "backbone" of the vector. Modern vectors may encompass additional features besides the transgene insert and a backbone: promoter, genetic marker, antibiotic resistance, reporter gene, targeting sequence, protein purification tag. Vectors called expression vectors (expression constructs) specifically are for the expression of the transgene in the target cell, and generally have control sequences.

HOST CELLS

In another aspect, the invention relates to an isolated host cell comprising a vector as defined above.

As used herein, the term "host cell" is intended to include any individual cell or cell culture that can be or has been recipient of vectors, exogenous nucleic acid molecules, and polynucleotides encoding the antibody construct of the present invention; and/or recipients of the antibody construct itself. The introduction of the respective material into the cell can be carried out by way of transformation, transfection and the like. The term "host cell" is also intended to include progeny or potential progeny of a single cell. Suitable host cells include prokaryotic or eukaryotic cells, and also include but are not limited to bacteria, yeast cells, fungi cells, plant cells, and animal cells such as insect cells and mammalian cells, *e.g.*, murine, rat, rabbit, macaque or human.

KITS

In another aspect, the invention relates to a kit comprising:

- an antibody or antigen-binding fragment thereof as defined above,
- a nucleic acid molecule coding said antibody or antigen-binding,
- a vector comprising said nucleic acid molecule, and/or
- a cell comprising said vector.

As a matter of convenience, the antibody of the present invention can be provided in a kit, i.e., a packaged combination of reagents.

In the context of the present invention, the term "kit" means two or more components (one of which corresponding to the antibody or antigen-binding thereof, the nucleic acid molecule, the vector or the cell of the invention) packaged together in a container, recipient or otherwise. A kit can hence be described as a set of products and/or utensils that are sufficient to achieve a certain goal, which can be marketed as a single unit.

The kit may comprise one or more recipients (such as vials, ampoules, containers, syringes, bottles, bags) of any appropriate shape, size and material containing the antibody construct of the present invention in an appropriate dosage for administration. The kit may additionally contain directions for use (e.g. in the form of a leaflet or instruction manual), means for administering the antibody construct of the present invention such as a syringe, pump, infuser or the like, means for reconstituting the antibody construct of the invention and/or means for diluting the antibody construct of the invention.

In an embodiment, the invention relates to a kit as defined above for a single-dose administration unit. The kit of the invention may also contain a first recipient comprising a dried/lyophilized antibody construct and a second recipient comprising an aqueous formulation. In certain embodiments of this invention, kits containing single-chambered and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes) are provided.

ANTIGENS

In an aspect, the invention relates to a polypeptide, in particular an antigen, comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E).

In an embodiment, the invention relates to a polypeptide, in particular an antigen, comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), said polypeptide having a size up to 300 amino acids.

In an embodiment, the polypeptide of the invention has a size between 50 and 300, preferably between 100 and 250 amino acids.

In an embodiment, the polypeptide of the invention has a size up to 50, 100, 150, 200, 250 or 300 amino acids.

In an embodiment, the invention relates to a polypeptide, in particular an antigen, comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E) and at least one epitope sequence of human SIRPa consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) or SEQ ID NO: 6 (YPQRLQLTWLE).

In an embodiment, the invention relates to a polypeptide, in particular an antigen, comprising or consisting of the epitope sequences of human SIRPa consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE).

In an embodiment, the invention relates to a polypeptide, in particular an antigen, comprising or consisting of at least one peptide selected from the group consisting of SEQ ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72, said polypeptide consisting of a sequence of up to 300 amino acids.

In an embodiment, the invention relates to a polypeptide, in particular an antigen, comprising or consisting of the peptide of amino acid sequence set forth in SEQ ID NO: 73 (YNQK) and/or the peptide of SIR amino acid sequence within SIRPa, said polypeptide consisting of a sequence of up to 300 amino acids.

METHOD OF MANUFACTURING AN ANTIBODY

In an aspect, the invention also relates to a method of manufacturing an antibody, in particular an antibody of the invention, comprising immunizing a non-human animal, in particular a non-human mammal, against at least one antigen as defined above, and in particular collecting the resulting serum from said immunised non-human animal to obtain antibodies directed against said antigen.

In particular, the invention also relates to a method of manufacturing an antibody comprising immunizing a non-human animal against an antigen comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), and in particular collecting the resulting serum from said immunised non-human animal to obtain antibodies directed against said antigen.

In particular, the invention also relates to a method of manufacturing an antibody comprising immunizing a non-human animal against an antigen comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E) and at least one epitope sequence of human SIRPa selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) or SEQ ID NO: 6 (YPQRLQLTWLE), and in particular collecting the resulting serum from said immunised non-human animal to obtain antibodies directed against said antigen.

In particular, the invention also relates to a method of manufacturing an antibody comprising immunizing a non-human animal against an antigen comprising or consisting of the epitope sequences of human SIRPa consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE), and in particular collecting the resulting serum from said immunised non-human animal to obtain antibodies directed against said antigen.

METHOD OF SELECTING AN ANTIBODY

In an aspect, the invention relates to a method of selecting an antibody of the invention, an antigen-binding fragment or mimetic of such an antibody, comprising or consisting of at least one of the following steps:

a. testing (e.g. according to a method describing in the Examples 1, 2 and 3) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to bind to SIRPa, in particular to an antigen as defined above,

b. testing (e.g. according to a method describing in the Examples 7 and 8) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to bind to SIRPb,

c. testing (e.g. according to a method describing in the Examples 9 and 10) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to bind to SIRPg,

d. testing (e.g. according to a method describing in the Examples 4 and 5) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to inhibit the binding of human CD47 to human SIRPa;

e. testing (e.g. according to a method describing in the Example 12) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to bind to T cells;

f. testing (e.g. according to a method describing in the Example 13) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to inhibit the T cells proliferation;

g. testing (e.g. according to a method describing in the Example 11) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to inhibit the binding of human CD47 to human SIRPg;

and optionally comprising the following step:

- selecting an antibody, an antigen-binding fragment or mimetic of such an antibody which specifically binds to SIRPa, in particular to an antigen as defined above, and which significantly inhibits the binding of CD47 to SIRPa, and which does not bind specifically to human SIRPg, and/or which does not bind specifically to human T-cells, and/or which does not significantly inhibit the proliferation of human T-cells, and/or which does not significantly inhibit the binding of human CD47 to human SIRPg; more particularly which specifically binds to SIRPa, in particular to an antigen as defined above, and which significantly inhibits the binding of CD47 to SIRPa, and which does not bind specifically to human SIRPg, and which does not bind specifically to human T-cells, and which does not significantly inhibit the proliferation of human T-cells, and which does not significantly inhibit the binding of human CD47 to human SIRPg.

The method of selecting an antibody of the invention can advantageously being performed further to the method of manufacturing an antibody according to the invention.

The following Figures and Examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

FIGURE LEGENDS

Figure 1. Binding analyses of anti-SIRPa antibodies by ELISA assay (human SIRPa-His coating and anti-human kappa detection).

Assessment by ELISA on immobilized SIRPa-His of chimeric (♦), HALA (□), HFLA (*), HFLB (+), HEFLA (▲), HEFLB (■), SIRP29 (Δ), Kwar23 (o) on **Figure A**; of HCLA (•), HCLB (x), HELA (◊), HELB (-) on **Figure B**; of HALB (-), HBLA (_), HBLB (▩) on **Figure C**. Revelation was performed with a donkey anti-human antibody and revealed by colorimetry at 450nm using TMB substrate. ED50 is the concentration of the indicated antibody to reach 50% of the signal in this assay. Binding of m18D5 clone (■) (n=4), SE5A5 commercial clone (▲) (n=7), 6G10 clone (▽) (n=3) and 12D7 clone (□) (n=4) on **Figure D**.

Figure 2. Affinity analysis by Biacore of anti-SIRP antibodies on human SIRPa recombinant protein.

SIRPa-His recombinant protein was immobilized onto a CM5 chip at 5µg/ml (500RU) and the indicated antibodies were added at different concentration. Values were measured after an association period (ka) of 3min followed by a dissociation period of 10 min (kd) to determine affinity constant (KD).

Figure 3. Binding analyses of anti-SIRPa antibodies on human monocytes (homozygote for SIRPa variant 1 (v1/v1)).

(A, B) Assessment by cytofluorometry on human monocytes v1/v1 (previously stained with human Fc Receptor Binding Inhibitor antibody) of chimeric (♦), HALA (□), HFLA (*), HFLB (+), HEFLA (▲), HEFLB (■), SIRP29 (Δ), Kwar23 (o). Revelation was performed with a PE labeled mouse anti-human Fc mAb on Cantoll cytometer, values corresponding to percentage of stained monocytes. ED50 is the concentration of the indicated antibody to reach 50% of the signal in this assay. **Figure A** corresponds to the percentage of monocytes v1/v1 stained. **Figure B** corresponds to the mean of fluorescence intensity (MFI) of monocytes v1/v1.

(C, D) Binding study of SIRPa antibodies on human monocytes by Flow cytometry (FACS): different anti-SIRPa antibodies were tested: m18D5 (■) (n=1), SE7C2 (▲)(n=2), 12D7 (□)(n=2), 6G10 (♦) (n=4) : **Figure C** represents the Mean Fluorescence Intensity (MFI) of the different antibodies over a dose response. **Figure D** represents the percentage of stained monocytes over antibody dose response. Statistical analysis were performed when it was possible.

(E, F, G) SIRPa variants binding in the population by anti-h SIRPa antibodies: The capacity of different anti-hSIRPa antibodies to bind SIRPa variants in the 32 volunteers was measured by FACS with a PE-

anti mouse IgG. All clones were tested at 10 µg/ml: m18D5 (■), 12D7 (▼), 6G10 (♦) and commercial antibodies SE5A5 (□), SE7C2 (Δ). **Figure E** represents the homozygote Variant 1 volunteers (n=16). **Figure F** represents the homozygote variant 2 volunteers (n=8). **Figure G** represents the heterozygote V1/V2 volunteers (n=8).

Figure 4. Competition of anti-SIRPa antibodies with CD47 on SIRPa.

(A) Assessment by ELISA on immobilized SIRPa-His of chimeric (♦), HFLA (*), HFLB (+), HEFLA (▲), HEFLB (■), SIRP29 (Δ), Kwar23 (o) at different concentrations incubated with constant concentration of biotinylated CD47-Fc (6µg/ml). Revelation was performed with streptavidin peroxidase to detect CD47 molecule and revealed by colorimetry at 450nm using TMB substrate. The results of a second experiment are given with the IC50 values. IC50 is the concentration of the indicated antibody to inhibit 50% of the signal in this assay.

(B) Antagonist activity study of anti-SIRPa antibodies on SIRPa-CD47 interaction by ELISA: The different anti-SIRPa antibodies were tested over a dose response: m18D5 clone (■)(n=1), commercial antibody SE5A5 (▲)(n=2) and m12D7 (□)(n=2). The figure represents the percentage of CD47 positive SIRPa-CD47 interactions measured by ELISA during a dose response of anti-hSIRPa antibodies.

Figure 5. Competition of anti-SIRPa antibodies with CD47 on human monocytes.

(A, B) Assessment by cytometry on human monocytes (v1/v1) of chimeric (♦), HFLA (*), HFLB (+), HEFLA (▲), HEFLB (■) at different concentrations incubated with constant concentration of biotinylated CD47-Fc (10 µg/ml). Revelation was performed with PhycoErythrin-streptavidin to detect CD47 molecule and revealed by CantoII cytometer. IC50 is the concentration of the indicated antibody to inhibit 50% of the signal in this assay. **Figure A** corresponds to the percentage of positive cells. **Figure B** corresponds to the mean of fluorescence intensity.

(C) Antagonist activity study of anti-SIRPa antibodies on Sirpa-CD47 interaction by FACS: The different anti-SIRPa antibodies were tested over a dose response: m18D5 clone (■)(n=1), commercial antibody SE7C2 (▲) (n=2) and m12D7 (□)(n=2). **Figure C** represents the percentage of CD47 positive cells measured by FACS after competition with anti-hSIRPa antibodies.

Figure 6. (A) Affinity analysis by Blitz of anti-SIRP antibody on human SIRPa recombinant protein pre-incubated or not with SP-D ligand. SIRPa-His recombinant protein was immobilized onto a NINTA biosensor at 10 µg/ml and the SP-D ligand was added at 100 µg/ml (saturating concentration). Then anti-SIRPa antibody was added at 20µg/ml and affinity values were deduced after an association

period (ka) of 120sec followed by a dissociation period of 120sec (kd) to determine affinity constant (KD). **(B)** Affinity analysis by Blitz of anti-SIRP antibody on human SIRPa recombinant protein pre-incubated with mouse 18D5 antibody. SIRPa-His recombinant protein was immobilized onto a NINTA biosensor at 10 µg/ml and the anti-SIRPa antibody was added at 20µg/ml (saturating concentration). Then SP-D ligand was added at 100 µg/ml and affinity values were deduced after an association period (ka) of 120sec followed by a dissociation period of 120sec (kd) to determine affinity constant (KD).

Figure 7. (A) Affinity analysis by Blitz of anti-SIRP antibodies on human SIRPb recombinant protein. SIRPb-His recombinant protein was immobilized onto a NINTA biosensor and the indicated antibodies were added at 20µg/ml. Values were deduced after an association period (ka) of 120sec followed by a dissociation period of 120sec (kd) to determine affinity constant (KD). **(B)** Binding analysis of anti-SIRP antibodies (human SIRPb-His coating and anti-human kappa detection). Assessment by ELISA on immobilized SIRPb-His of HEFLB (■), SIRP29 (Δ), Kwar23 (o), B4B6 (♦) and IgG4 Ab control (※). Revelation was performed with a donkey anti-human antibody with the exception of B4B6 revealed with a mouse antibody and revealed by colorimetry at 450nm using TMB substrate.

Figure 8. (A) Affinity analysis by Blitz of anti-SIRP antibodies on human SIRPg recombinant protein. SIRPg-His recombinant protein was immobilized onto a NINTA biosensor and the indicated antibodies were added at 10 µg/ml. Values were deduced after an association period (ka) of 120sec followed by a dissociation period of 120sec (kd) to determine affinity constant (KD). **(B)** Binding analysis by ELISA assay of anti-SIRP antibodies on SIRPg (human SIRPg-His coating and anti-human kappa detection). Assessment by ELISA on immobilized SIRPg-His of HEFLB (■), SIRP29 (Δ), Kwar23 (o), LSB2-20 (●) and IgG4 Ab control (※). Revelation was performed with a donkey anti-human antibody and revealed by colorimetry at 450nm using TMB substrate.

Figure 9. Affinity analysis by Blitz of CD47 on human SIRPg recombinant protein pre-incubated with anti-SIRP antibodies. SIRPg-His recombinant protein was immobilized onto a NINTA biosensor at 10 µg/ml and the indicated antibodies were added at 20µg/ml (saturating concentration). Then, CD47Fc was added at 100 µg/ml and affinity values were deduced after an association period (ka) of 120sec followed by a dissociation period of 120sec (kd) to determine affinity constant (KD).

Figure 10. Geometric mean fluorescence intensity measured by flow cytometry on **(A)** peripheral human CD3+ T cells, **(B)** red blood cells or **(C)** platelets after staining with different monoclonal antibodies and revealing with secondary anti-IgG fluorescent antibody.

Figure 11. Percentage of positive peripheral human CD3+ T cells after staining with different monoclonal antibodies. The table indicates the value of % of positive cells in duplicate experiments.

Figure 12. Human T cells isolated from peripheral blood mononuclear cells from healthy volunteers were stimulated with **(A) (C)** anti-CD3+ anti-CD28 beads at a 1:1 ratio for 3 days or **(B) (D)** with allogeneic dendritic cells (DC) at a 5 T cell : 1 DC ratio for 5 days or **(E)** with different concentrations of tuberculin unpurified protein derivative (PPD) for 5 days. Antibodies were added at day 0 of the culture. Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Figure 13. Mouse CD8+ T cells were isolated from splenocytes of naive mice. CD8 T cells were stimulated with anti-CD3+ anti-CD28 beads at a 1:1 ratio for 3 days. Antibodies were added at day 0 of the culture. Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Figure 14. Human T cells isolated from peripheral blood mononuclear cells from healthy volunteers were stimulated with allogeneic dendritic cells (DC) at a 5 T cell : 1 DC ratio for 5 days. Antibodies were added at day 0 of the culture. Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Figure 15. (A) Anti-tumor effect of anti-SIRPa (P84 clone) i.p. administration three times a week for 4 weeks (300µg/injection) in combination or not with two injection (day 4 & 8) of anti-4-1-BB mAb (3H3 clone, 100 µg/injection) or with injections (twice a week) of anti-PDL-1 (10F.9G2 clone, 200 µg/injection, treatment during 4 weeks) in an orthotropic model of murine hepatoma (2.5.10^6 of Hepa 1.6 cells injected through the portal vein on day 0). Mice were considered cured when they survived three times longer than the time necessary to all control mice died. **(B)** Tumor infiltrating cells were analyzed at day 13 after tumor inoculation. **(C)** Tumor infiltrating cells and spleen cells were analyzed at day 13 after tumor inoculation. **(D)** Mice previously cured in the hepatoma model by anti-SIRPa + anti-4-1BB injection or SIRPa mutant mice treated with anti-4-1BB were rechallenged by Hepa 1.6 cells injection in the spleen (2.5.10^6 cells/mouse). Naive mice were injected in parallel in the same route in order to compare tumor development with rechallenged mice. Mice were then left untreated. **(E)** Mice previously cured in the hepatoma model by anti-SIRPa + anti-4-1BB were rechallenged by Hepa 1.6 cells injection in the spleen (2.5.10^6 cells/mouse) and their spleen was harvested 30 days after rechallenge. Splenocytes and T-cell splenocytes were isolated. Naive mice were injected intravenously with vehicle, whole splenocytes (10.10^6/mouse) or T-cell purified from

splenocytes (2.5.10^6/mouse) and all received Hepa1.6 cell injection in the spleen (2.5.10^6 cells/mouse). Mice were then left untreated and considered cured when they survived three times longer than the time necessary to all control mice died.

Figure 16. Mice previously cured in the hepatoma model by anti-SIRPa + anti-PDL1 injection were rechallenged by Hepa 1.6 cells injection in the spleen (2.5.10^6 cells/mouse). Naive mice were injected in parallel in the same route in order to compare tumor development with rechallenged mice. Mice were then left untreated.

Figure 17. Anti-tumor effect of anti-SIRPa (P84 clone) i.p. administration three times a week for 4 weeks (200µg/injection) in an orthotopic model of murine mammary carcinoma (0.25.10^6 of 4T1 cells injected in the mammary gland). The tumor development was evaluated by measuring the diameter of the tumor and calculated according to the formula : =(0,52*(d^2))^1,5. Mice were euthanized when tumor development was nearly 1000 mm^3 according to the ethical guidelines.

Figure 18. Immune cells phenotype analysis in spleen, tumor and lymph nodes of mice treated with anti-SIRPa (P84 clone) or ctrl mAb i.p. three times a week for two weeks (200µg/injection) in an orthotropic model of murine mammary carcinoma (0.25.10^6 of 4T1 cells injected in the mammary gland). Immune cell analysis were performed two weeks after tumor inoculation.

Figure 19. Anti-SIRPa (P84 clone), anti-CD47 (MIAP410 clone) and irrelevant isotype control were administered intraperitoneally at day 0 and day 2 at 12mg/kg in C57Bl/6 mice. Blood samples were collected at day 0 and day 3 in EDTA containing tubes and blood count was performed with a XS-800i hematology analyzer (Sysmex). The level of hemoglobin (left) and the percentage of hematocrit (right) were evaluated at day 3. The dotted-lines represent normal range values in the C57Bl/6 mice for each parameter.

Figure 20. (Up) Flow cytometry analysis of anti-SIRPa (HEFLB, grey) and anti-CD47 (B6H12, black) mAb as compared to a control mAb (dotted line) on human platelets freshly isolated from the blood of healthy donors. (Bottom) Human platelet aggregation measurement using optical aggregometer in the presence of a 50 µg/ml of control mAb, anti-SIRPa (HEFLB), anti-CD47 (B6H12) or anti-integrin αIIb as positive control of inhibitor of aggregation. Antibodies were evaluated on non-activated and ADP-activated platelets.

Figure 21. Allogeneic CD4+ T cells were cultured in a 1:1 ratio with CD14+ myeloid cells extracted from **(A)** fresh or **(B)** frozen ovarian cancer ascites with 10 µg/ml of control antibody (white), anti-SIRPa HEFLB (black) or anti-CD47 mAb (grey). Proliferation was measured at day 5 by ^{3}H-thymidine incorporation. **(C)** Alternatively, allogeneic T cells were cultured in a 5:1 ratio with allogeneic dendritic cells and different ratio of CD14+ myeloid cells extracted from ovarian cancer ascites in the presence of 10µg/ml of antibody as in **(A)**.

EXAMPLES

In the following Examples, the antibody "18D5" (or "m18D5") corresponds to the mouse antibody 18D5, the "chimeric" antibody corresponds to the chimeric mouse/human 18D5 antibody, and the antibodies "HALA, HALB, HBLA, HBLB, HCLA, HCLB, HELA, HELB, HFLA, HFLB, HEFLA and HEFLB" correspond to specific humanized 18D5 variants. The antibodies 6G10 and 12D7 belong to the Applicant; these antibodies have been obtained by the same method than m18D5 and are used as control. These control antibodies are IgG2a mouse monoclonal anti-human SIRPa antibodies.

In addition, commercial antibodies were used for comparison. The first one is an anti-SIRPa antibody, named SE7C2 (Santa Cruz sc-23863); the second antibody is an antibody able to recognize both SIRP α /β and is named SE5A5 (BioLegend BLE323802); and the third one is an anti-human SIRPa antibody named Kwar23 (Creative Biolabs). An anti-human SIRPa antibody named SIRP29 from University of Toronto described in the PCT application WO2013056352 was also used for comparison.

Example 1. Binding analyses of the anti-SIRPa antibodies on SIRPa by ELISA

Method: The binding activity of the anti-SIRPa antibodies was assessed by ELISA. For the ELISA assay with the chimeric antibody, the humanized antibodies, SIRP29 and Kwar23, a recombinant hSIRPa (Sino Biologicals, Beijing, China; reference 11612-H08H) was immobilized on plastic at 0.5µg/ml in carbonate buffer (pH9.2) and the purified antibody was added to measure binding. After incubation and washing, peroxidase-labeled donkey anti-human IgG (Jackson Immunoresearch; USA; reference 709-035-149) was added and revealed by conventional methods.

For the ELISA assay with the mouse antibodies, a recombinant hSIRPa (Sino Biologicals, Beijing, China; reference 10975-H08H) was immobilized on plastic at 0.5µg/ml in carbonate buffer (pH9.2) and the purified antibody was added to measure binding. After incubation and washing, peroxidase-labeled goat anti-mouse Fc chain (Jackson Immunoresearch; reference 115-036-071) was added and revealed by conventional methods.

Results: As shown in **Figure 1A, 1B and 1C**, the binding activity of the different anti-SIRPa antibodies on SIRPa as measured by ELISA showed effective concentrations (EC50) of 2.9 ng/ml for the chimeric antibody, 3.9 ng/ml for HALA, 5.1 ng/ml for HFLA, 4.0 ng/ml for HFLB, 7.1 ng/ml for HEFLA, 4.4 ng/ml HEFLB in a first experiment, 4.06 ng/ml for the chimeric antibody, 5.60 ng/ml for HCLA, 5.59 ng/ml for HCLB, 4.61 ng/ml for HELA, 4.13 ng/ml for HELB in a second experiment, and 2.74 ng/ml for the chimeric antibody, 2.53 ng/ml for HALB, 2.68 ng/ml for HBLA, 2.95 ng/ml for HBLB in a third experiment. Those results indicate that the antibodies of the invention tested are good SIRPa binders by ELISA as compared to other known anti-SIRPa antibodies SIRP29 (3.7 ng/ml) and Kwar23 (3.3

ng/ml). Those results indicate that the epitope recognized by all the antibodies of the invention is accessible when SIRPa is coated on a plastic well.

As shown in **Figure 1D**, the binding activity of different anti-SIRPa antibodies on SIRPa as measured by ELISA showed an effective dose (ED50) of 0.16nM (24ng/ml) for SE5A5 and 0.06nM (9ng/ml) for the clone m18D5. The clones 6G10 and 12D7 did not seem to bind SIRPa by ELISA assay. Those results indicate that the clone m18D5 is a good SIRPa binder by ELISA compared to a commercial antibody and indicate that the epitope recognized by this clone is accessible when SIRPa is coated on a plastic well compared to clones 6G10 and 12D7.

Example 2. Biosensor affinity measurement of the anti-SIRPa antibodies for SIRPa

Method: Recombinant hSIRPa (Sino Biologicals, Beijing, China; reference 11612-H08H) was immobilized into a CM5 sensor chip (GeHealthcare; France) at 5µg/ml (500RU) and antibodies were applied at different concentrations with a flow rate of 40µl/min. Analysis was performed with a BIAcore 3000 (Biacore, GeHealthcare). Values were measured after an association period (ka) of 3min followed by a dissociation period of 10 min (kd) to determine affinity constant (KD).

Results: As shown in **Figure 2**, the antibodies of the invention have a strong affinity (KD) for SIRPa (from 1.93e-10 M to 3.67e-10 M), which is equivalent to the affinity of the known anti-SIRPa antibodies SIRP29 and Kwar23 and better than the affinity of the commercial anti-SIRPa antibodies SE7C2 and SE5A5.

Example 3. SIRPa binding assay on human monocytes by cytofluorometry

Method: To measure the binding of the anti-SIRPa antibodies on human monocytes, human Fc Receptor Binding Inhibitor (BD pharmingen; USA; reference 564220) was first added for 30min at room-temperature to block human Fc receptors on human monocytes to reduce background. Then, an antibody was incubated for 30min at 4°C, and washed before stained 30min at 4°C with PE-labelled anti-human IgG Fc (Biolegend; USA; reference 409303). For the mouse antibodies, a PE-labelled anti-mouseIgG (Jackson Immunoresearch; reference 715-116-151) was used. Samples were analyzed on BD LSRII or Canto II cytofluorometer.

Results: As shown in **Figure 3**, the results indicate a strong binding of the anti-SIRPa antibodies of the invention on human monocytes and a binder binding (as measured with the MFI (Median Fluorescent Intensity)) that the known anti-SIRPa antibodies Kwar23, SE7C2 and SE5A5.

Example 4. Competitive analysis between CD47 and the anti-SIRPa antibodies by antagonist ELISA assay

Method: For competitive ELISA assay, recombinant hSIRPa (Sino Biologicals, Beijing, China; reference 11612-H08H) was immobilized on plastic at 0.5µg/ml in carbonate buffer (pH9.2). For the chimeric antibody, the humanized antibodies, SIRP29 and Kwar23, a purified antibody (at different concentrations) was mixed with 6µg/ml final (fix concentration) of biotinylated Human CD47Fc (AcroBiosystems interchim; France; reference: #CD7-H82F6) to measure competitive binding for 2h at 37°C. After incubation and washing, peroxidase-labeled streptavidin (Vector laboratoring; USA; reference SA-5004) was added to detect Biotin-CD47Fc binding and revealed by conventional methods.

For the mouse antibodies, a purified antibody (at different concentrations) was mixed with 0.04µg/ml of CD47Fc (Sino Biologicals, Beijing, China; reference 12283-H02H) to measure competitive binding for 2h at 37°C. After incubation and washing, peroxidase-labeled donkey anti-human Fc chain (Jackson Immunoresearch; reference 709-035-149) was added to detect CD47Fc binding and revealed by conventional methods.

Results: As shown in **Figure 4**, the antibodies of the invention have an antagonist activity on the SIRPa-CD47 interaction. In particular, it is observed that the chimeric antibody, HFLA, HFLB, HEFLA and HEFLB have a better antagonist activity as compared to the antagonist activity of SIRP29 and the commercial anti-SIRPa antibody SE5A5.

Example 5. Competitive analysis between CD47 and the humanized anti-SIRPa antibodies on human monocytes by antagonist cytofluorometry assay

Method: To measure the competition between CD47 and the humanized anti-SIRPa antibodies on human monocytes, a purified antibody was added on monocytes for 15min at 4°C, then mixed with 5µg/ml final of biotinylated Human CD47Fc (AcroBiosystems interchim; France; reference: #CD7-H82F6) and incubated for 30min at 4°C to measure competitive binding antibody. After incubation and washing, PE-labelled streptavidin (BDBiosciences; USA; reference 554061) was added for 15min at 4°C to detect Biotin-CD47Fc binding and analyzed on BD LSRII or Canto II cytofluorometer.

To measure the competition between CD47 and the mouse anti-hSIRPa antibodies on human monocytes, a purified antibody was added on monocytes for 15min at 4°C, then mixed with 5µg/ml final of CD47Fc (Sino Biologicals, Beijing, China; reference 12283-H02H) and incubated for 15 min at 4°C to measure competitive binding antibody. After incubation and washing, FITC-labelled anti-human Fc (Beckman Coulter; reference IM1627) was added for 15min at 4°C to detect CD47Fc binding and analyzed on BD LSRII or Canto II cytofluorometer.

Results: As shown in **Figure 5**, the antibodies of the invention have an antagonist activity on SIRPa-CD47 interaction on human monocytes.

Example 6. Blitz method competition with SP-D

Method: This method was performed with a Blitz (Forté Bio; USA; reference C22-2 No 61010-1). Condition A: SIRPa + Anti-SIRPa antibody + Surfactant Protein D (SP-D). In a first step, SIRPa (His) recombinant protein (Sino Biologicals, Beijing, China; reference 11612-H08H) was immobilized at 10 µg/ml by histidine tail into a Ni-NTA biosensor (Forté Bio; USA; reference 18-0029) for 30 seconds. In a second step, anti-SIRPa antibodies were added at 20µg/mL (saturating concentration) for 120 seconds. Then, human SP-D (R et D Systems; USA; reference 1920-SP-050) was associated at 100 µg/mL, in competition with anti-SIRPa antibodies, for 120 seconds. The dissociation of SP-D was made in kinetics buffer for 120 seconds. Analysis of data was made with the Blitz pro 1.2 software, which calculated association constant (ka) and dissociation constant (kd) and determined the affinity constant KD (ka/kd).

Condition B: SIRPa + Surfactant Protein D (SP-D) + Anti-SIRPa antibody. In a first step, Sirp-a (His) recombinant protein (Sino Biologicals, Beijing, China; reference 11612-H08H) was immobilized at 10 µg/ml by histidine tail into a Ni-NTA biosensor (Forté Bio; USA; reference 18-0029) for 30 seconds. In a second step, human SP-D (R et D Systems; USA; reference 1920-SP-050) was added at 100 µg/mL for 120 seconds. Then, anti-SIRPa antibodies were associated at 20µg/mL (saturating concentration) for 120 seconds. The dissociation of anti-SIRPa antibody was made in kinetics buffer for 120 seconds. Analysis data was made with the Blitz pro 1.2 software, which calculated association constant (ka) and dissociation constant (kd) and determined the affinity constant KD (ka/kd).

Results: As shown in **Figure 6**, the binding of the anti-SIRPa antibody 18D5 does not block the binding of SP-D to SIRPa and the binding of SP-D does not block the binding of 18D5 to SIRPa. Thus, the antibody of the invention does not inhibit the interaction between SIRPa and SP-D.

Example 7. Affinity of the anti-SIRPa antibodies for SIRPb by Blitz method

Method: This method was performed with a Blitz (Forté Bio; USA; reference C22-2 No 61010-1). Recombinant hSIRPb-His (Antibodies-online; USA; reference ABIN3077231) was immobilized at 10 µg/ml by histidine tail into a Ni-NTA biosensor (Forté Bio; USA; reference 18-0029) for 30 seconds. Then, an anti-SIRPa antibody was associated at 20µg/mL for 120 seconds. The dissociation of anti-SIRPa antibody was made in kinetics buffer for 120 seconds. Analysis of data was made with the Blitz pro 1.2 software, which calculated association constant (ka) and dissociation constant (kd) and determined the affinity constant KD (ka/kd).

Results: As shown in **Figure 7A**, the antibodies of the invention have a lower affinity for SIRPb as compared to SIRPa. In particular, it is noted that the chimeric antibody, HFLA, HFLB, HEFLA, HEFLB have a reduced affinity for SIRPb as compared to SIRP29 and Kwar23.

Example 8. ELISA binding of anti-SIRP antibodies on SIRPb

Method: For activity ELISA assay, recombinant hSIRPb-His (Antibodies-online; USA; reference ABIN1466557) was immobilized on plastic at 1µg/ml in carbonate buffer (pH9.2) and a purified antibody was added to measure binding. After incubation and washing, peroxidase-labeled donkey anti-human IgG (Jackson Immunoresearch; USA; reference 709-035-149) was added and revealed by conventional methods.

Results: As shown in **Figure 7B**, the anti-SIRPa antibodies have a low affinity for SIRPb. It must be indicated that the revelation was performed with a donkey anti-human antibody for all antibodies except for B4B6 (revealed with a mouse antibody), which may explain that the signal obtained for the anti-SIRPb antibody B4B6 is lower than the signal obtained for the anti-SIRPa antibodies.

Example 9. Affinity analysis of the anti-SIRPa antibodies for SIRPg by Blitz method

Method: This method was performed with a Blitz (Forté Bio; USA; reference C22-2 No 61010-1). Recombinant hSIRPg-His (Sino Biologicals, Beijing, China; reference 11828-H08H) was immobilized at 10 µg/ml by histidine tail into a Ni-NTA biosensor (Forté Bio; USA; reference 18-0029) for 30 seconds. Then, an anti-SIRPa antibody was associated at 20µg/mL for 120 seconds. The dissociation of anti-SIRPa antibody was made in kinetics buffer for 120 seconds. Analysis of data was made with the Blitz pro 1.2 software, which calculated association constant (ka) and dissociation constant (kd) and determined the affinity constant KD (ka/kd).

Results: As shown in **Figure 8A**, the anti-SIRPa antibodies of the invention have a low affinity for SIRPg. This affinity is slightly weaker than the affinity of the known anti-SIRPa antibodies SIRP29 and Kwar23. However, the kinetics properties differ between anti-SIRPa antibodies, SIRP29 and Kwar23, with a high dissociation rate constant (Kd) for anti-SIRPa antibodies as compared to SIRP29 and Kwar23. In particular, HFLB has the lowest affinity for SIRPg with a KD value of 1.036e-7 M that equals to a 2-log difference as compared to the KD values of SIRP29 and Kwar23.

Example 10. ELISA binding of the anti-SIRP antibodies on SIRPg

Method: For activity ELISA assay, hSIRPg-His (Sino Biologicals, Beijing, China; reference 11828-H08H) was immobilized on plastic at 1µg/ml in carbonate buffer (pH9.2) and purified antibody were added to measure binding. After incubation and washing, peroxidase-labeled donkey anti-human IgG (Jackson Immunoresearch; USA; reference 709-035-149) was added and revealed by conventional methods.

Results: As shown in **Figure 8B**, the anti-SIRPa antibody HEFLB does not bind SIRPg while the known anti-SIRPa antibodies SIRP29 and Kwar23 show a significant binding to SIRPg.

Example 11. Blitz method competition with CD47 for SIRPg: SIRPg + anti-SIRPa antibody + CD47

Method: This method was performed with a Blitz (Forté Bio; USA; reference C22-2 No 61010-1). In a first step, hSIRPg-His (Sino Biologicals, Beijing, China; reference 11828-H08H) was immobilized at 10 µg/ml by histidine tail into a Ni-NTA biosensor (Forté Bio; USA; reference 18-0029) for 30 seconds. In a second step, an anti-SIRPa antibody was added at 20µg/mL (saturating concentration) for 120 seconds. Then, human CD47Fc ((Sino Biologicals, Beijing, China; reference 12283-H02H) was associated at 100 µg/mL, in competition with anti-SIRPa antibodies, for 120 seconds. The dissociation of CD47Fc was made in kinetics buffer for 120 seconds. Analysis data was made with the Blitz pro 1.2 software, which calculated association constant (ka) and dissociation constant (kd) and determined the affinity constant KD (ka/kd).

Results: As shown in **Figure 9**, the anti-SIRPa HEFLB of the invention does not compete with the binding of CD47 to SIRPg. At the opposite, the other known antibodies SIRP29 and, in particular, kwar23 compete with the binding of CD47 to SIRPg.

Example 12. Binding to blood cells by flow cytometry

Method: The experiment was realized to analyze the binding of the anti-SIRPa antibodies on human blood cells. CD3-positive T lymphocytes, red blood cells and platelets were extracted from purified blood from healthy volunteers. Cells were then stained for 30min at 4°C with 10 micrograms/ml of each tested antibody, washed and then stained with a secondary fluorescent anti-IgG antibody for another 30min at 4°C. After washes, cells were analyzed on a CANTO II (BD Bioscience) flow cytometer.

Results: As shown in **Figure 10**, the T cells, the red blood cells and the platelets are positive for CD47, which is expressed ubiquitously, and they were stained with the B6H12 antibody. The SIRP29 and the Kwar23 antibodies, like LSB2.20 (specific anti-SIRPγ antibody), bind to T cells that are known to express SIRPγ. However, the anti-SIRPa humanized 18D5 antibody does not bind to the T cells (same results obtained with four different 18D5 humanized variants tested). Red blood cells and platelets do not express SIRPa and, thus, they were not revealed with the humanized 18D5 antibody and the other anti-SIRPa antibodies. This result shows the specificity of the humanized 18D5 antibody for SIRPa on live cells as compared to the known anti-SIRPa antibodies.

As shown in **Figure 11**, the T-cells are not stained by the humanized 18D5 antibody (same results obtained with five different 18D5 humanized variants tested) and with the chimeric 18D5 (data not shown) whereas more than 70% of T cells are stained by SIRP29 and Kwar23.

Example 13. Human CD3+ T cell proliferation

Method: hPBMC were isolated from buffy coat of healthy volunteers. CD4 or CD8 T cells were selected by positive selection using an AutoMACS (Miltenyi) and plated in 96-round well plate (50 000 cells/well). The proliferative signals were provided by either anti-CD3/anti-CD28 coated microbeads (LifeTechnologies) at a 1 bead for 1 T cell ratio during three days, or allogeneic mature dendritic cells generated in vitro at a 5 T cell for 1 mDC during 5 days or with different concentrations of tuberculin unpurified protein derivative (PPD) for 5 days. Antibodies targeting the SIRPa/CD47 pathway were added from the beginning of the proliferation test at a saturating concentration (10 µg/mL). Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Results: As shown in **Figure 12**, the anti-SIRPa antibody HALA and HEFLB variants do not inhibit the T cell proliferation when T cells are stimulated with anti-CD3+ anti-CD28 beads **(A) (C)** or with allogenic dendritic cells **(B) (D)** or with PPD **(E)**, whereas the anti-SIRPa Kwar23 inhibits T cell proliferation when T cells are stimulated with allogenic dendritic cells. As expected, the anti-CD47 antibodies and the anti-SIRPg antibody are inhibitors of the T cell proliferation.

Example 14. Mouse CD8+ T cell proliferation

Method: Splenocytes were isolated from naive mice. CD8 T cells were selected by positive selection using an AutoMACS (Miltenyi) and plated in 96-round well plate (50 000 cells/well). The proliferative signals were provided by anti-CD3/anti-CD28 coated microbeads (LifeTechnologies) at a 1 bead for 1 T cell ratio during three days. A mouse anti-SIRPa antibody (P84) and an anti-CD47 antibody (MIAP310) targeting the SIRPa/CD47 pathway were added from the beginning of the proliferation test at a saturating concentration (10 µg/mL). Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Results: As shown in **Figure 13**, there is no inhibition of the anti-SIRPa or anti-CD47 antibody on the proliferation of mouse T cells. This result is explained by the fact that mice does not express the SIRPg gene. Thus, mice can be used as a model to predict the *in vivo* effects of a specific anti-SIRPa antibody that does not bind SIRPg. In contrast, anti-CD47 or non-selective anti-SIRPa antibodies *in vivo* preclinical efficacy, in particular on adaptive immunity and generation of memory T lymphocytes, is not predictive of human situation.

Example 15. Human T cell proliferation

Method: hPBMC were isolated from buffy coat of healthy volunteers. CD4 or CD8 T cells were selected by positive selection using an AutoMACS (Miltenyi) and plated in 96-round well plate (50 000 cells/well). The proliferative signals were provided by either anti-CD3/anti-CD28 coated microbeads (LifeTechnologies) at a 1 bead for 1 T cell ratio during three days, or allogeneic mature dendritic cells generated in vitro at a 5 T cell for 1 mDC during 5 days. Antibodies were added from

the beginning of the proliferation test at a saturating concentration (5µg/mL for anti-CD47 and anti-SIRPa antibodies and 2,5µg/mL for the anti-PD-1/PD-L1 antibodies and the recombinant 4-1BBL). Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Results: As shown in **Figure 14**, the anti-PD-1/PD-L1 antibodies and the recombinant 4-1BBL have a boost effect on the proliferation of human T cells, while anti-CD47 has a negative effect on the proliferation of human T cells. In particular, the anti-CD47 antibody prevents the human T-cell immunostimulatory efficacy of anti-PD-1/PD-L1 or 4-1BB agonist agents. The anti-SIRPa HEFLB has no significant effect on the proliferation of T cells.

Example 16. Anti-tumor effects in mice

Method: Mice were anesthetized with a cocktail of xylazine/ketamine. After a laparotomy, tumoral Hepa 1.6 cells were injected through the portal vein ($2,5.10^6$ cells/100 µL) in PBS. The treatment was started 4 days after tumor injection. The agonistic anti-4-1BB monoclonal antibody (3H3) was injected two times at d4 and d8 after Hepa 1.6 cells (Hepatocarninoma cells, HCC) injection intraperitoneally in PBS (100 µg/injection). The anti-PDL1 monoclonal antibody was injected twice a week during 4 weeks intraperitoneally in PBS (200µg/injection). The antagonistic anti-SIRPa antibody (P84) was injected three time a week during four weeks intraperitoneally in PBS (300µg/injection).

The anti-tumor response was evaluated in the orthotopic model of HCC thirteen days after the tumor inoculation. At this time, the tumor and the spleen were collected in order to phenotype the immune cells that infiltrated the tumor or in the systemic way. Splenocytes and non-parenchymal cells (NPC) of the liver which are the infiltrating immune cells were stained with four different mixes for flow cytometry acquisition.

Results: As shown in **Figure 15A**, the anti-SIRPa antibody alone significantly prolongs survival in a fraction of mice (28%). In combination with anti-4-1BB or anti-PDL1 antibodies, anti-SIRPa antibody allows a very high response rate of mice surviving even after treatment withdrawal.

As shown in **Figure 15B**, the combination of anti-SIRPa with a co-stimulatory agent (e.g. anti-4-1BB) or T-cell checkpoint inhibitor (e.g. anti-PDL1) modifies the tumor microenvironment by decreasing the regulatory and immunosuppressive immune cells (Tregs, Mo-MDSC) while increasing accumulation of effector memory CD8+ T cells in combination with anti-4-1BB. The Mo-MDSC are characterized by a high expression of Ly6C and no Ly6G among the CD11b positive- and MHC class II negative-population.

As shown in **Figure 15C**, the combination of anti-SIRPa with a co-stimulatory agent (e.g. anti-4-1BB) or T-cell checkpoint inhibitor (e.g. anti-PDL1) modifies the cell composition of the tumor microenvironment and in periphery in the spleen, by decreasing the frequency of immature and naïve B cells while increasing accumulation of memory and plasmablast cells. Similarly, accumulation

of cytolytic (CD27-negative) NK cells is induced the tumor and periphery by the anti-SIRPa combination with anti-41BB or anti-PDL1.

Altogether, anti-SIRPa modifies the tumor and peripheral immunity in particular adaptive (T-cell, Tregs, B-cells) and innate (MDSC, Macrophages, NK cells) immune cells contributing to tumor elimination and long-term protection.

Example 17. Anti-tumor effects in mice previously cured

Method: Mice previously cured in the hepatoma model by anti-SIRPa + anti-4-1BB injection or SIRPa mutant mice treated with anti-4-1BB were rechallenged by Hepa 1.6 cells injection in the spleen (2.5.10^6 cells/mouse). Mice were anesthetized with 3% of isoflurane in the air. After incision on the flank of the mice and isolation of the spleen, tumoral Hepa 1.6 cells were injected into the spleen ($2,5.10^6$ cells/50µL) in PBS. Naive mice were injected in parallel in the same route in order to compare tumor development with rechallenged mice.

Results: As shown in **Figure 15D**, all the cured mice survived when rechallenged and, at the opposite, all naïve mice died. This result demonstrates that memory T cells were induced under anti-SIRPa therapy or absence of SIRPa signals (SIRPa mutant mice) and still persist on the long-term in cured mice.

Example 18. Anti-tumor effects of T-cell splenocytes or whole splenocytes collected from mice previously cured

Method: Cured anti-SIRPa + anti-4-1BB rechallenged mice were euthanized and the spleen was collected. After red blood cell lysis, splenocytes were extracted and CD3 positive T cells were isolated from a part of splenocytes with an AutoMACS. After anesthesia, mice were injected with either T-cell splenocytes ($2,5.10^6$ cells/100 µL) or whole splenocytes (10.10^6 cells/100 µL) or excipient alone (PBS) intravenously. All mice received Hepa 1.6 cells through the portal vein as described previously ($2,5.10^6$ cells/100 µL).

Results: As shown in **Figure 15E**, the splenocytes and isolated T lymphocytes collected from cured mices has a high positive effect on the survival of mice. This results indicate that memory T cells are present in the splenocytes of the cured mice after treatment of the hepatoma and are responsible on the long-term adaptive immune memory.

Example 19. Anti-tumor effects in mice previously cured

Method: Mice previously cured in the hepatoma model by anti-SIRPa + anti-PDL-1 injection were rechallenged by Hepa 1.6 cells injection in the spleen (2.5.10^6 cells/mouse). Mice were anesthetized with 3% of isoflurane in the air. After incision on the flank of the mice and isolation of

the spleen, tumoral Hepa 1.6 cells were injected into the spleen ($2,5.10^6$ cells/50µL) in PBS. Naive mice were injected in parallel in the same route in order to compare tumor development with rechallenged mice.

Results: As shown in **Figure 16**, all the cured mice survived when rechallenged and, at the opposite, all naïve mice died. This result suggests that memory T cells are still present in cured mice. This result demonstrates that memory T cells were induced under anti-SIRPa therapy and still persist on the long-term in cured mice.

Example 20. Effects of the growth of a tumor in a mammary carcinoma model

Method: Mice were anesthetized with 3% of isoflurane in the air. Mice were shaved on the abdomen and 4T1 cells were injected in the mammary gland with an insulinic syringe (30 Gauges) in 50µL of PBS. The antagonistic anti-SIRPa antibody (P84) or a control antibody was injected three time a week during four weeks intraperitoneally in PBS (200µg/injection).

Results: As shown in **Figure 17**, the anti-SIRPa antibody significantly ($p<0.01$) reduces the growth of the tumor in the mammary carcinoma model as compared to a control antibody.

Figure 18 shows the immune cell analysis two weeks after inoculation. Anti-SIRPa has a positive effect on myeloid and non-myeloid cells (T and NK cells) both in tumor and in periphery (spleen) with a dramatic decrease of Tregs and accumulation of memory T cells.

Example 21. Effects of SIRPa antibodies on the concentration of hemoglobin and on the hematocrit

Method: Anti-SIRPa (P84 clone), anti-CD47 (MIAP410 clone) and irrelevant isotype control were administered intraperitoneally at day 0 and day 2 at 12mg/kg in C57Bl/6 mice. Blood samples were collected at day 0 and day 3 in EDTA containing tubes and blood count was performed with a XS-800i haematology analyzer (Sysmex). The level of hemoglobin (left) and the percentage of hematocrit (right) were evaluated at day 3.

Results: As shown in **Figure 19**, the anti-SIRPa antibody has no toxic effect on the concentration of hemoglobin and on the hematocrit. At the opposite, the anti-CD47 antibody induces a decrease of the concentration of hemoglobin and of the hematocrit in accordance with anemia observed during phase 1 in man.

Example 22. Platelet aggregation

Method: Blood was collected from healthy donor volunteers into Vacuette collection tubes (Greiner Bio-One) buffered with sodium citrate. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were obtained by centrifugation for 10 minutes at 200g and 15 minutes at 3 500g, respectively. The working PRP was adjusted to 3.10^8 platelets.L^{-1}. *Inhibition Assays:* mAb were pre-incubated with PRP

for a final concentration of 40 or 50 $\mu g.mL^{-1}$ test antibodies. After 3 minutes without stirring, platelet aggregation was initiated with ADP 5µM addition. Aggregation was determined by measuring the transmission of light through the sample at 37°C with continuous stirring using a standard optical aggregometer (TA-8V Thrombo-Aggregometer, SD Innovation SAS, Frouard, France). The transmission of PPP was set as 100%. Aggregation was recorded under stirring for a total of 5 minutes. *Induction Assays:* Platelet aggregation was directly initiated by mAb addition ($50\mu g.mL^{-1}$). Aggregation was recorded under stirring for a total of max. 10 minutes.

Results: As shown in **Figure 20**, in contrast to anti-CD47 antibodies, anti-SIRPa antibodies does not bind to human red blood cells or platelets. Consequently, anti-CD47 induces *in vitro* human platelets aggregation while anti-SIRPa antibodies does not. Similarly, anti-SIRPa antibodies does not disturb reversible ADP-induced human platelets aggregation while anti-integrin alpha 2b completely abrogates it.

Example 23. Proliferation of allogeneic T cells by SIRPa-blocking CD14+ cells from a cancer ovarian ascitis

Method: Allogeneic CD4 T cells were isolated by positive selection using an AutoMACS (Miltenyi) from hPBMC of a buffy coat of a healthy volunteer. CD4 were plated in 96-round well plate (50 000 cells/well). CD14+ cells were isolated by the same method from the ascitis of a cancer ovarian patient. The CD14+ cells were plated with the allogeneic CD4 T cells at a 1:1 ratio for 5 days. In some conditions, human LPS-matured allogeneic monocyte-derived dendritic cells (moDC) were added at a 1:5 ratio to stimulate T cells and analyzed the immunosuppressive action of different ratio of CD14+ MDSC purified from the ascite. Antibodies targeting the SIRPa/CD47 pathway were added from the beginning of the proliferation test at a saturating concentration (10µg/mL). Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Results: As shown in **Figures 21A and 21B**, Fresh and frozen human myeloid cells (TAM) purified from ovarian cancer ascites are hypo-stimulating allogeneic human T lymphocytes. In contrast to anti-CD47 antibodies, anti-SIRPa antibodies modifies myeloid cells properties allowing human T-cell activation and proliferation.

As shown in **Figure 21C**, human myeloid cells (MDSC) purified from ovarian cancer ascites can suppress human T-cell proliferation induced by allogeneic moDC at 1:1 and 2:1 myeloid to T-cell ratio. In contrast to anti-CD47 antibodies, anti-SIRPa antibodies does not potentiates the immunosuppression induced by human MDSC.

CLAIMS

1. An anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least one peptide of amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6 within human SIRPa,

in particular, that specifically binds to the peptide of amino acid sequence set forth in SEQ ID NO: 3 within human SIRPa and to at least another peptide of amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6 within human SIRPa; and/or

that specifically binds to a conformational epitope comprising at least one peptide selected from the group consisting of SEQ ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72 within human SIRPa.

2. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to claim 1, that specifically binds to the peptides of amino acid sequence set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6 within human SIRPa; and/or

that specifically binds to a conformational epitope consisting of the peptide of amino acid sequence set forth in SEQ ID NO: 73 and the peptide of SIR amino acid sequence within human SIRPa.

3. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to claims 1 or 2, which comprises:

a) a heavy chain variable domain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain variable domain comprising LCDR1, LCDR2 and LCDR3,

wherein:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14,

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 15 or SEQ ID NO: 16,

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 17 SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20,

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21,

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23.

4. An anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which comprises:

a) a heavy chain variable domain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain variable domain comprising LCDR1, LCDR2 and LCDR3,

wherein:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14,

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 15 or SEQ ID NO: 16,

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 17 SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20,

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21,

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23.

5. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 4, which is an anti-SIRPa antagonist antibody, which inhibits the binding of human CD47 to human SIRPa.

6. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 5, which does not specifically bind to human SIRPg.

7. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 6, which does not specifically bind to human T-cells, in particular CD3+ T-cells.

8. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 7, which does not inhibit the proliferation of human T-cells.

9. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 8, which does not inhibit the binding of human CD47 to human SIRPg.

10. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 9, which comprises:

- a heavy chain variable domain comprising or consisting of the amino acid sequence 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 and SEQ ID NO: 30, and/or

- a light chain variable domain comprising or consisting of the amino acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32 and SEQ ID NO: 33,

in particular, which comprises:

- a light chain variable domain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 33, and

- a heavy chain variable domain comprising or consisting of the amino acid sequence 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 and SEQ ID NO: 30, in particular SEQ ID NO: 29, SEQ ID NO: 30, and more particularly SEQ ID NO: 30.

11. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 10, which comprises:

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 24, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 31,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 25, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 25, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 26, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 26, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 27, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 27, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 28, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 28, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 29, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 29, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 30, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 30, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33.

12. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 11, wherein said antibody is a humanized monoclonal antibody, in particular wherein the antibody light chain constant domain is derived from a human kappa light chain constant domain, more particularly wherein the light chain constant domain consists of the sequence of SEQ ID NO: 35, and wherein the antibody heavy chain constant domain is derived from a human IgG1, IgG2, IgG3, or IgG4 heavy chain constant domain, in particular from a human IgG4 heavy chain constant domain, more particularly wherein the antibody heavy chain constant domain consists of the sequence with SEQ ID NO: 34.

13. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 12, or

an anti-human SIRPa antagonist antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of human CD47 to human SIRPa, and which does not bind specifically to human SIRPg, and/or which does not bind specifically to human T-cells, and/or which does not inhibit the proliferation of human T-cells, and/or which does not inhibit the binding of human CD47 to human SIRPg,

for use as a medicament.

14. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 12, or

an anti-human SIRPa antagonist antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of human CD47 to human SIRPa, and which

does not bind specifically to human SIRPg, and/or which does not bind specifically to human T-cells, and/or which does not inhibit the proliferation of human T-cells, and/or which does not inhibit the binding of human CD47 to human SIRPg,

for use in the prevention or treatment of a disease selected from the group consisting of a cancer (in particular inflammatory cancer and cancer with infiltrated myeloid cells, particularly with infiltrated MDSCs and/or TAM cells), an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (in particular Pseudomonas and CMV), fibrosis, atherosclerosis, obesity, type II diabetes and a transplant dysfunction, or for use in vaccination.

15. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 14, for its use according to claim 14, wherein said anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic is administered to a patient presenting a SIRPa-positive tumor.

16. A pharmaceutical composition comprising at least one anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 14, and a pharmaceutically acceptable carrier.

17. A combination product comprising:

- at least one anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 14, and
- at least one second therapeutic agent selected from the group consisting of chemotherapeutic agents, radiotherapy agents, immunotherapeutic agents, cell therapy agents, antibiotics and probiotics; in particular immunotherapeutic agents selected from the group consisting of checkpoint blocker or activator of adaptive immune cells, particularly selected from the group consisting of anti-PDL1, anti-PD1, anti-CTLA4, anti-CD137, anti-CD2, anti-CD28, anti-CD40, anti-HVEM, anti-BTLA, anti-CD160, anti-TIGIT, anti-TIM-1/3, anti-LAG-3, anti-2B4, and anti-OX40, anti-CD40 agonist, CD40-L, TLR agonists, anti-ICOS, ICOS-L and B-cell receptor agonists,

for simultaneous, separate or sequential use as a medicament.

18. An isolated nucleic acid molecule encoding an antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 12.

19. A vector comprising a nucleic acid molecule according to claim 18.

20. An isolated host cell comprising a nucleic acid molecule according to claim 18 and/or a vector according to claim 19.

21. A polypeptide, particularly an antigen, comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3,

in particular an antigen comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3 and at least one epitope sequence of human SIRPa selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6, more particularly an antigen comprising or consisting of the epitope sequences of human SIRPa consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6,

said polypeptide consisting of a sequence of up to 300 amino acids.

22. A method of manufacturing an antibody as defined in any one of claims 1 to 12 comprising immunizing a non-human animal, in particular a non-human mammal, against at least one antigen as defined in claim 21 and/or against at least one antigen comprising or consisting of at least one peptide selected from the group consisting of SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73 and the peptide of SIR amino acid sequence, said antigen consisting of a sequence of up to 300 amino acids, and in particular collecting the resulting serum from said immunised non-human animal to obtain antibodies directed against said antigen.

23. An *in vitro* or *ex vivo* method to determine a SIRPa positive cells in a subject from a biological sample previously obtained from said subject, comprising:

i) determining *in vitro* the expression and/or the level of expression of SIRPa, in a biological sample previously obtained from said subject using the anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 14.

24. An *in vitro* or *ex vivo* method of diagnosis, in particular a method of diagnostic suitable for use in personalized medicine, more particularly in a companion diagnosis, wherein an anti-SIRPa antibody or an antigen-binding fragment thereof or an antigen-binding mimetic according to any one of claims 1 to 14 is used for the detection of SIRPa+ cells in a sample previously obtained from a subject and optionally for the quantification of the expression of SIRPa.

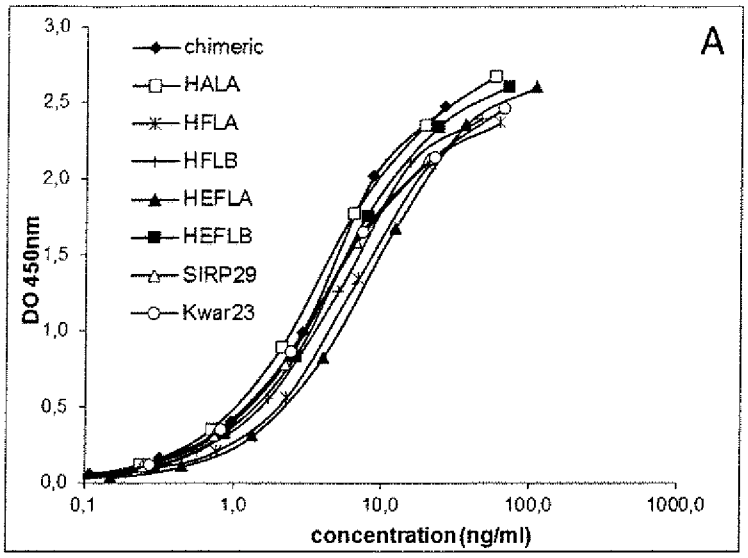
25. Use of an anti-SIRPa antibody or an antigen-binding fragment thereof or an antigen-binding mimetic according to any one of claims 1 to 14, in the manufacture of a medicament suitable for use in a diagnostic test, in particular for use in personalized medicine, or in a companion diagnostic test.

26. *In vitro* or *ex vivo* use of at least one anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 14 in a method wherein SIRPa is used as a biomarker that is predictive for the response to a treatment in a subject, in particular in a cancer subject.

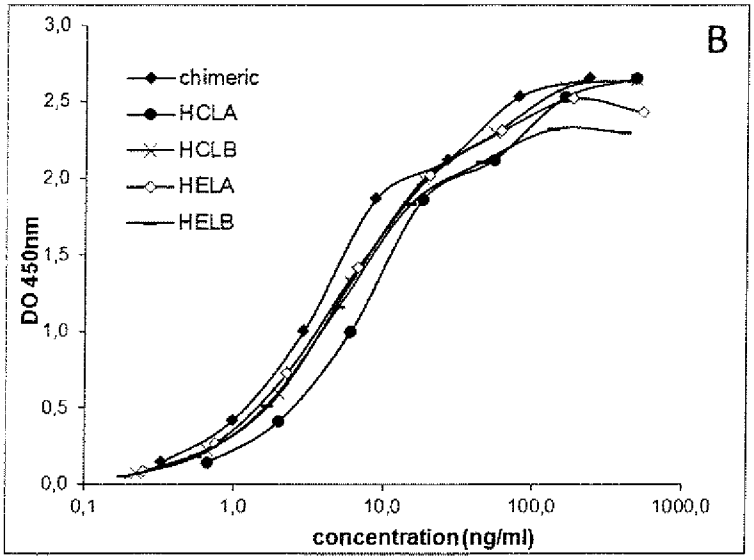
27. An *in vitro* or *ex vivo* method of predicting the response of a cancer subject to a treatment, in particular with anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 14, comprising:

- determining the expression level of SIRPa in a tumor sample previously obtained from a subject, in particular with anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 14, and
- comparing the expression level of SIRPa to a value representative of an expression level of SIRPa in a non-responding subject population,

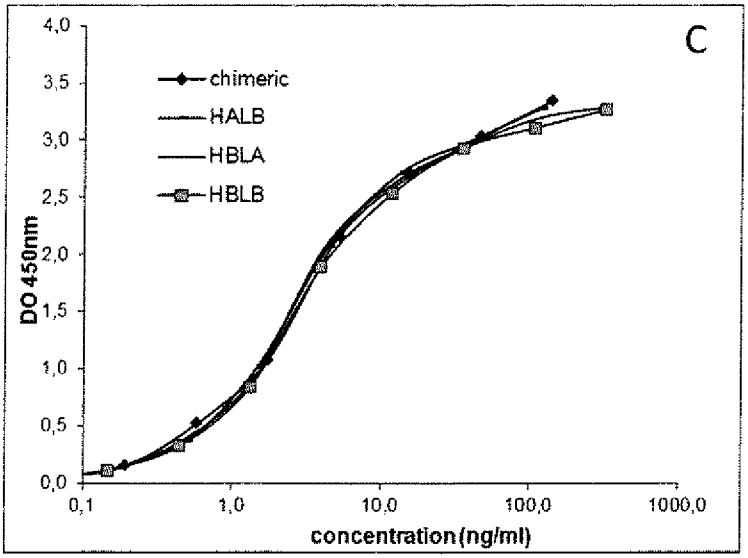
wherein a higher expression level of SIRPa in the tumor sample of the subject is indicative for a patient who will respond to the treatment.



	EC50 (ng/ml)
chimeric	2,9
HALA	3,9
HFLA	5,1
HFLB	4,0
HEFLA	7,1
HEFLB	4,4
SIRP29	3,7
Kwar23	3,3



	EC50 (ng/ml)
chimeric	4,06
HCLA	5,60
HCLB	5,59
HELA	4,61
HELB	4,13



	EC50 (ng/ml)
chimeric	2,74
HALB	2,53
HBLA	2,68
HBLB	2,95

FIGURE 1 (start)

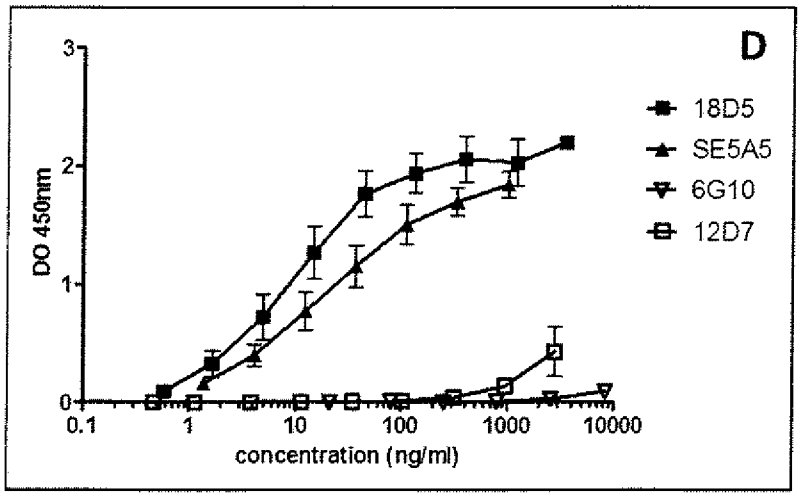
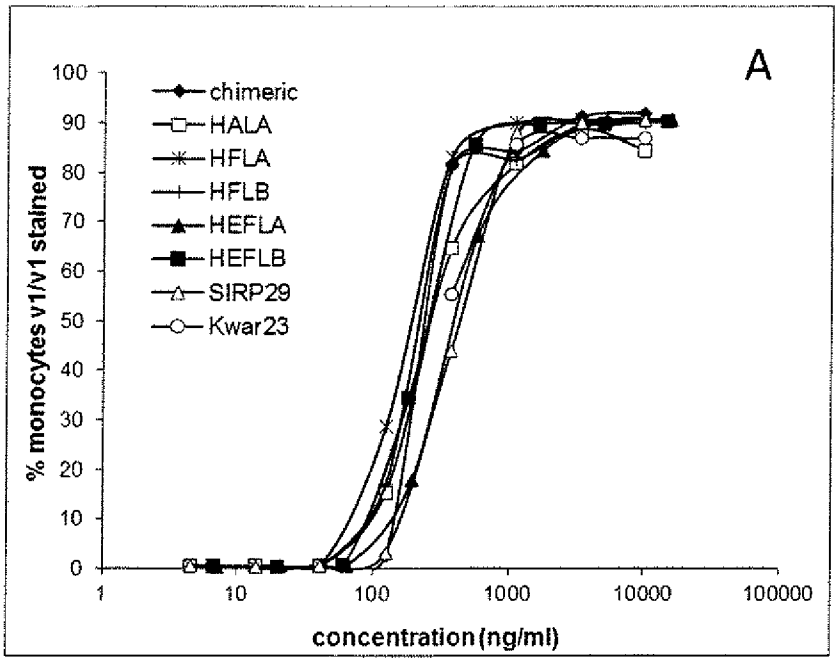


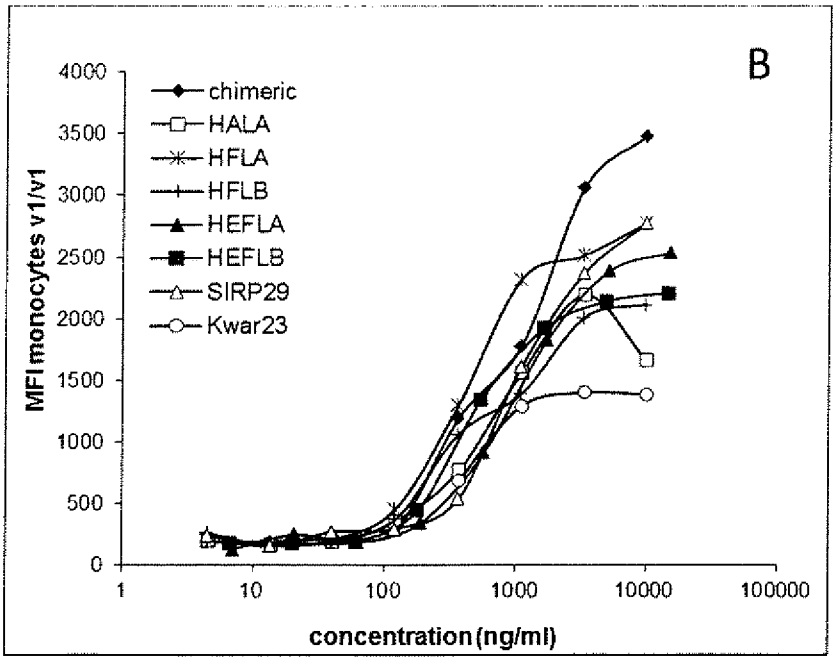
FIGURE 1 (end)

	Association (ka) (1/Ms)	Dissociation (kd) (1/s)	Affinity (KD) (M)
mouse 18D5	6,23e4	1,20e-5	**1,93e-10**
chimeric	1,49e5	5,14e-5	**3,44e-10**
HFLA	8,57e5	1,86e-4	**2,17e-10**
HFLB	7,91e5	2,49e-4	**3,15e-10**
HEFLA	5,59e5	1,48e-4	**2,64e-10**
HEFLB	6,54e5	1,89e-4	**2,89e-10**
SIRP29	4,15e4	1,01e-5	**2,43e-10**
Kwar23	2,44e5	8,95e-5	**3,67e-10**
SE7C2	5,98e3	0,01	**1,67e-6**
SE5A5	2,45e4	9,49e-4	**3,87e-8**

FIGURE 2

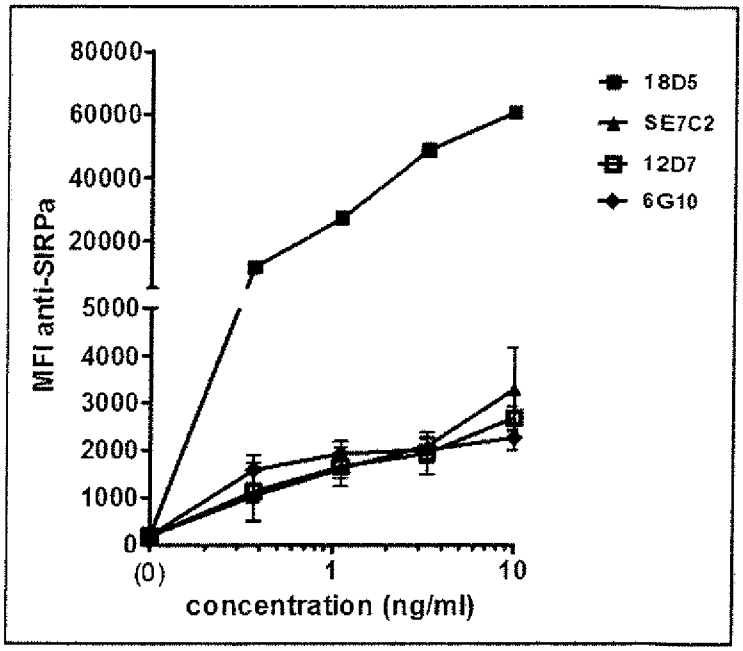


% stained	EC50 (ng/ml)
chimeric	225
HALA	242
HFLA	174
HFLB	201
HEFLA	356
HEFLB	208
SIRP29	376
Kwar23	260



MFI	EC50 (ng/ml)
chimeric	780
HALA	590
HFLA	328
HFLB	467
HEFLA	858
HEFLB	449
SIRP29	960
Kwar23	378

FIGURE 3 (start)



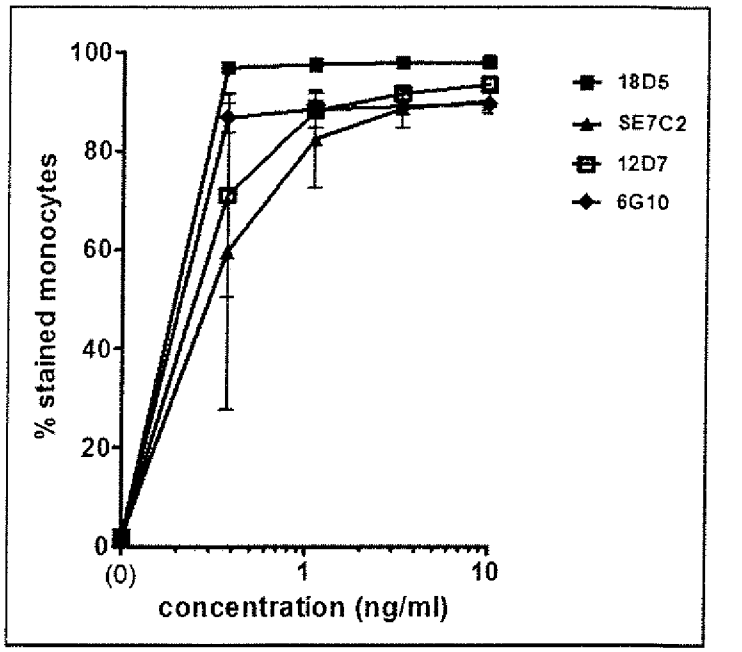


FIGURE 3 (middle)

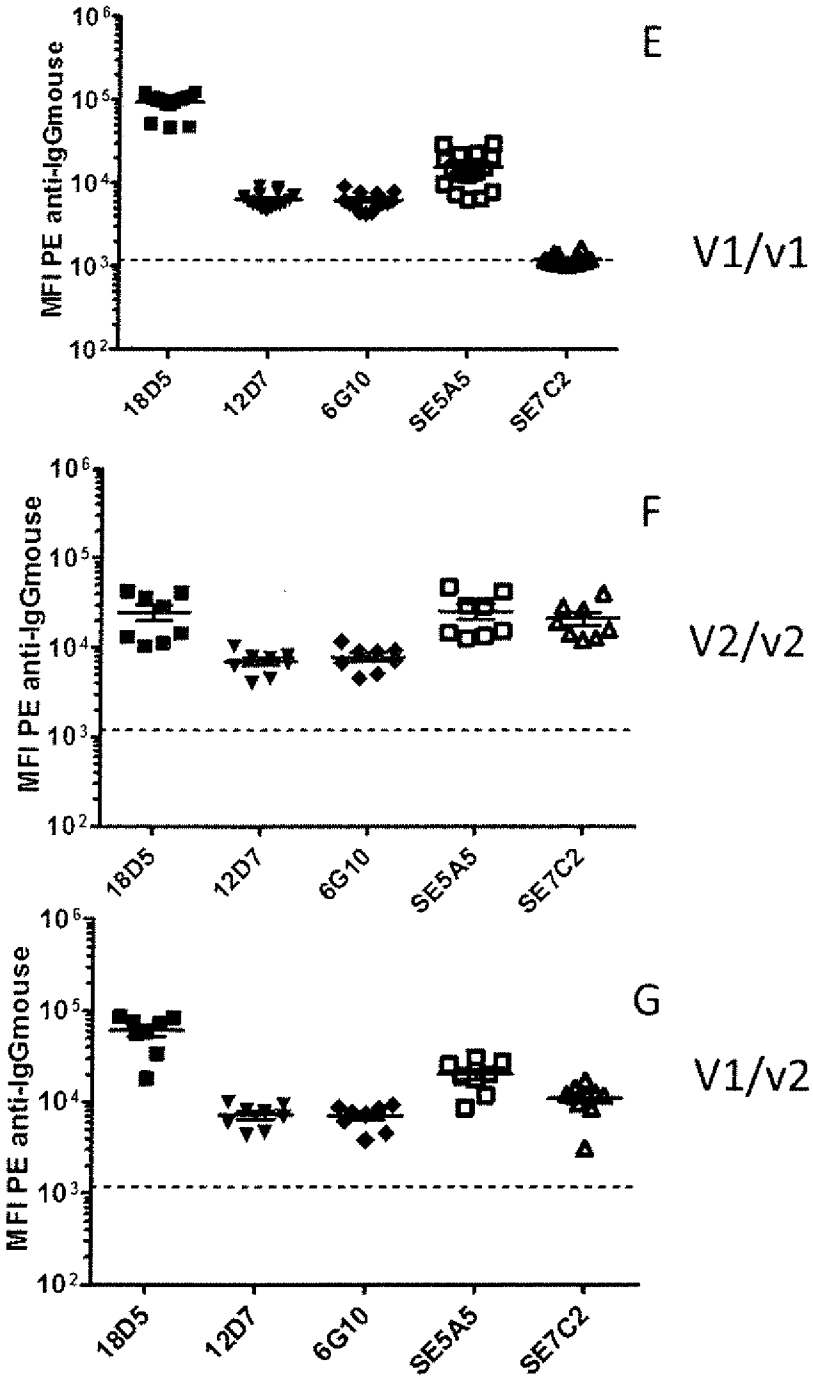
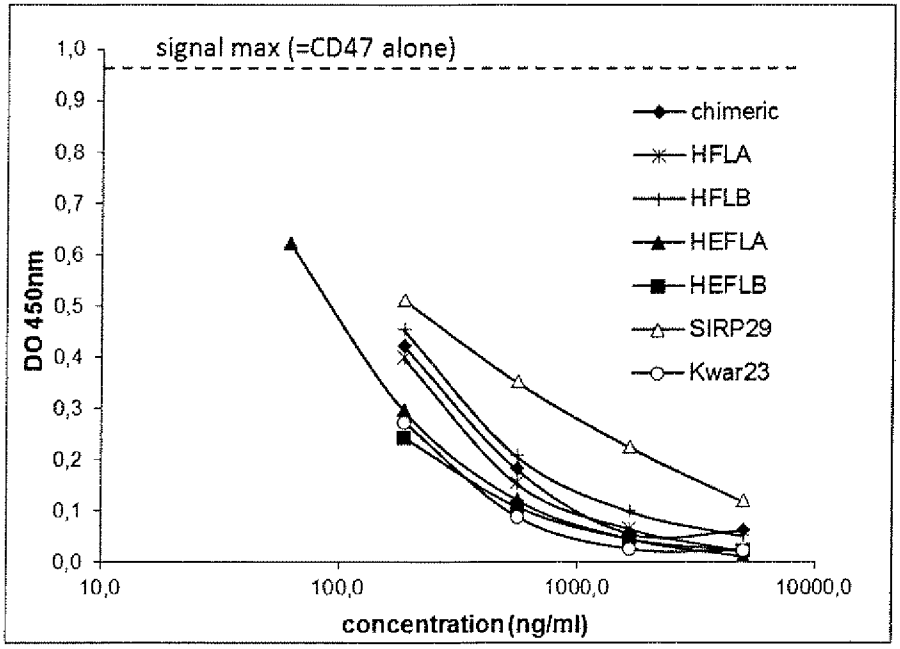

E
MFI PE anti-IgGmouse
10^6
10^5
10^4
10^3
10^2
18D5
12D7
6G10
SE5A5
SE7C2
V1/v1
F
MFI PE anti-IgGmouse
V2/v2
G
MFI PE anti-IgGmouse
V1/v2

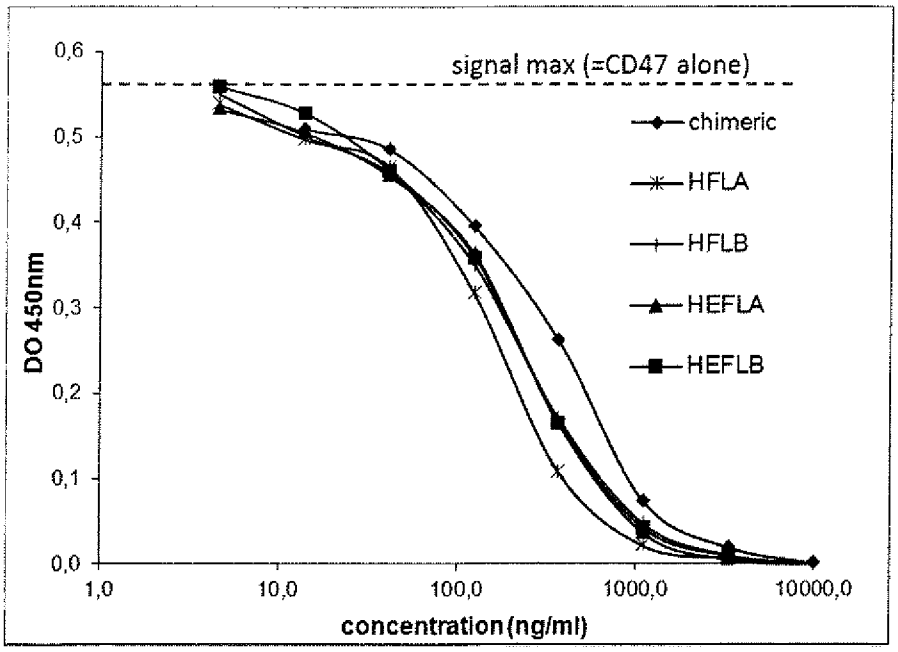
FIGURE 3 (End)

A

First experiment



Second experiment



	IC50 (ng/ml)
chimeric	303,8
HFLA	146,2
HFLB	182,7
HEFLA	190,6
HEFLB	186,3

FIGURE 4 (start)

B

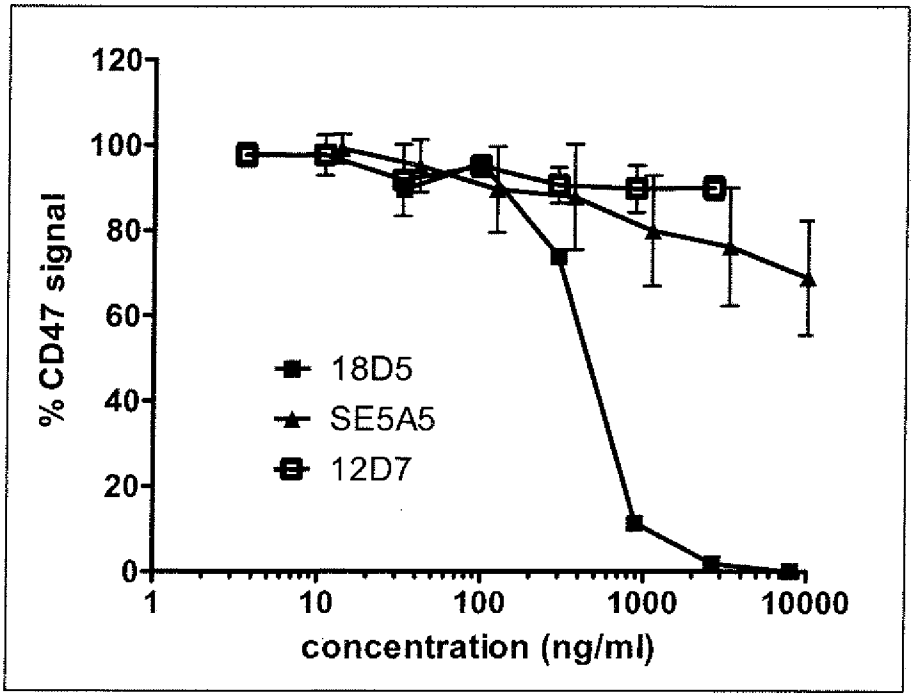
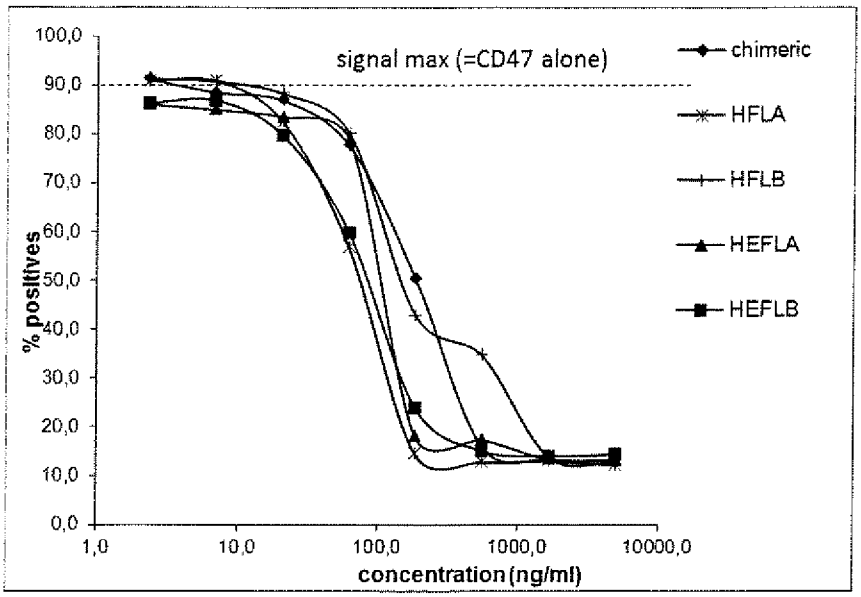


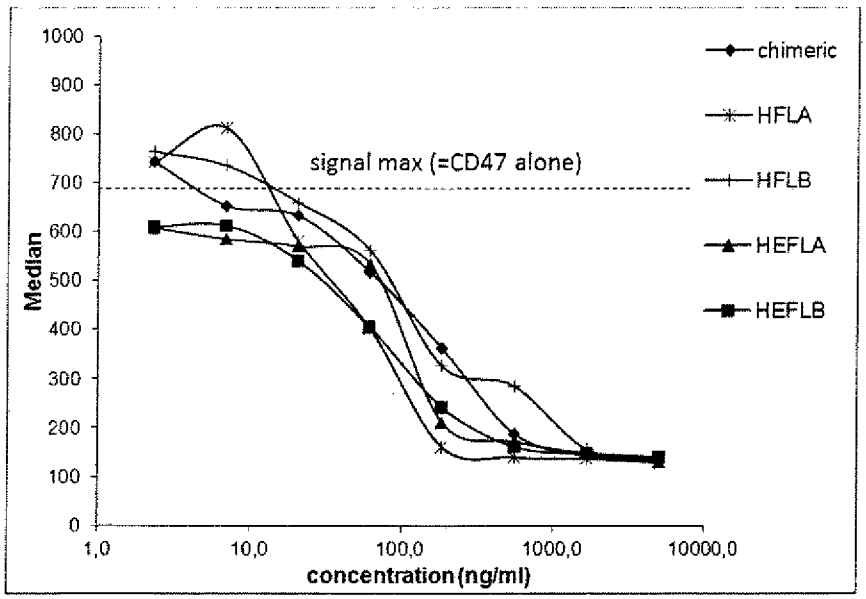
FIGURE 4 (end)

A



	IC50 (ng/ml)
chimeric	171,83
HFLA	64,09
HFLB	149,73
HEFLA	104,56
HEFLB	73,49

B



	IC50 (ng/ml)
chimeric	199,9
HFLA	75,1
HFLB	169,9
HEFLA	117,6
HEFLB	90,1

FIGURE 5 (start)

C

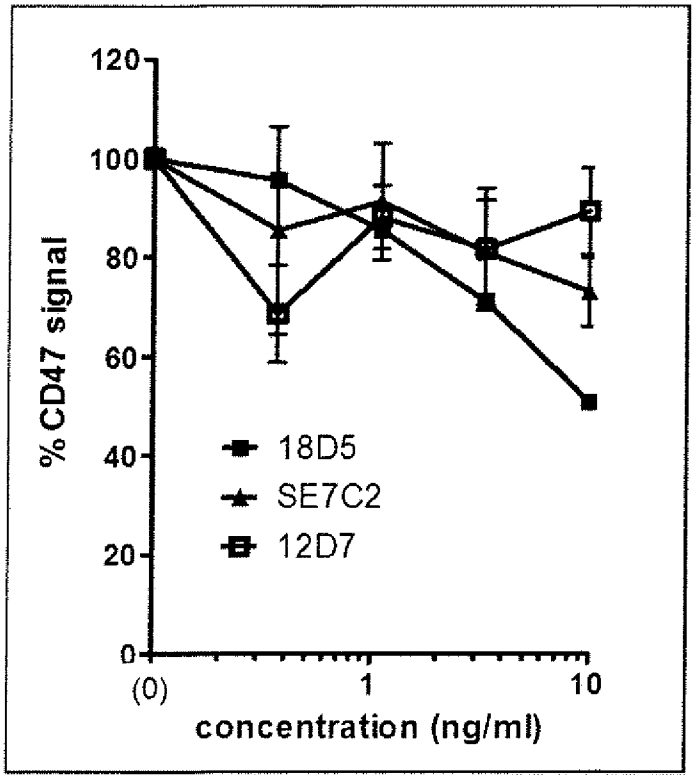


FIGURE 5 (end)

A	Association (ka) (1/Ms)	Dissociation (kd) (1/s)	Affinity (KD) (M)
mouse 18D5	1,58e4	1,76e-3	1,11e-7
mouse 18D5 with SP-D	3,797e5	1,088e-2	2,865e-8

B	Association (ka) (1/Ms)	Dissociation (kd) (1/s)	Affinity (KD) (M)
SP-D	2,465e3	1,078e-2	4,372e-6
SPD with mouse 18D5	3,272e3	2,405e-3	7,35e-7

FIGURE 6

A

	Association (ka) (1/Ms)	Dissociation (kd) (1/s)	Affinity (KD) (M)
Chimeric	8,837e4	1,385e-3	1,568e-8
HALA	1,328e5	8,504e-4	6,403e-9
HFLA	7,447e4	1,919e-3	2,576e-8
HFLB	6,694e4	1,738e-3	2,596e-8
HEFLA	9,827e4	1,221e-3	1,243e-8
HEFLB	1,939e4	1,711e-3	8,69e-8
SIRP29	1,078e5	8,114e-4	7,531e-9
kwar23	2,863e5	7,935e-4	2,772e-9

B

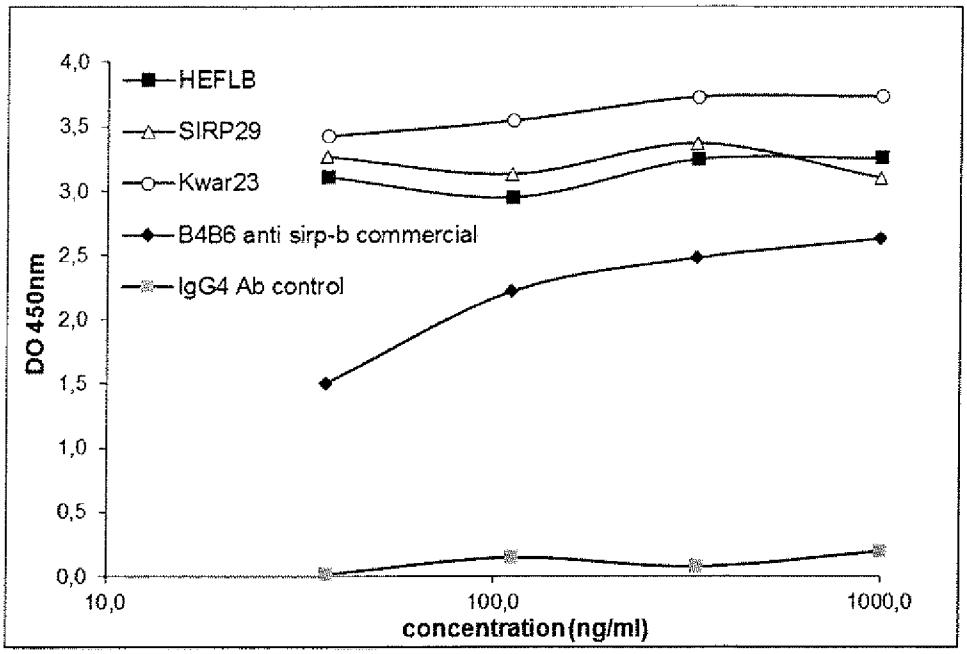


FIGURE 7

A

	Association (ka) (1/Ms)	Dissociation (kd) (1/s)	Affinity (KD) (M)
Chimeric	4,93e4	3,194e-3	6,479e-8
HALA	1,539e5	7,093e-3	4,609e-8
HFLA	2,477e5	1,18e-2	4,764e-8
HFLB	1,191e5	1,234e-2	1,036e-7
HEFLA	2,173e5	1,14e-2	5,244e-8
HEFLB	1,92e5	1,193e-2	6,215e-8
SIRP29	1,36e5	7,2e-4	5,296e-9
kwar23	3,57e5	7,648e-4	2,142e-9

B

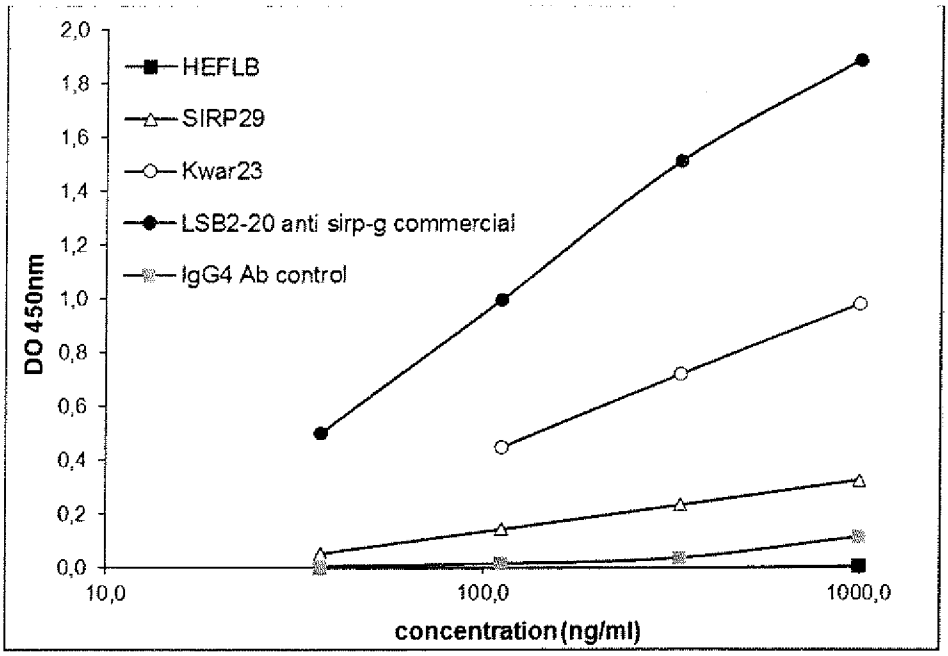


FIGURE 8

	Association (ka) (1/Ms)	Dissociation (kd) (1/s)	Affinity (KD) (M)
CD47 alone	2,193e4	1,309e-2	5,969e-7
HEFLB +CD47	5,372e4	5,334e-2	9,928e-7
SIRP29 +CD47	2,118e4	5,525e-2	2,572e-6
Kwar23 +CD47	4,369e2	1,259e-1	2,88e-4

FIGURE 9

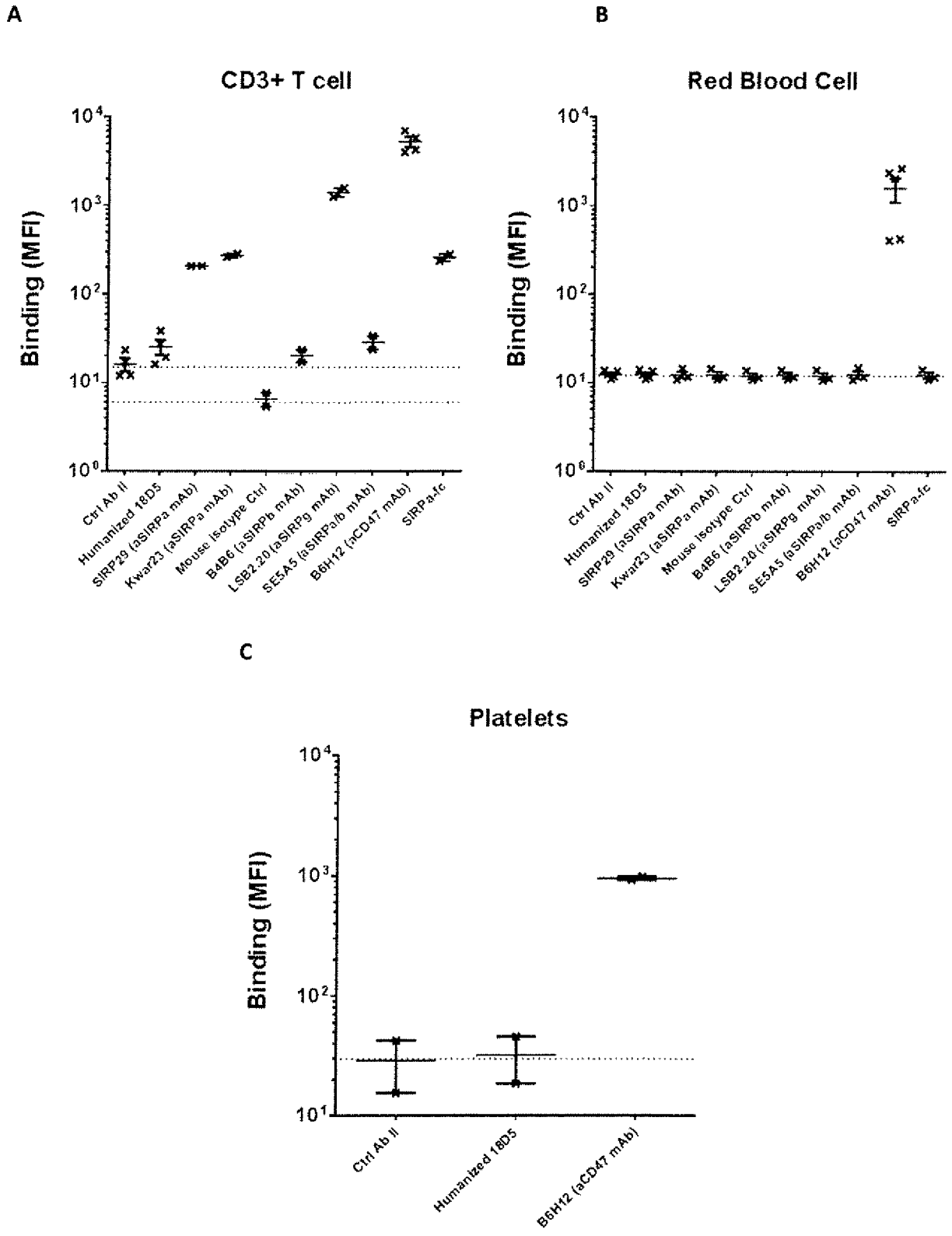
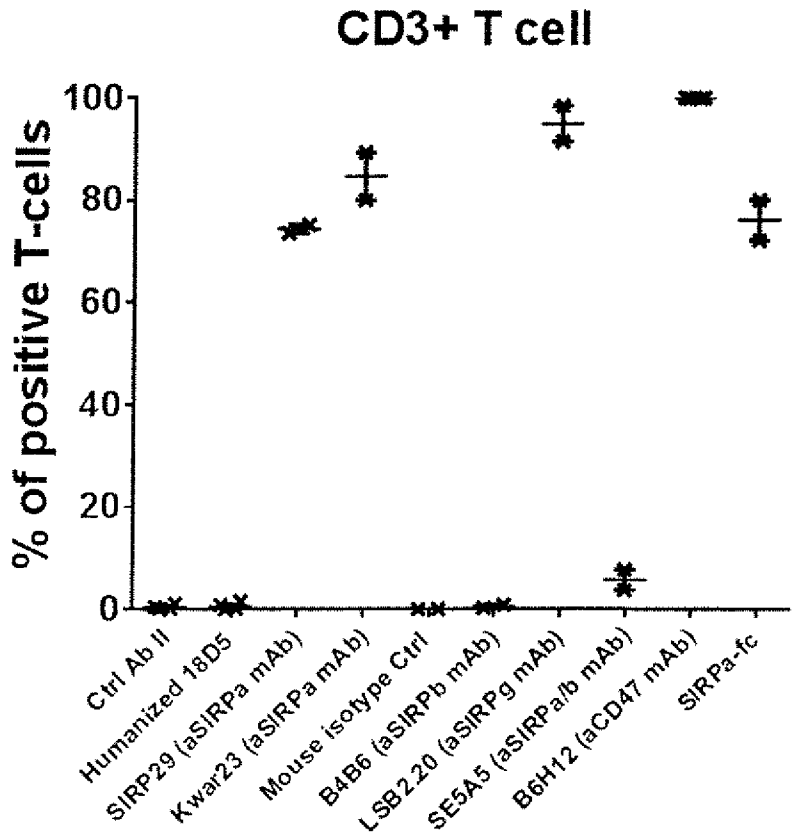

A
CD3+ T cell
Binding (MFI)
10^4
10^3
10^2
10^1
10^0
Ctrl Ab II
Humanized 18D5
SIRP29 (aSIRPa mAb)
Kwar23 (aSIRPa mAb)
Mouse Isotype Ctrl
B4B6 (aSIRPb mAb)
LSB2.20 (aSIRPg mAb)
SE5A5 (aSIRPa/b mAb)
B6H12 (aCD47 mAb)
SIRPa-fc
B
Red Blood Cell
Binding (MFI)
10^4
10^3
10^2
10^1
10^0
Ctrl Ab II
Humanized 18D5
SIRP29 (aSIRPa mAb)
Kwar23 (aSIRPa mAb)
Mouse Isotype Ctrl
B4B6 (aSIRPb mAb)
LSB2.20 (aSIRPg mAb)
SE5A5 (aSIRPa/b mAb)
B6H12 (aCD47 mAb)
SIRPa-fc
C
Platelets
Binding (MFI)
10^4
10^3
10^2
10^1
Ctrl Ab II
Humanized 18D5
B6H12 (aCD47 mAb)

FIGURE 10



	Ctrl Ab II	Humanized 18D5	SIRP29 (aSIRPa mAb)	Kwar23 (aSIRPa mAb)	Mouse isotype Ctrl	B4B6 (aSIRPb mAb)	LSB2.20 (aSIRPg mAb)	SE5A5 (aSIRPa/b mAb)	B6H12 (aCD47 mAb)	SIRPa-fc
Exp 1 HV1	0,45	1,54	73,5	80	0	0,87	91,4	3,72	100	72,2
Exp 1 HV2	1,12	0,84	75,2	89,3	0,016	0,25	98,4	7,69	100	80,1
Exp 2 HV1	0,057	0,016							99,9	
Exp 2 HV2	0,076	0,058							99,9	

FIGURE 11

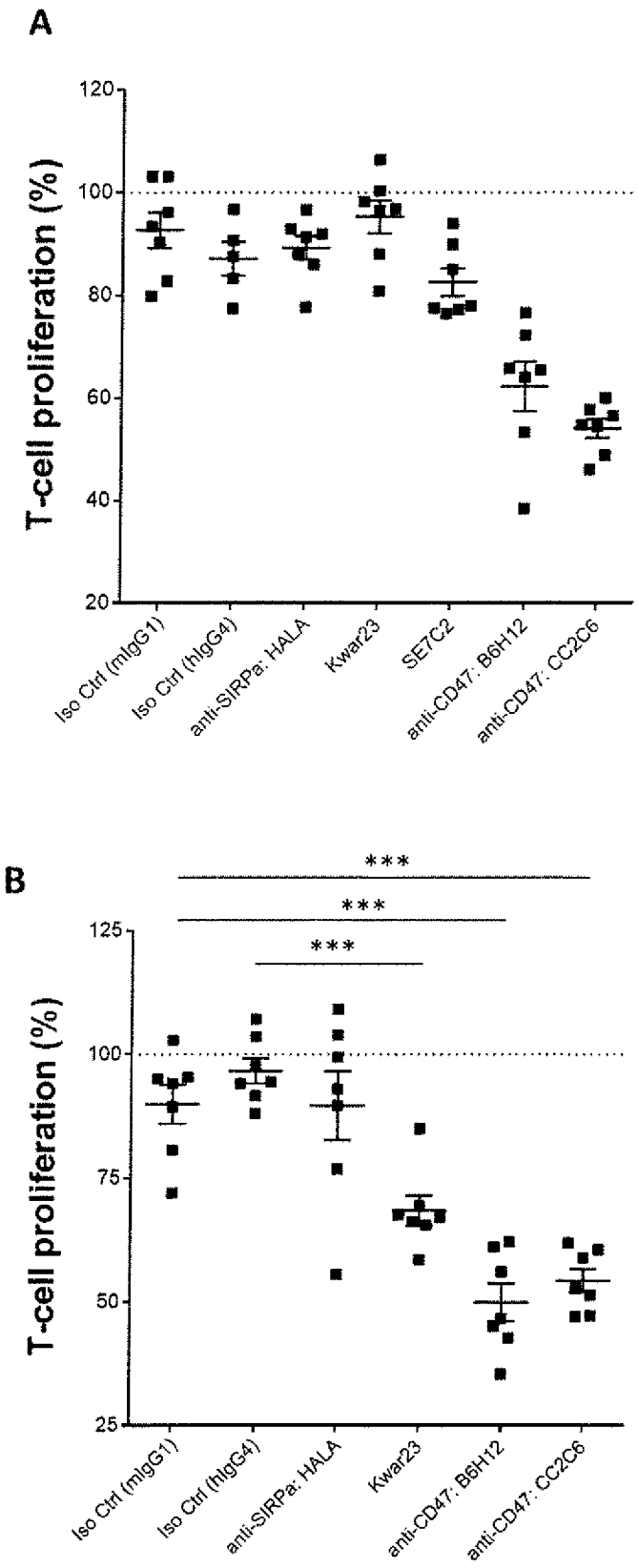


FIGURE 12 (start)

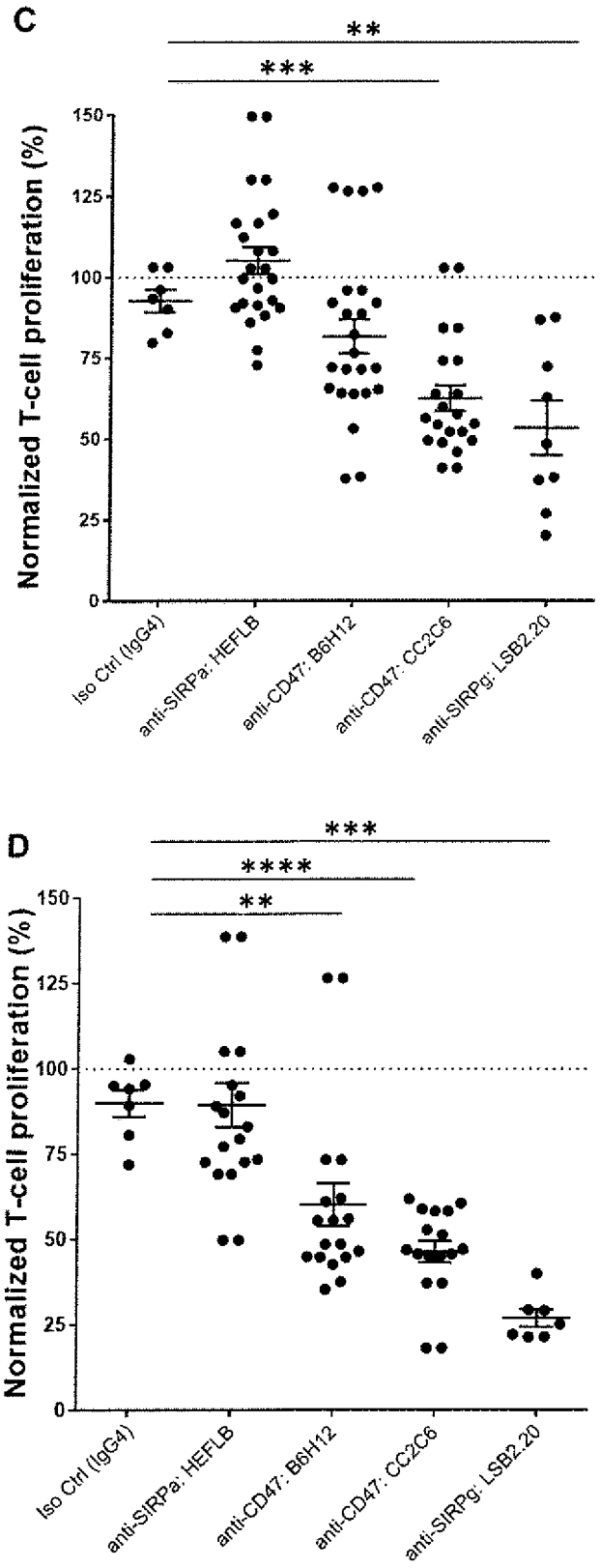


FIGURE 12 (middle)

E

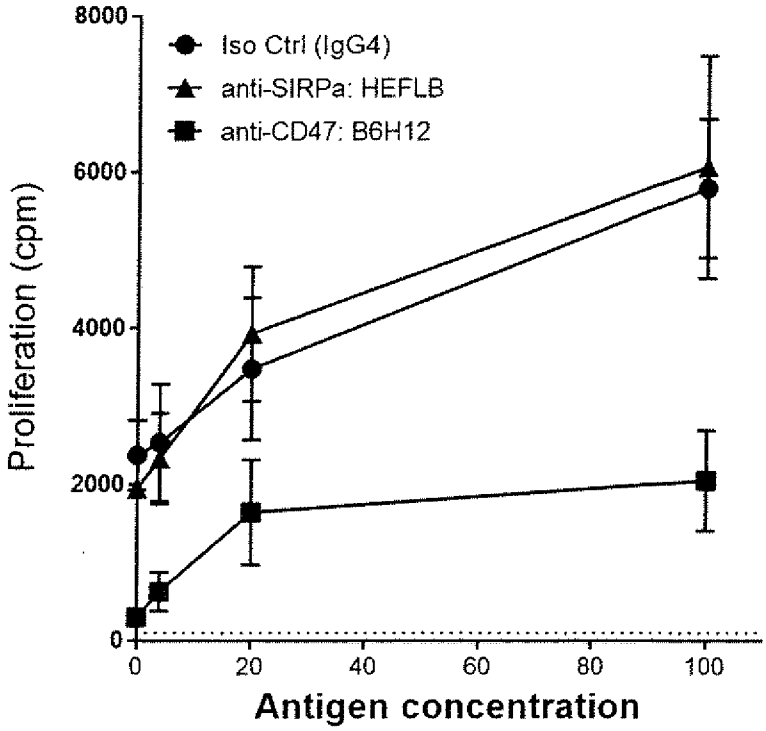


FIGURE 12 (end)

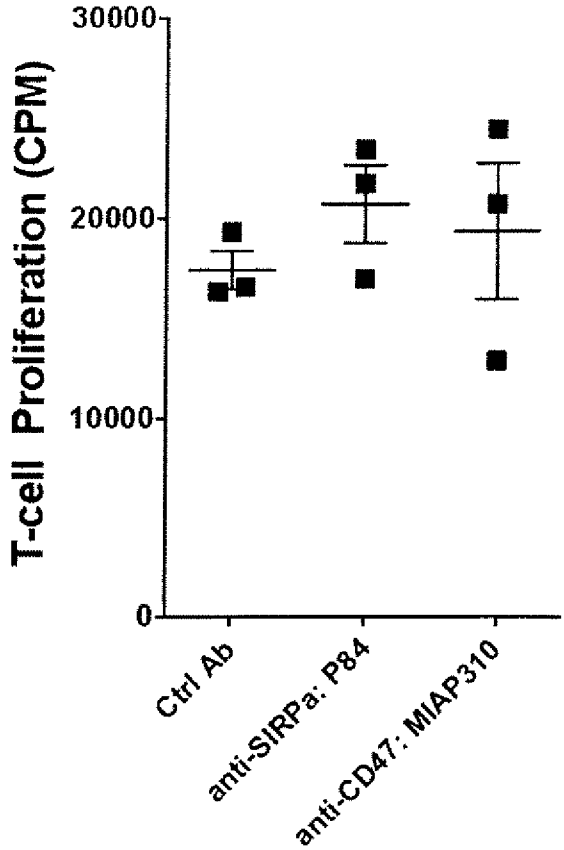
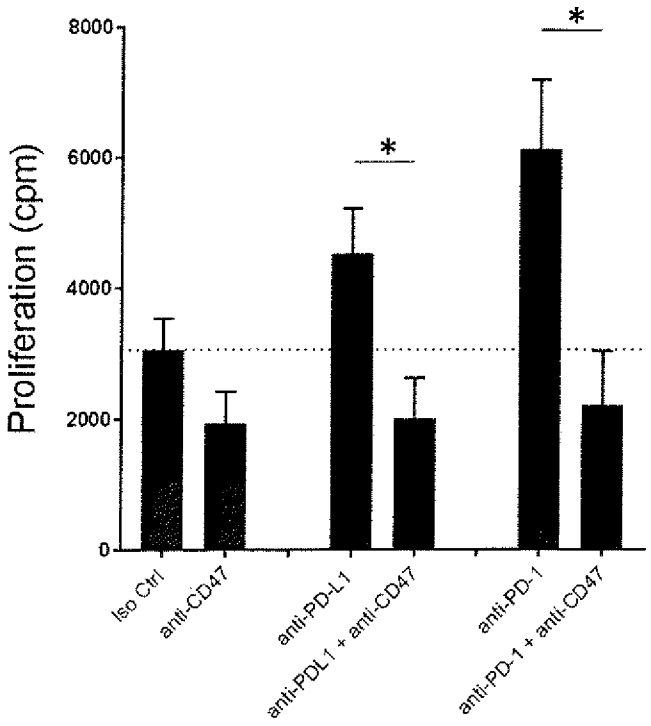


FIGURE 13


Proliferation (cpm)
8000
6000
4000
2000
0
*
*
Iso Ctrl
anti-CD47
anti-PD-L1
anti-PDL1 + anti-CD47
anti-PD-1
anti-PD-1 + anti-CD47

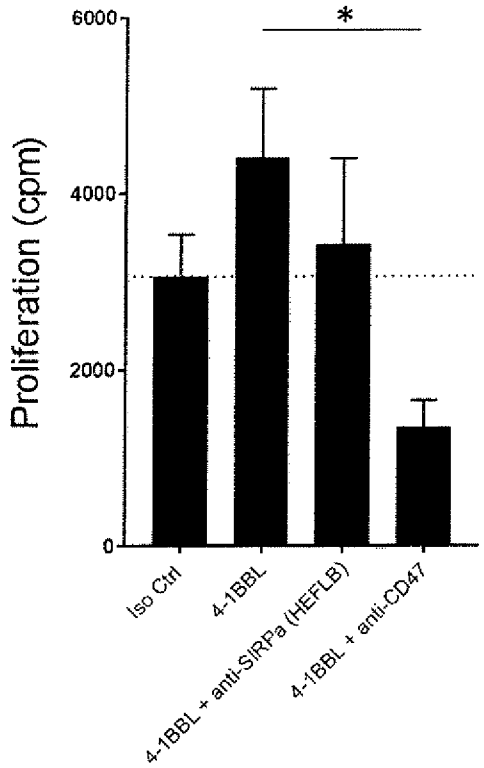

Proliferation (cpm)
6000
4000
2000
0
*
Iso Ctrl
4-1BBL
4-1BBL + anti-SIRPa (HEFLB)
4-1BBL + anti-CD47

FIGURE 14

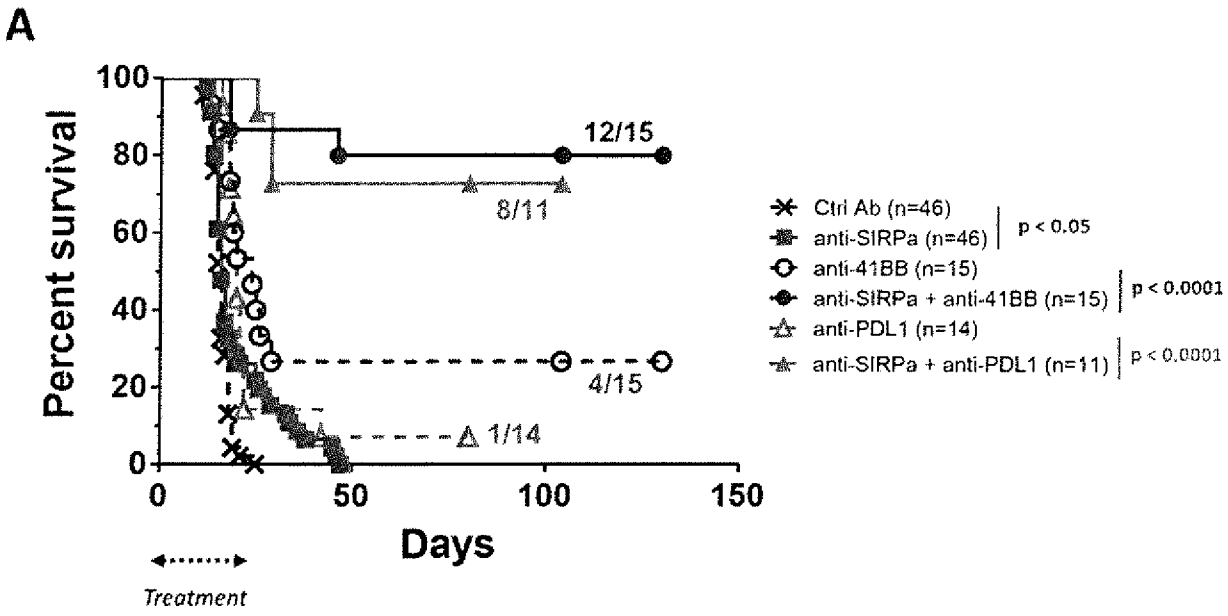
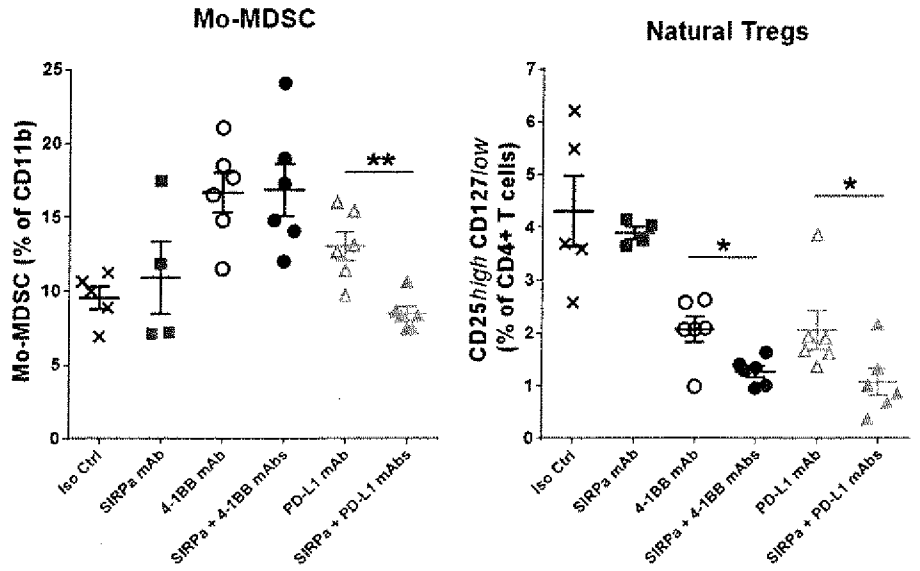


FIGURE 15 (start)

B Reduced regulatory cells



Increased effector T cells

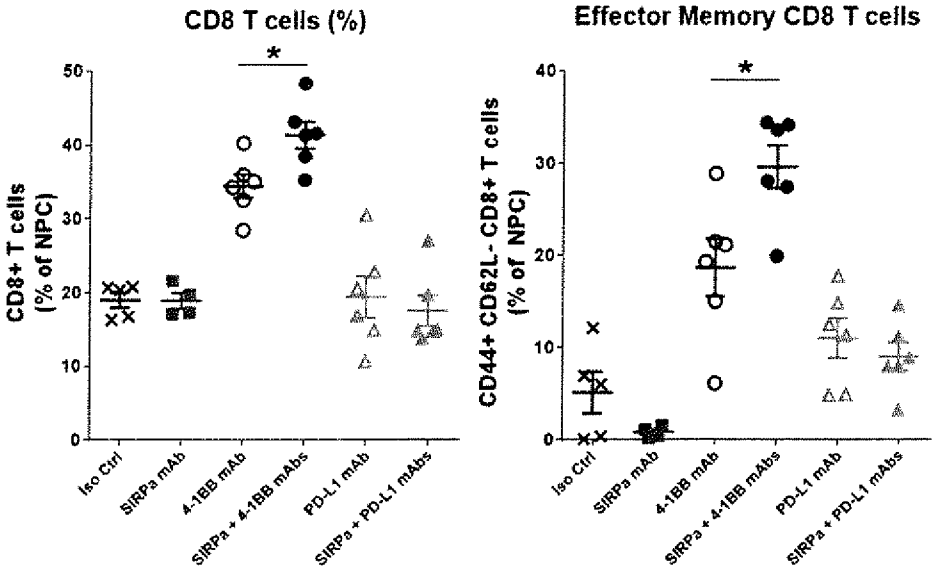


FIGURE 15 (middle)

C

Tumor HCC

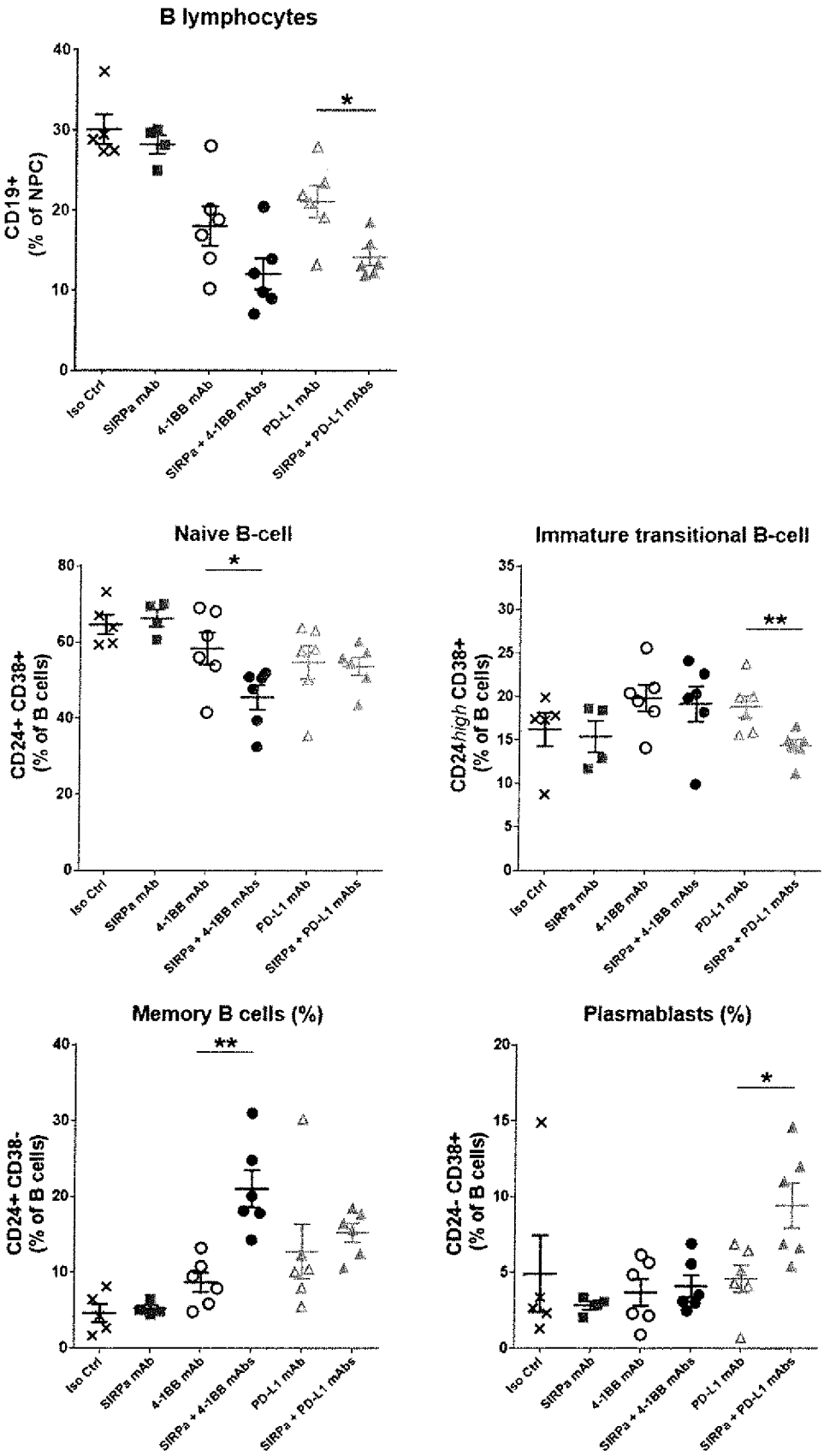


FIGURE 15 (middle)

Periphery (spleen)

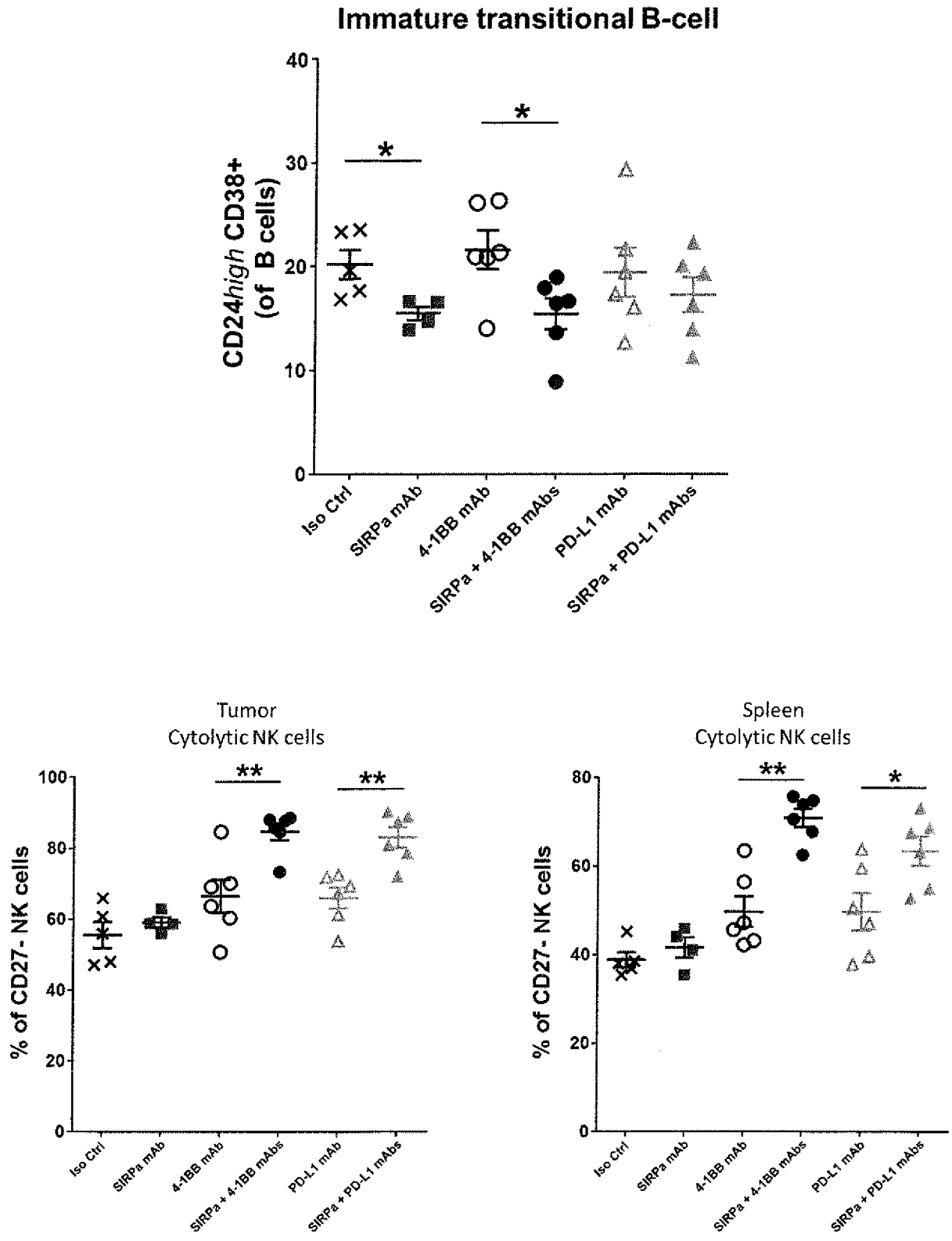


FIGURE 15 (middle)

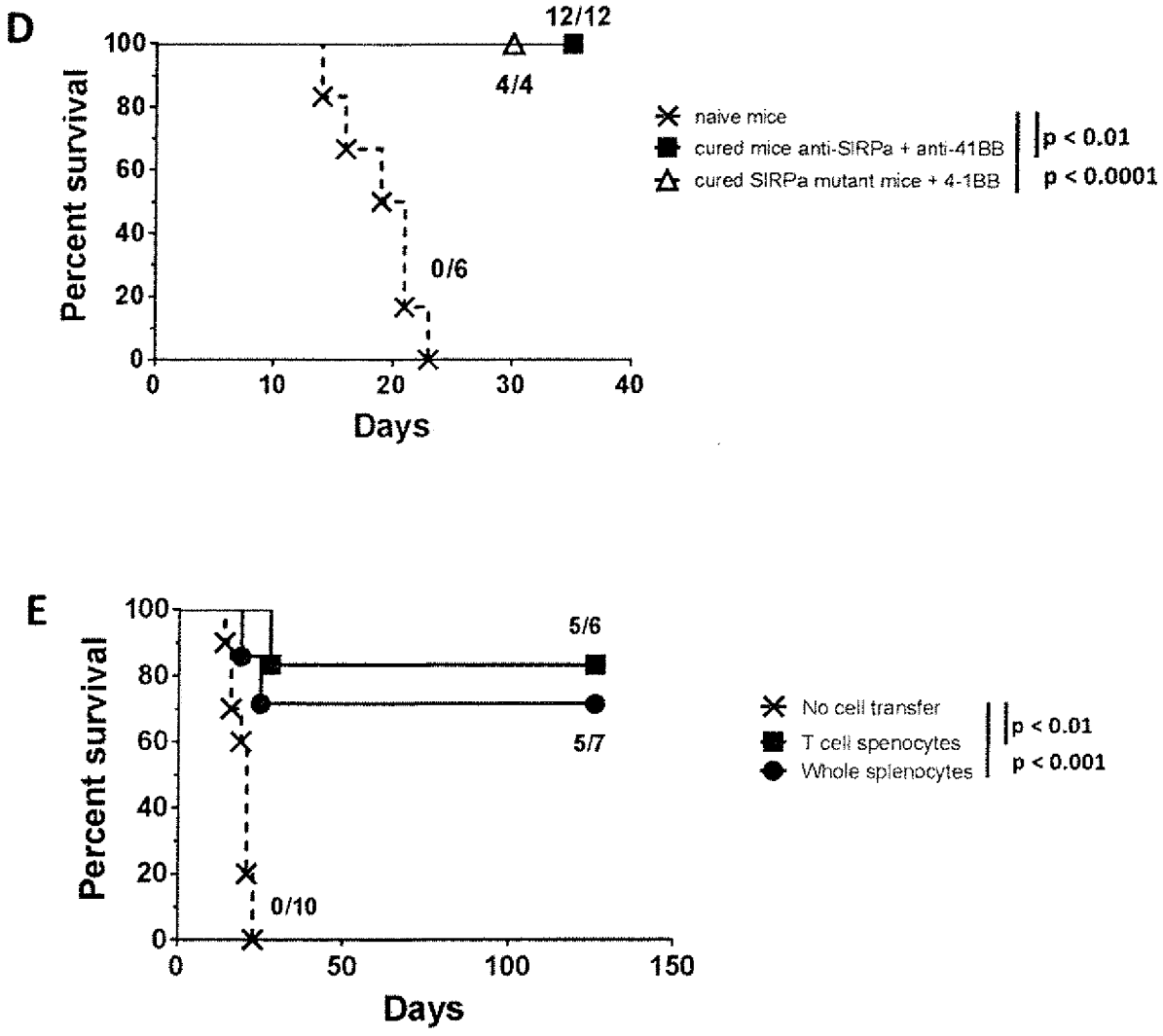


FIGURE 15 (end)

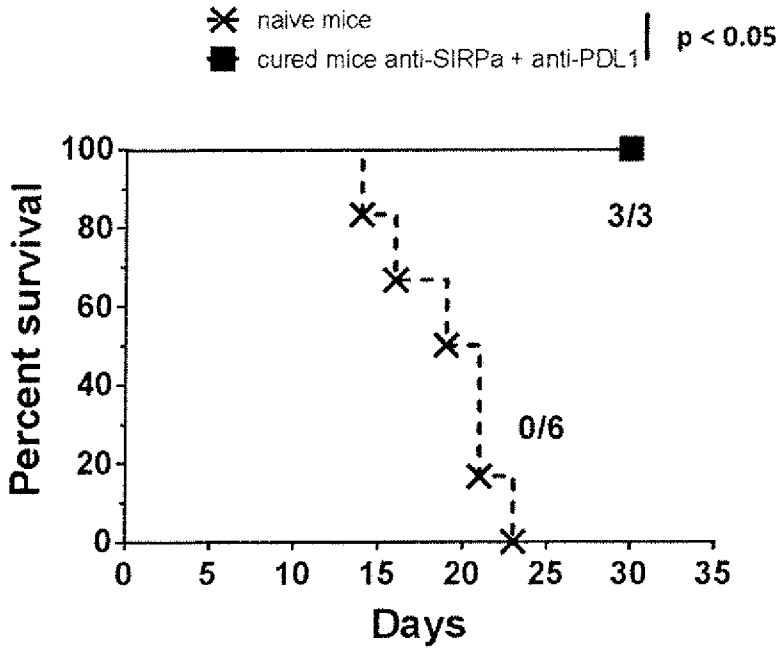


FIGURE 16

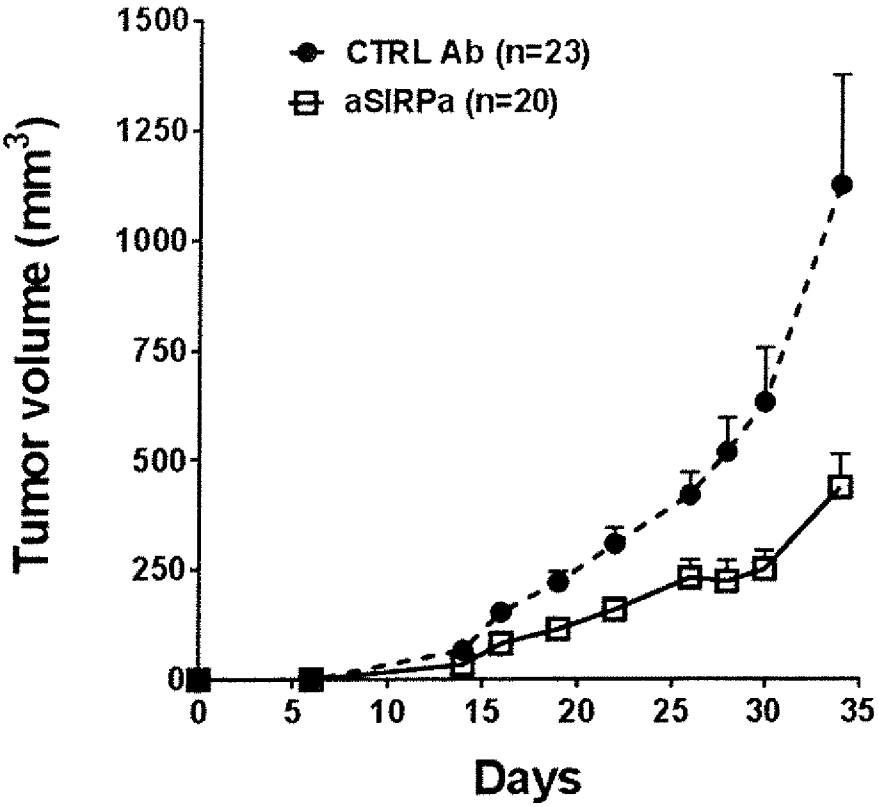


FIGURE 17

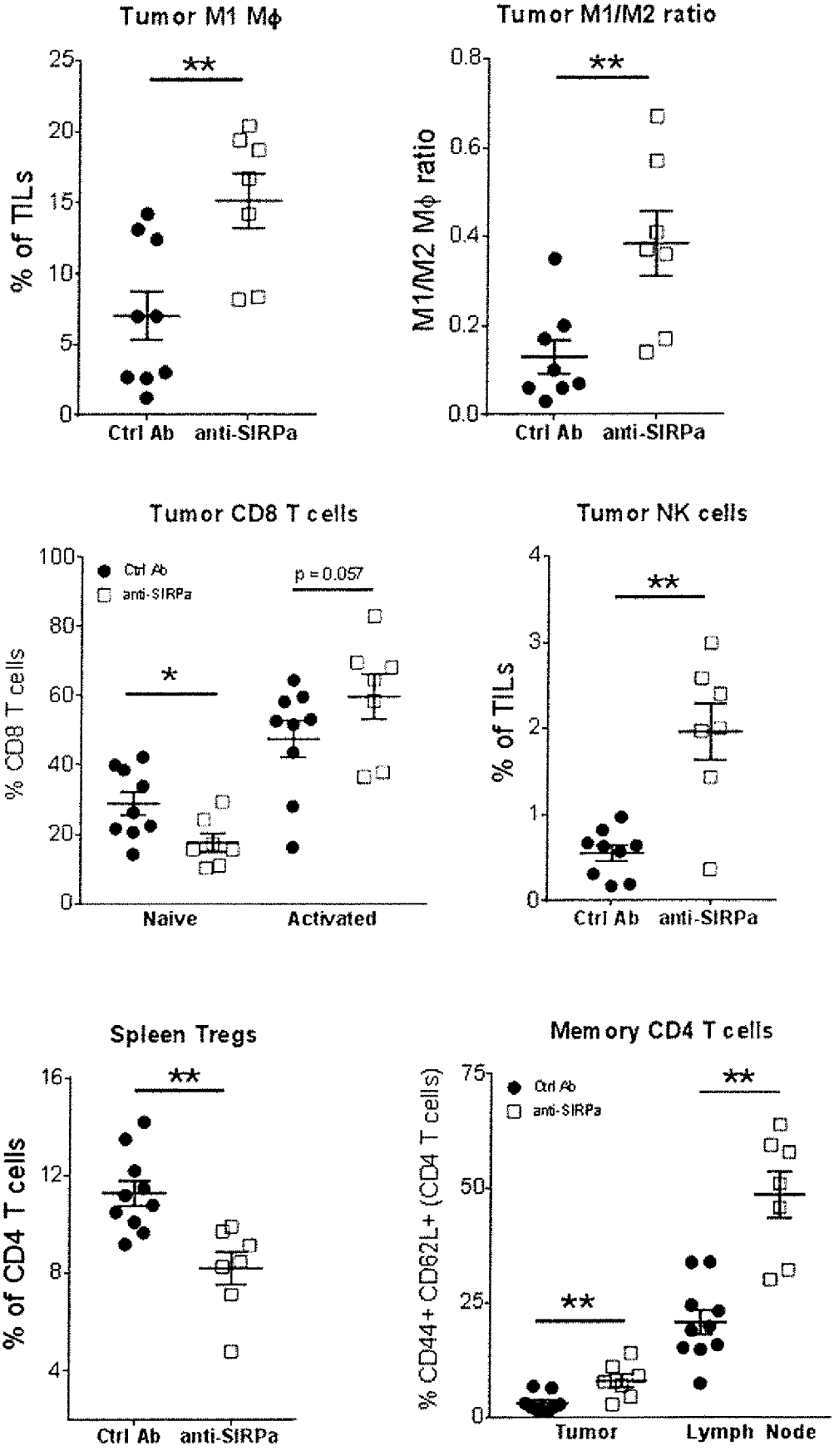
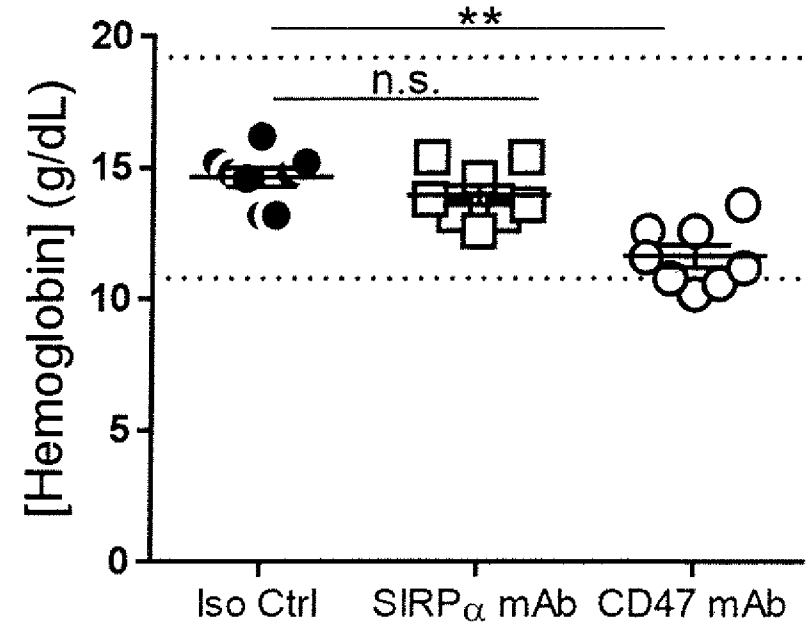

Tumor M1 Mϕ
% of TILs
25
20
15
10
5
0
**
Ctrl Ab
anti-SIRPa
Tumor M1/M2 ratio
M1/M2 Mϕ ratio
0.8
0.6
0.4
0.2
0.0
**
Ctrl Ab
anti-SIRPa
Tumor CD8 T cells
% CD8 T cells
100
80
60
40
20
0
Ctrl Ab
anti-SIRPa
p = 0.057
*
Naive
Activated
Tumor NK cells
% of TILs
4
3
2
1
0
**
Ctrl Ab
anti-SIRPa
Spleen Tregs
% of CD4 T cells
16
12
8
4
**
Ctrl Ab
anti-SIRPa
Memory CD4 T cells
% CD44+CD62L+ (CD4 T cells)
75
50
25
0
Ctrl Ab
anti-SIRPa
**
**
Tumor
Lymph Node

FIGURE 18


**
n.s.
20
15
10
5
0
[Hemoglobin] (g/dL)
Iso Ctrl
SIRPα mAb
CD47 mAb

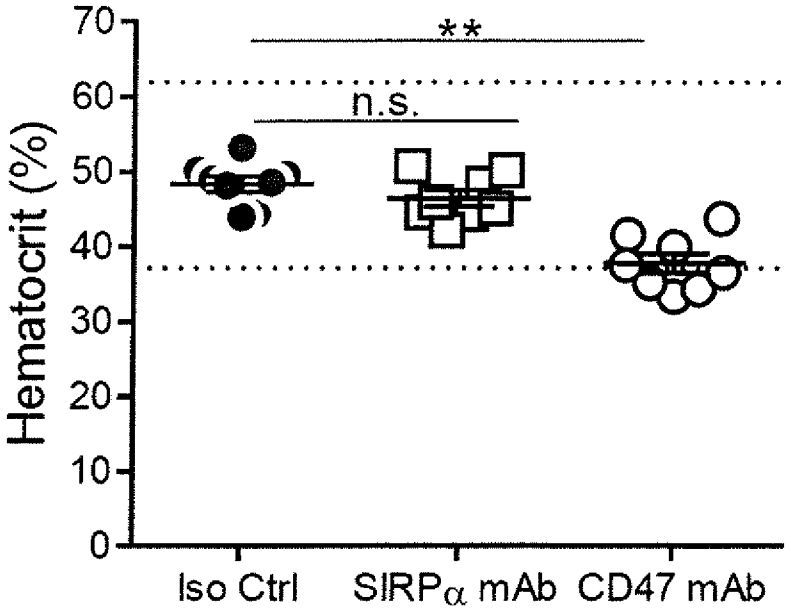

**
n.s.
70
60
50
40
30
20
10
0
Hematocrit (%)
Iso Ctrl
SIRPα mAb
CD47 mAb

FIGURE 19

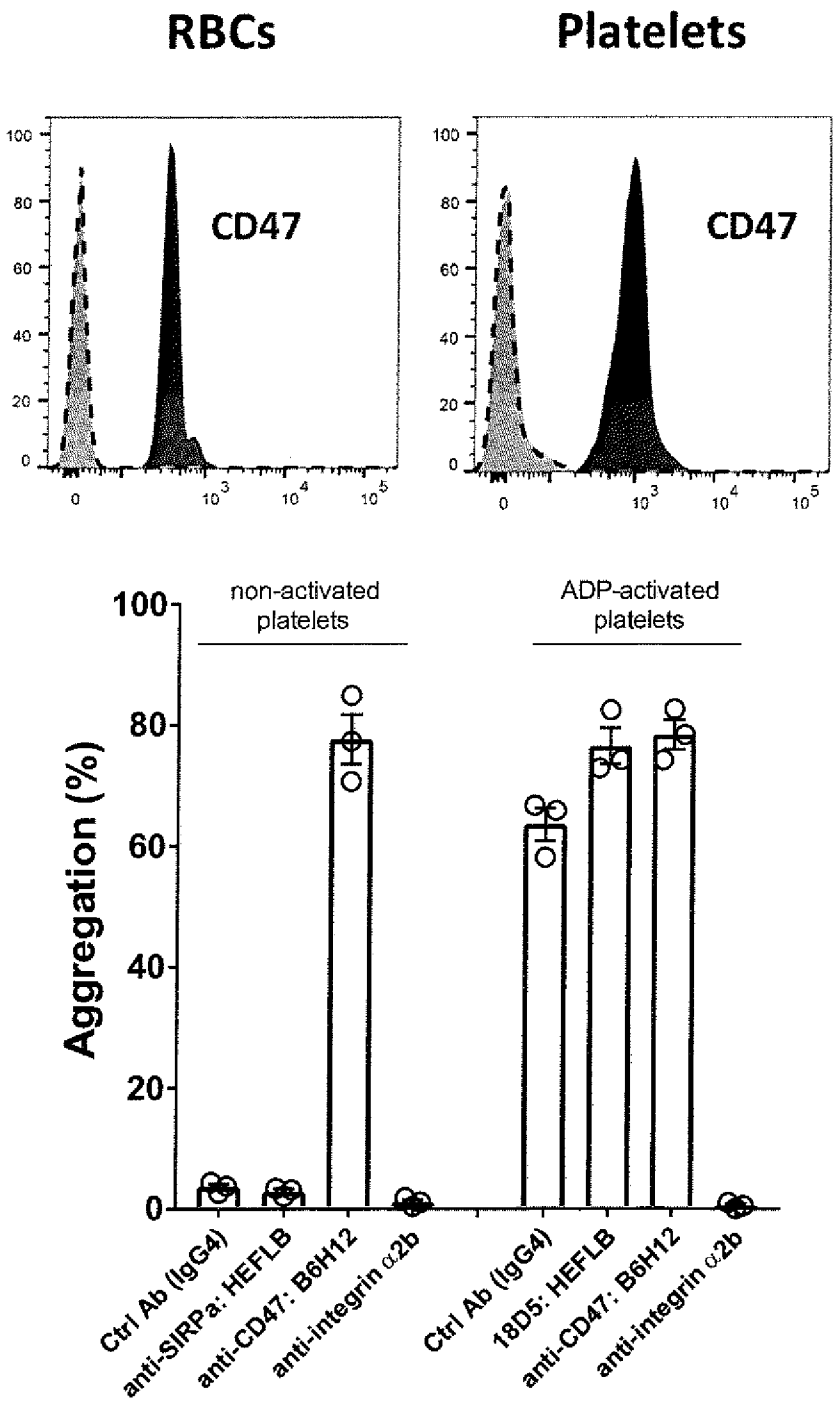

RBCs
Platelets
CD47
CD47
100
80
60
40
20
0
0
10^3
10^4
10^5
non-activated platelets
ADP-activated platelets
Aggregation (%)
Ctrl Ab (IgG4)
anti-SIRPa: HEFLB
anti-CD47: B6H12
anti-integrin α2b
Ctrl Ab (IgG4)
18D5: HEFLB
anti-CD47: B6H12
anti-integrin α2b

FIGURE 20

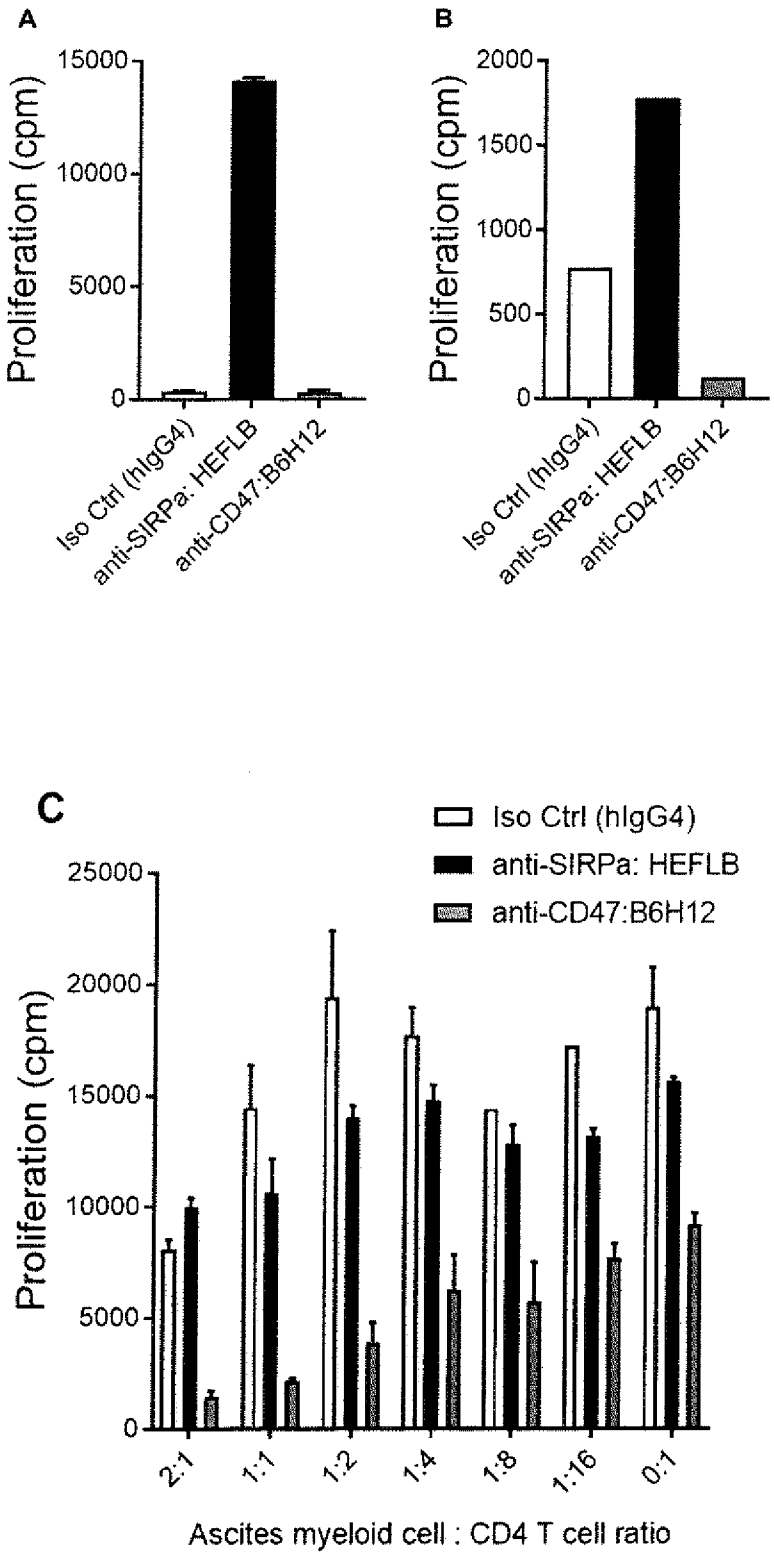

A
Proliferation (cpm)
15000
10000
5000
0
Iso Ctrl (hIgG4)
anti-SIRPa: HEFLB
anti-CD47:B6H12
B
Proliferation (cpm)
2000
1500
1000
500
0
Iso Ctrl (hIgG4)
anti-SIRPa: HEFLB
anti-CD47:B6H12
C
Iso Ctrl (hIgG4)
anti-SIRPa: HEFLB
anti-CD47:B6H12
Proliferation (cpm)
25000
20000
15000
10000
5000
0
2:1
1:1
1:2
1:4
1:8
1:16
0:1
Ascites myeloid cell : CD4 T cell ratio

FIGURE 21

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DOCUMENT MADE AVAILABLE UNDER THE PATENT COOPERATION TREATY (PCT)

International application number:		**PCT/EP2017/059071**
International filing date:		**14 April 2017 (14.04.2017)**
Document type:		**Certified copy of priority document**
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Patentanmeldung Nr.	**Patent application No.**	**Demande de brevet n°**

17305182.2 / EP17305182

The organization code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP17305182.

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le President de l'Office européen des brevets
p.o.



V. Joseph

Anmeldung Nr:
Application no.: 17305182.2
Demande no :

Anmeldetag:
Date of filing: 17.02.17
Date de dépôt :

Anmelder / Applicant(s) / Demandeur(s):

Ose Immunotherapeutics
22 Boulevard Benoni Goullin
44200 Nantes/FR

Bezeichnung der Erfindung / Title of the invention / Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, oder falls die Anmeldung in einer Nicht-Amtssprache des EPA eingereicht wurde, siehe Beschreibung bezüglich ursprünglicher Bezeichnung.
If no title is shown, or if the application has been filed in a non-EPO language, please refer to the description for the original title.
Si aucun titre n'est indiqué, ou si la demande a été déposée dans une langue autre qu'une langue officielle de l'OEB, se référer à la description pour le titre original.)

NEW ANTI-SIRPa ANTIBODIES AND THEIR THERAPEUTIC APPLICATIONS

In Anspruch genommene Priorität(en) / Priority(Priorities) claimed / Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen / State/Date/File no. / Pays/Date/Numéro de dépôt:

Am Anmeldetag benannte Vertragstaaten / Contracting States designated at date of filing / Etats contractants désignés lors du dépôt:

AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

NEW ANTI-SIRPa ANTIBODIES AND THEIR THERAPEUTIC APPLICATIONS

The invention pertains to the field of immunotherapy. The present invention provides new anti-SIRPa antibodies able to specifically decreases the interaction between SIRPa and CD47 without affecting the interaction between SIRPg and CD47.

Signal regulatory protein alpha or SIRPa (also designated as SIRPα, CD172a or SHPS-1), is expressed on monocytes, most subpopulations of tissue macrophages, granulocytes, subsets of dendritic cells in lymphoid tissues, some bone marrow progenitor cells, and to varying levels on neurons, with a notably high expression in synapse-rich areas of the brain, such as the granular layer of the cerebellum and the hippocampus. SIRPa is the prototypic member of the SIRP paired receptor family of closely related SIRP proteins. The gene coding for human SIRPa is a polymorphic gene and several variants were described in human population. The most common protein variants are SIRPa v1 and v2 (accession numbers NP_542970 (P78324) and CAA71403). The polymorphisms in human SIRP lead to changes in surface-exposed amino acids, but this does not affect binding to CD47.

The SIRPa interaction with CD47 is largely described and provides a downregulatory signal that inhibits host cell phagocytosis. CD47 is widely expressed at lower levels by most healthy cells but it is also overexpressed in some cancer cells. Therefore, CD47 functions as a "don't-eat-me" signal. Both CD47 and SIRPa also engage in many other interactions. One of the best characterized physiological functions of the CD47-SIRPa interaction is its role in the homeostasis of hematopoietic cells, in particular red blood cells and platelets. Because CD47 serves as a "don't-eat-me" signal and, as such, is an important determinant of host cell phagocytosis by macrophages, the potential contribution of CD47-SIRPa interaction in cancer cell clearance has been intensely investigated in recent years.

The SIRPa/CD47 pathway is nowadays subject to different pharmaceutical developments to enhance macrophages phagocytosis. In fact, like infected cells, cancer cells carry aberrant cargo such as unfamiliar proteins or normal proteins at abnormal levels, yet these cells frequently subvert innate immune control mechanisms by concurrently over-expressing immunoregulatory molecules. It is becoming clear that one such mechanism involves CD47, a protein of "self" expressed by normal cells. CD47 interacts with SIRPa and leads to the transmission of a "don't eat me" signal to phagocytic macrophages, which then leave target cells unaffected. Over-expression of CD47 by cancer cells renders them resistant to macrophages, even when the cancer cells are coated with therapeutic antibodies, and correlates with poor clinical outcomes in numerous solid and hematological cancers. In experimental models, in particular human tumor-xenograft models in immunodeficient mice, blockade of the CD47/SIRPa pathway was very effective to promote tumor elimination by macrophages and to decrease cancer cell dissemination and metastasis formation. Blockade of the CD47/SIRPa pathway, by enhancing antibody-dependent phagocytosis by macrophages, has been

described to synergize with depleting therapeutic anticancer antibodies such as Trastuzumab (anti-Her2), Cetuximab (anti-EGFR), Rituximab (anti-CD20) and Alemtuzumab (anti-CD52).

However, CD47 also engages with another member of the SIRP family, SIRP-gamma (also designated as SIRPg, SIRPγ, CD172g or SIRP beta 2) that is present at the surface of T cells and not on myeloid cells. It has been shown that the SIRPg-CD47 interaction mediates cell-cell adhesion, enhances superantigen-dependent T-cell-mediated proliferation and co-stimulates T-cell activation (Piccio et al., Blood, 105:6, 2005).

Due to the similarity of sequences between SIRPa and SIRPg, in particular in the region that interacts with CD47, the anti-SIRPa antibodies disclosed in the prior art also bind SIRPg and have undesirable effects in humans such as an inhibition of the proliferation of T-cells and a decrease of the immune response. Such side effects could not be predicted since the tests of the known antibodies were performed in mice models, which do not possess the SIRPg gene, and thus such side effects were absent.

Consequently, there is a need for anti-SIRPa antibodies able to specifically antagonize the SIRPa-CD47 interaction without affecting the SIRPg-CD47 interaction.

Here, the Inventors provide new anti-SIRPa antibodies, in particular humanized antibodies, that antagonize the SIRPa-CD47 interaction but do not specifically bind SIRPg and, thus, do not affect the SIRPg-CD47 interaction. These new antibodies selected on the basis of these characteristics are particularly promising for numerous therapeutic applications, in particular for the treatment of cancer including inflammatory cancers and cancers with infiltrated myeloid cells (in particular with infiltrated MDSCs and/or TAM cells).

SIRPa ANTIBODIES (WITH EPITOPES)

In an aspect, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least one peptide comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

As used herein, the term "antibody" comprises polyclonal antibodies, monoclonal antibodies or recombinant antibodies.

As used herein, a "monoclonal antibody" is intended to refer to a preparation of antibody molecules, antibodies which share a common heavy chain and common light chain amino acid sequence, in contrast with "polyclonal" antibody preparations which contain a mixture of antibodies of different amino acid sequence. Monoclonal antibodies can be generated by several known technologies like phage, bacteria, yeast or ribosomal display, as well as by classical methods exemplified by

hybridoma-derived antibodies. Thus, the term "monoclonal" is used to refer to all antibodies derived from one nucleic acid clone.

The antibodies of the present invention include recombinant antibodies. As used herein, the term "recombinant antibody" refers to antibodies which are produced, expressed, generated or isolated by recombinant means, such as antibodies which are expressed using a recombinant expression vector transfected into a host cell; antibodies isolated from a recombinant combinatorial antibody library; antibodies isolated from an animal (*e.g.* a mouse) which is transgenic due to human immunoglobulin genes; or antibodies which are produced, expressed, generated or isolated in any other way in which particular immunoglobulin gene sequences (such as human immunoglobulin gene sequences) are assembled with other DNA sequences. Recombinant antibodies include, for example, chimeric and humanized antibodies.

As used herein, a "chimeric antibody" refers to an antibody in which the sequence of the variable domain derived from the germline of a mammalian species, such as a mouse, have been grafted onto the sequence of the constant domain derived from the germline of another mammalian species, such as a human.

As used herein, a "humanized antibody" refers to an antibody in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

As used herein, an "antigen-binding fragment of an antibody" means a part of an antibody, *i.e.* a molecule corresponding to a portion of the structure of the antibody of the invention, that exhibits antigen-binding capacity for SIRPa, possibly in its native form; such fragment especially exhibits the same or substantially the same antigen-binding specificity for said antigen compared to the antigen-binding specificity of the corresponding four-chain antibody. Advantageously, the antigen-binding fragments have a similar binding affinity as the corresponding 4-chain antibodies. However, antigen-binding fragment that have a reduced antigen-binding affinity with respect to corresponding 4-chain antibodies are also encompassed within the invention. The antigen-binding capacity can be determined by measuring the affinity between the antibody and the target fragment. These antigen-binding fragments may also be designated as "functional fragments" of antibodies.

Antigen-binding fragments of antibodies are fragments which comprise their hypervariable domains designated CDRs (Complementary Determining Regions) or part(s) thereof encompassing the recognition site for the antigen, *i.e.* the extracellular domain of SIRPa, thereby defining antigen recognition specificity.

Each Light and Heavy chain variable domains (respectively VL and VH) of a four-chain immunoglobulin has three CDRs, designated VL-CDR1 (or LCDR1), VL-CDR2 (or LCDR2), VL-CDR3 (or LCDR3) and VH-CDR1 (or HCDR1), VH-CDR2 (or HCDR2), VH-CDR3 (or HCDR3), respectively.

The skilled person is able to determine the location of the various regions/domains of antibodies by reference to the standard definitions in this respect set forth, including a reference numbering system, a reference to the numbering system of KABAT or by application of the IMGT "collier de perle" algorithm. In this respect, for the definition of the sequences of the invention, it is noted that the delimitation of the regions/domains may vary from one reference system to another. Accordingly, the regions/domains as defined in the present invention encompass sequences showing variations in length or localization of the concerned sequences within the full-length sequence of the variable domains of the antibodies, of approximately +/- 10%.

Based on the structure of four-chain immunoglobulins, antigen-binding fragments can thus be defined by comparison with sequences of antibodies in the available databases and prior art, and especially by comparison of the location of the functional domains in these sequences, noting that the positions of the framework and constant domains are well defined for various classes of antibodies, especially for IgGs, in particular for mammalian IgGs. Such comparison also involves data relating to 3-dimensional structures of antibodies.

For illustration purpose of specific embodiments of the invention, antigen binding fragments of an antibody that contain the variable domains comprising the CDRs of said antibody encompass Fv, dsFv, scFv, Fab, Fab', F(ab')2. Fv fragments consist of the VL and VH domains of an antibody associated together by hydrophobic interactions; in dsFv fragments, the VH:VL heterodimer is stabilised by a disulphide bond; in scFv fragments, the VL and VH domains are connected to one another via a flexible peptide linker thus forming a single-chain protein. Fab fragments are monomeric fragments obtainable by papain digestion of an antibody; they comprise the entire L chain, and a VH-CH1 fragment of the H chain, bound together through a disulfide bond. The F(ab')2 fragment can be produced by pepsin digestion of an antibody below the hinge disulfide; it comprises two Fab' fragments, and additionally a portion of the hinge region of the immunoglobulin molecule. The Fab' fragments are obtainable from F(ab')2 fragments by cutting a disulfide bond in the hinge region. F(ab')2 fragments are divalent, *i.e.* they comprise two antigen binding sites, like the native immunoglobulin molecule; on the other hand, Fv (a VHVL dimmer constituting the variable part of Fab), dsFv, scFv, Fab, and Fab' fragments are monovalent, *i.e.* they comprise a single antigen-binding site. These basic antigen-binding fragments of the invention can be combined together to obtain multivalent antigen-binding fragments, such as diabodies, tribodies or tetrabodies. These multivalent antigen-binding fragments are also part of the present invention.

As used herein, the term "bispecific" antibodies refers to antibodies that recognize two different antigens by virtue of possessing at least one region (*e.g.* derived from a variable region of a first antibody) that is specific for a first antigen, and at least a second region (*e.g.* derived from a variable region of a second antibody) that is specific for a second antigen. A bispecific antibody specifically

binds to two target antigens and is thus one type of multispecific antibody. Multispecific antibodies, which recognize two or more different antigens, can be produced by recombinant DNA methods or include, but are not limited to, antibodies produced chemically by any convenient method. Bispecific antibodies include all antibodies or conjugates of antibodies, or polymeric forms of antibodies which are capable of recognizing two different antigens. Bispecific antibodies include antibodies that have been reduced and reformed so as to retain their bivalent characteristics and to antibodies that have been chemically coupled so that they can have several antigen recognition sites for each antigen such as BiME (Bispecific Macrophage Enhancing antibodies), BiTE (bispecific T cell engager), DART (Dual affinity retargeting); DNL (dock-and-lock), DVD-Ig (dual variable domain immunoglobulins), HAS (human serum albumin), kih (knobs into holes).

Accordingly, bispecific antibodies of the invention are directed against SIRPa and a second antigen.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that is bispecific.

Several researches to develop therapeutic antibodies had led to engineer the Fc regions to optimize antibody properties allowing the generation of molecules that are better suited to the pharmacology activity required of them. The Fc region of an antibody mediates its serum half-life and effector functions, such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP). Several mutations located at the interface between the CH2 and CH3 domains, such as T250Q/M428L and M252Y/S254T/T256E + H433K/N434F, have been shown to increase the binding affinity to FcRn and the half-life of IgG1 *in vivo*. However, there is not always a direct relationship between increased FcRn binding and improved half-life. One approach to improve the efficacy of a therapeutic antibody is to increase its serum persistence, thereby allowing higher circulating levels, less frequent administration and reduced doses. Engineering Fc regions may be desired to either reduce or increase the effector function of the antibody. For antibodies that target cell-surface molecules, especially those on immune cells, abrogating effector functions is required. Conversely, for antibodies intended for oncology use, increasing effector functions may improve the therapeutic activity. The four human IgG isotypes bind the activating Fcγ receptors (FcγRI, FcγRIIa, FcγRIIIa), the inhibitory FcγRIIb receptor, and the first component of complement (C1q) with different affinities, yielding very different effector functions. Binding of IgG to the FcγRs or C1q depends on residues located in the hinge region and the CH2 domain. Two regions of the CH2 domain are critical for FcγRs and C1q binding, and have unique sequences in IgG2 and IgG4.

As used herein, a "modified antibody" corresponds to a molecule comprising an antibody or an antigen-binding fragment thereof, wherein said monoclonal antibody or functional fragment thereof is associated with a functionally different molecule. A modified antibody of the invention may be

either a fusion chimeric protein or a conjugate resulting from any suitable form of attachment including covalent attachment, grafting, chemical bonding with a chemical or biological group or with a molecule, such as a PEG polymer or another protective group or molecule suitable for protection against proteases cleavage *in vivo*, for improvement of stability and/or half-life of the antibody or functional fragment. With similar techniques, especially by chemical coupling or grafting, a modified antibody can be prepared with a biologically active molecule, said active molecule being for example chosen among toxins, in particular Pseudomonas exotoxin A, the A-chain of plant toxin ricin or saporin toxin, especially a therapeutic active ingredient, a vector (including especially a protein vector) suitable for targeting the antibody or functional fragment to specific cells or tissues of the human body, or it may be associated with a label or with a linker, especially when fragments of the antibody are used. PEGylation of the antibody or functional fragments thereof is a particular interesting embodiment as it improves the delivery conditions of the active substance to the host, especially for a therapeutic application. PEGylation can be site specific to prevent interference with the recognition sites of the antibodies or functional fragments, and can be performed with high molecular weight PEG. PEGylation can be achieved through free cysteine residues present in the sequence of the antibody or functional fragment or through added free Cysteine residues in the amino sequence of the antibody or functional fragment.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that is modified.

The macromolecules of the invention comprise antibodies and fragments thereof but also comprise artificial proteins with the capacity to bind antigens mimicking that of antibodies, also termed herein antigen-binding antibody mimetic. Such proteins comprise affitins and anticalins. Affitins are artificial proteins with the ability to selectively bind antigens. They are structurally derived from the DNA binding protein Sac7d, found in *Sulfolobus acidocaldarius*, a microorganism belonging to the archaeal domain. By randomizing the amino acids on the binding surface of Sac7d, *e.g.* by generating variants corresponding to random substitutions of 11 residues of the binding interface of Sac7d, an affitin library may be generated and subjecting the resulting protein library to rounds of ribosome display, the affinity can be directed towards various targets, such as peptides, proteins, viruses and bacteria. Affitins are antibody mimetics and are being developed as tools in biotechnology. They have also been used as specific inhibitors for various enzymes (Krehenbrink *et al.*, J. mol. Biol., 383:5, 2008). The skilled person may readily develop anticalins with the required binding properties using methods know in the art, in particular as disclosed in patent application WO2008068637 and the above-cited publication, in particular the generation of phage display and/or ribosome display libraries and their screening using an antigen as dislosed herein. Anticalins are artificial proteins that are able to bind to antigens, either to proteins or to small molecules. They are antibody mimetic derived from human

lipocalins which are a family of naturally binding proteins. Anticalins are about eight times smaller with a size of about 180 amino acids and a mass of about 20 kDa (Skerra, Febs J., 275:11, 2008). Anticalin phage display libraries have been generated which allow for the screening and selection, in particular of anticalins with specific binding properties. The skilled person may readily develop affitins with the required binding properties using methods know in the art, in particular as disclosed in EP patent EP1270725 B1 , US patent US8536307 B2, (Schlehuber and Skerra, Biophys. Chem., 96:2-3, 2002) and the above-cited publication, in particular the generation of phage display and/or ribosome display libraries and their screening using an antigen as dislosed herein. Anticalins and affitins may both be produced in a number of expression system comprising bacterial expressin systems. Thus, the invention provides affitins, anticalins and other similar antibody mimetics with the features of the antibodies described herein, in particular with regard to the binding to SIRPa, the inhibition of the interaction between SIRPa and CD47, the non-binding to SIRPg, the non binding to T cells, the non inhibition of the proliferation of T cells, the non inhibition of the interaction between SIRPg and CD47 all of which are contemplated as macromolecules of the invention.

All the embodiments disclosed herein for antibodies or fragments thereof are transposed *mutatis mutandis* to the macromolecules of the invention, in particular to antigen-binding antibody mimetics.

As used herein, the term "epitope" means the part of an antigen to which the antibody binds. The epitopes of protein antigens can be divided into two categories, conformational epitope and linear epitope. A conformational epitope corresponds to discontinuous sections of the antigen's amino acid sequence. A linear epitope corresponds to a continuous sequence of amino acids from the antigen.

In the invention, the peptides that are present within SIRPa and that are bound by the anti-SIRPa antibodies are constitutive of the epitope specifically recognized by these antibodies.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least two, three, four or five peptides comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to the peptide comprising or consisting of amino acid sequence SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E) within SIRPa and to at least one peptide comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to the peptides comprising or consisting of amino acid sequence SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least one peptide comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 7 (GRELIYNQKEGH), SEQ ID NO: 8 (KFRKGSPDDVE), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to the peptides of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 7 (GRELIYNQKEGH), SEQ ID NO: 8 (KFRKGSPDDVE), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least one peptide comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 9 (ARELIYNQKEGH), SEQ ID NO: 10 (KFRKGSPDTE), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE).

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to the peptides of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 9 (ARELIYNQKEGH), SEQ ID NO: 10 (KFRKGSPDTE), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least one peptide comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 11 (GRELIYN), DVE, SEQ ID NO: 12 (HTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to the peptides of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 11 (GRELIYN), DVE, SEQ ID NO: 12

(HTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least one peptide comprising or consisting of amino acid sequence selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 13 (ARELIYN), SEQ ID NO: 12 (HTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that specifically binds to the peptides of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 13 (ARELIYN), SEQ ID NO: 12 (HTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE) within SIRPa.

As used herein, the term "SIRPa" refers to a SIRPa protein from a mammal species, preferably a human SIRPa (e.g. accession numbers NP_542970 (P78324) and CAA71403).

As used herein, the term "anti-SIRPa antibody" refers to an antibody which specifically binds to SIRPa, in particular to a human SIRPa.

The specific binding between the antibody or antigen-binding fragment thereof of the invention and the epitope (or the region comprising the epitope) implies that the antibody exhibits appreciable affinity for the epitope (the region comprising the epitope) on a particular protein or antigen (here SIRPa). "Appreciable affinity" includes binding with an affinity of about 10^{-9} M (KD) or stronger. Preferably, binding is considered specific when the binding affinity is between 10^{-9} M and 10^{-12} M, optionally between 10^{-9} M and 10^{-10} M, in particular 10^{-10} M. Whether a binding domain specifically reacts with or binds to a target can be tested readily by, inter alia, comparing the reaction of said binding domain with a target protein or antigen with the reaction of said binding domain with proteins or antigens other than the target protein.

The affinity can be determined by various methods well known from the one skilled in the art. These methods include, but are not limited to, Biacore Analysis, Blitz analysis and Scatchard plot.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that has a KD value inferior to 10^{-9} M, preferably inferior to 10^{-10} M for SIRPa, more preferably inferior to 1.10^{-11} M, particularly by Biacore Analysis.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that decreases the interaction between SIRPa and CD47.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that partially or fully, in particular fully, inhibits the binding of CD47 to SIRPa, in particular of human CD47 to human SIRPa.

Such an antibody of the invention specifically binds SIRPa and antagonizes the interaction between SIRPa and CD47.

In particular, the anti-SIRPa antagonist antibody of the invention is capable of reducing or inhibiting the binding of CD47 to SIRPa by at least 50%, 60%, 70%, preferably 80%, more preferably 90% or most preferably 100%, as compared to a negative control molecule, in a binding assay.

In particular, the anti-SIRPa antagonist antibody of the invention is capable of reducing or inhibiting the binding of CD47 to SIRPa by from 50% to 100%, preferably from 60% to 90%, more preferably from 70% to 80%, as compared to a negative control molecule, in a binding assay.

Methods for determining antibody specificity and affinity by competitive inhibition are known in the art (see, e.g., Harlow *et al.*, Antibodies:A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1998); Colligan *et al.*, Current Protocols in Immunology, Green Publishing Assoc., NY (1992; 1993); Muller, Meth. Enzym., 92:589-601 (1983)) and described in the examples below.

These methods include, but are not limited to, Biacore Analysis, Blitz analysis, flow cytometry and ELISA assay.

In an embodiment, the invention relates to an anti-SIRPa antibody as defined above that has an IC50 lower than 500 ng/ml, in particular lower than 400 ng/ml, 300 ng/ml, more particularly lower than 200 ng/ml, as determined in a competitive SIRPa binding assay between CD47 and the anti-SIRPa antibody by ELISA.

In an embodiment, the invention relates to an anti-SIRPa antibody as defined above that has an IC50 lower than 500 ng/ml, in particular lower than 400 ng/ml, 300 ng/ml, more particularly lower than 200 ng/ml, lower than 150 ng/ml and even more particularly lower than 100 ng/ml as determined by competition cytometry assay on human monocytes between CD47 and the anti-SIRPa antibody.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not specifically bind to SIRPg, preferably to human SIRPg.

Such an antibody of the invention does not affect or does not prevent the interaction between SIRPg and CD47.

As used herein, the term "SIRPg" relates to a signal regulatory protein gamma (also designated SIRP gamma, CD172g or SIRP beta 2), from a mammal species, preferably a human SIRPg.

A reference sequence of the human SIRPg protein, used in the examples of the present application, corresponds to the sequence associated to the Accession number Q9P1W8.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that has a KD value superior to 10^{-9} M, preferably superior to 10^{-8} M, more preferably superior to 10^{-7} M for SIRPg, in particular by Blitz analysis.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not significantly inhibit, antagonize, the binding of CD47 to SIRP-g, that does not significantly compete with the binding of CD47 to SIRPg.

This antagonist effect can be determined using the methods as defined the examples of the present application.

In the invention, it can be considered that an antibody (or antigen-binding fragment thereof or antigen-binding antibody mimetic) does not antagonize the binding of CD47 to SIRPg if said antibody (or antigen-binding fragment thereof or antigen-binding antibody mimetic) induces no increase, or induces an increase inferior to 1 log, of the KD value of CD47 in a SIRPg binding competitive assay by Blitz.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not specifically bind to T-cells, in particular to CD3+ T-cells.

In particular, the anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention does not bind to T-cells from mammal species, in particular to human T-cells.

In particular, it is considered that an anti-SIRPa antibody (or antigen-binding fragment thereof or antigen-binding antibody mimetic) does not specifically bind to human T cells if, in a population of human T cells isolated from PBMC from a healthy donor, less than 10%, preferably less than 5%, more preferably less than 2%, most preferably less than 1% of the population of human T cells are recognized by said anti-SIRPa antibody (or antigen-binding fragment thereof or antigen-binding antibody mimetic).

This effect can be measured by the methods as described in the examples of the present application.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not significantly inhibit the proliferation of T-cells, in particular CD3+ T-cells, preferably from mammal species and more preferably of human T cells.

In particular, it is considered that an anti-SIRPa antibody does not significantly inhibit the proliferation of T-cells if the proliferation of T-cells is reduced by less than 30%, preferably less than

20%, more preferably less than 10%, most preferably less than 5% as compared with a negative control.

The proliferation of T-cells can be determined by various methods. For example, the proliferation of T-cells can be measured by incorporation of H^3-thymidine as described in the examples of the present application.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not significantly inhibit, antagonize, the binding of the surfactant proteins to SIRP-a, that does not significantly compete with the binding of the surfactant proteins to SIRPa.

As used herein, the "surfactant proteins" are collagen-containing C-type (calcium dependent) lectins, which contribute significantly to surfactant homeostasis and pulmonary immunity (for review, see Kishore *et al.*, Surfactant proteins SP-A and SP-D: structure, function and receptors, Mol Immunol, 43(9), 1293-315, 2006).

As used herein, the term "surfactant protein" refers to a surfactant protein from a mammal species, preferably a human surfactant protein.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not inhibit the binding of the human surfactant protein D (SP-D) to SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not inhibit the binding of the human surfactant protein A (SP-A) to SIRPa.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that does not antagonize the interaction between the surfactant proteins and SIRPa.

The competition between SIRPa and surfactant proteins can be determined by competitive assay using methods well known from the one skilled in the art. These methods include, but are not limited to, Biacore Analysis, Blitz analysis and ELISA assay.

In the invention, it can be considered that an antibody (or antigen-binding fragment thereof or antigen-binding antibody mimetic) does not antagonize the binding of a surfactant protein to SIRPa if said antibody (or antigen-binding fragment thereof or antigen-binding antibody mimetic) induces no increase, or induces an increase inferior to 1 log, of the KD value of the surfactant protein in a SIRPa binding competitive assay by Blitz.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that weakly binds, or does not specifically bind to SIRPb.

As used herein, the term "SIRPb" refers to a SIRPb protein (also designated as SIRPβ, signal-regulatory protein beta-1, SIRP-beta-1, CD172 antigen-like family member B or CD172b) from a mammal species, preferably a human SIRPb.

A reference sequence of the human SIRPb protein, used in the examples of the present application, corresponds to the sequence associated to the Accession number Q5TFQ8-1.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that has KD value superior to 10^{-9} M, preferably superior to 10^{-8} M for SIRPb, in particular by Blitz analysis.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 15 (NIDPSDSDTHYNQKFKD) or SEQ ID NO: 16 (NIDPSDSDTHYSPSFQG),

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 17 (GGTGTMAWFAY) SEQ ID NO: 18 (GGTGTLAWFAY), SEQ ID NO: 19 (GGTGTMAYFAY) or SEQ ID NO: 20 (GGTGTLAYFAY),

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23 (FQGTHVPYT).

18D5 / Variant A /Variant B

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 15 (NIDPSDSDTHYNQKFKD),

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 17 (GGTGTMAWFAY),

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23 (FQGTHVPYT).

Variant C

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 16 (NIDPSDSDTHYSPSFQG),

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 17 (GGTGTMAWFAY),

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23 (FQGTHVPYT).

Variant E

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 16 (NIDPSDSDTHYSPSFQG),

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 18 (GGTGTLAWFAY),

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23 (FQGTHVPYT).

Variant F

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 16 (NIDPSDSDTHYSPSFQG),

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 19 (GGTGTMAYFAY),

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23 (FQGTHVPYT).

Variant EF

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 16 (NIDPSDSDTHYSPSFQG),

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 20 (GGTGTLAYFAY),

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23 (FQGTHVPYT).

The anti-SIRPa antibodies of the invention may have a heavy chain variable region comprising the amino acid sequence of HCDR1 and/or HCDR2 and/or HCDR3 of the human antibodies as provided herein; and/or a light chain variable region comprising the amino acid sequence of LCDR1 and/or LCDR2 and/or LCDR3 of the human antibodies as provided herein.

In an embodiment, the antibody comprises an amino acid sequence variant of one or more of the CDRs of the provided human antibodies, which variant comprises one or more amino acid insertion(s) within or adjacent to a CDR residue and/or deletion(s) within or adjacent to a CDR residue and/or substitution(s) of CDR residue(s) (with substitution(s) being the preferred type of amino acid alteration for generating such variants).

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

- a heavy chain variable domain comprising or consisting of the amino acid sequence selected from SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, and/or

- a light chain variable domain comprising or consisting of the amino acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32 and SEQ ID NO: 33.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

- a heavy chain variable domain comprising or consisting of the amino acid sequence 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 and SEQ ID NO: 30, and

- a light chain variable domain comprising or consisting of the amino acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32 and SEQ ID NO: 33.

The sequences of the specific variable domains are given in Table 1 below.

Heavy chain variable domain of the wild-type antibody **(18D5)**	QVQLQQPGAELVRPGSSVKLSCKASGYTFTSYWVHWVKQ RPIQGLEWIGNIDPSDSDTHYNQKFKDKASLTVDKSSSTAY MQLSSLTFEDSAVYYCVRGGTGTMAWFAYWGQGTLVTVS A	SEQ ID NO: 24
Heavy chain variable domain of humanized variant **(HA)**	EVQLVQSGAEVKKPGESLRISCKASGYTFTSYWVHWVRQM PGKGLEWIGNIDPSDSDTHYNQKFKDHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLVTVSS	SEQ ID NO: 25
Heavy chain variable domain of humanized variant **(HB)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYNQKFKDHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLVTVSS	SEQ ID NO: 26
Heavy chain variable domain of humanized variant **(HC)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLVTVSS	SEQ ID NO: 27
Heavy chain variable domain of humanized variant **(HE)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTLAWFAYWGQGTLVTVSS	SEQ ID NO: 28
Heavy chain variable domain of humanized variant **(HF)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYW VHWVRQMPGKGLEWMGNIDPSDSDTHYSPSFQGHVTLS VDKSISTAYLQLSSLKASDTAMYYCVRGGTGTMAYFAYWG QGTLVTVSS	SEQ ID NO: 29
Heavy chain variable domain of humanized variant **(HEF)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTLAYFAYWGQGTLVTVSS	SEQ ID NO: 30
Light chain of the wild-type antibody **(18D5)**	DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSYGNTYLYWY LQKPGQSPKLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRVE AEDLGVYFCFQGTHVPYTFGSGTKLEIK	SEQ ID NO: 31
Light chain variable domain of humanized variant A **(LA)**	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSYGNTYLYWY QQRPGQSPRLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYFCFQGTHVPYTFGGGTKVEIK	SEQ ID NO: 32
Light chain variable domain of humanized variant **(LB)**	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSYGNTYLYWF QQRPGQSPRLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYYCFQGTHVPYTFGGGTKVEIK	SEQ ID NO: 33

Table 1. Examples of heavy chain variable domains and light chain variable domains of antibodies according to the invention.

The sequences of the variable domains of the antibodies exemplified in the present invention can be deduced from the combinations of the sequences shown in Table 2.

Antibody	Heavy chain variable domain	Lightchain variable domain
18D5	SEQ ID NO: 24	SEQ ID NO: 31
HALA	SEQ ID NO: 25	SEQ ID NO: 32
HALB	SEQ ID NO: 25	SEQ ID NO: 33
HBLA	SEQ ID NO: 26	SEQ ID NO: 32
HBLB	SEQ ID NO: 26	SEQ ID NO: 33
HCLA	SEQ ID NO: 27	SEQ ID NO: 32
HCLB	SEQ ID NO: 27	SEQ ID NO: 33
HELA	SEQ ID NO: 28	SEQ ID NO: 32
HELB	SEQ ID NO: 28	SEQ ID NO: 33
HFLA	SEQ ID NO: 29	SEQ ID NO: 32
HFLB	SEQ ID NO: 29	SEQ ID NO: 33
HEFLA	SEQ ID NO: 30	SEQ ID NO: 32
HEFLB	SEQ ID NO: 30	SEQ ID NO: 33

Table 2. Heavy chain variable domain and light chain variable domain of specific antibodies according to the invention.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises a light chain variable domain comprising or consisting of the amino acid sequence SEQ ID NO: 33.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

- a light chain variable domain comprising or consisting of the amino acid sequence SEQ ID NO: 33, and

- a heavy chain variable domain comprising or consisting of the amino acid sequence 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 and SEQ ID NO: 30,

preferably

- a heavy chain variable domain comprising or consisting of the amino acid sequence selected from the group consisting of SEQ ID NO: 29 and SEQ ID NO: 30,

more preferably

- a heavy chain variable domain comprising or consisting of the amino acid sequence selected from the group consisting of SEQ ID NO: 30,

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that comprises:

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 24, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 31,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 25, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 25, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 26, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 26, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 27, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 27, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 28, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 28, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 29, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 29, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 30, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 30, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33.

In the invention, the antibodies can be produced with any heavy chain and light chain constant domains.

In one embodiment, the anti-human SIRPa antibody of the invention is a humanized monoclonal antibody, in particular wherein the antibody light chain constant domain is derived from a human kappa light chain constant domain, more particularly wherein the light chain constant domain consists of the sequence of SEQ ID NO: 35, and wherein the antibody heavy chain constant domain is derived from a human IgG1, IgG2, IgG3, or IgG4 heavy chain constant domain, in particular from a human IgG4 heavy chain constant domain, more particularly wherein the antibody heavy chain constant domain consists of the sequence with SEQ ID NO: 34.

Heavy chain constant domain (IgG4m-S228P) SEQ ID NO: 34	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPGK
Light chain constant domain (CLkappa) SEQ ID NO: 35	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Table 3. Examples of a heavy chain constant domain and a light chain constant domain suitable for the antibodies according to the invention.

In an embodiment, the invention relates to an anti-SIRPa antibody as defined above that comprises:

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 36, and

- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 43,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 37, and

- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 44,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 37, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 45,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 38, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 44,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 38, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 45,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 39, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 44,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 39, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 45,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 40, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 44,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 40, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 45,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 41, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 44,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 41, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 45,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 42, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 44,

or

- a heavy chain comprising or consisting of the amino acid sequence SEQ ID NO: 42, and
- a light chain comprising or consisting of the amino acid sequence SEQ ID NO: 45.

Heavy chain of the wild-	QVQLQQPGAELVRPGSSVKLSCKASGYTFTSYWVHWVKQ RPIQGLEWIGNIDPSDSDTHYNQKFKDKASLTVDKSSSTAY	SEQ ID NO: 36

type antibody **(18D5)**	MQLSSLTFEDSAVYYCVRGGTGTMAWFAYWGQGTLVTVS AASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPP CPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGL PSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSPGK	
Heavy chain of humanized variant **(HA)**	EVQLVQSGAEVKKPGESLRISCKASGYTFTSYWVHWVRQM PGKGLEWIGNIDPSDSDTHYNQKFKDHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSPGK	SEQ ID NO: 37
Heavy chain of humanized variant **(HB)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYNQKFKDHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSPGK	SEQ ID NO: 38
Heavy chain of humanized variant **(HC)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD	SEQ ID NO: 39

	GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSPGK	
Heavy chain of humanized variant **(HE)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTLAWFAYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSPGK	SEQ ID NO: 40
Heavy chain of humanized variant **(HF)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTMAYFAYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSPGK	SEQ ID NO: 41
Heavy chain of humanized variant **(HEF)**	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQM PGKGLEWMGNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYL QLSSLKASDTAMYYCVRGGTGTLAYFAYWGQGTLVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCP PCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY TQKSLSLSPGK	SEQ ID NO: 42
Light chain of the wild-type antibody **(18D5)**	DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSYGNTYLYWY LQKPGQSPKLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRVE AEDLGVYFCFQGTHVPYTFGSGTKLEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 43
Light chain variable	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSYGNTYLYWY	SEQ ID NO: 44

domain of humanized variant A **(LA)**	QQRPGQSPRLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYFCFQGTHVPYTFGGGTKVEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC	
Light chain variable domain of humanized variant **(LB)**	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSYGNTYLYWF QQRPGQSPRLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYYCFQGTHVPYTFGGGTKVEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 45

Table 4. Heavy chain and light chain sequences of specific antibodies according to the invention.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above that is able to induce the differentiation of monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, wherein said non suppressive cells secrete pro-inflammatory cytokines such as IL6, IL12 and TNF, and no or low level of anti-inflammatory cytokines such as IL10 and TGFβ.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, wherein said non suppressive cells express iNOS.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, wherein said non suppressive cells do not express the MHC Class II markers and express the markers CD80-CD86.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, wherein said non suppressive cells express at least one marker of the natural killer (NK) cells.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, wherein said antibody or antigen-binding fragment thereof is able to inhibit M2 polarization of macrophages and/or favors pro-inflammatory M1-type macrophages.

The antibodies of the invention can modify the macrophage polarization in order to induce a pro-inflammatory environment, *i.e.* they can inhibit the anti-inflammatory signal provided by M2-type macrophages and/or favor the pro-inflammatory signal provided by M1-type macrophages This

approach allows to reestablish an inflammatory environment favorable to the action of the T effector cells, in particular in eliminating the cancer cells.

SIRPa ANTIBODIES

In another aspect, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that comprises:

a) a heavy chain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain comprising LCDR1, LCDR2 and LCDR3,

wherein said CDRs are defined as follows:

- HCDR1 comprising or consisting of a peptide of the amino acid sequence SEQ ID NO: 14 (SYWVH),

- HCDR2 comprising or consisting of a peptide of the amino acid sequence SEQ ID NO: 15 (NIDPSDSDTHYNQKFKD) or SEQ ID NO: 16 (NIDPSDSDTHYSPSFQG),

- HCDR3 comprising or consisting of a peptide of the amino acid sequence SEQ ID NO: 17 (GGTGTMAWFAY), SEQ ID NO: 18 (GGTGTLAWFAY), SEQ ID NO: 19 (GGTGTMAYFAY) or SEQ ID NO: 20 (GGTGTLAYFAY),

- LCDR1 comprising or consisting of a peptide of the amino acid sequence SEQ ID NO: 21 (RSSQSLVHSYGNTYLY),

- LCDR2 comprising or consisting of a peptide of the amino acid sequence SEQ ID NO: 22 (RVSNRFS), and

- LCDR3 comprising or consisting of a peptide of the amino acid sequence SEQ ID NO: 23 (FQGTHVPYT).

The embodiments recited for the antibodies recited in the first aspect of the invention are repeated *mutadis mutandis* to the antibodies recited in the second aspect of the invention.

The invention also relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg, in particular which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

APPLICATIONS

In another aspect, the invention relates to:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

for use as a medicament.

The present invention also relates to a method of treatment in a subject in need thereof comprising administering to said subject an effective amount of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

The present invention also relates to the use of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

in the manufacture of a medicament.

In another aspect, the invention relates to:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or

which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

for use in the treatment of any condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

As defined herein, "a condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells" corresponds to a cancer including inflammatory cancers and cancers with infiltrated myeloid cells (in particular with infiltrated MDSCs and/or TAM cells), an infectious disease, a trauma, an auto-immune disease (such as rheumatoid arthritis, type 1 diabetes, lupus, psoriasis), a vaccination, a chronic inflammatory diseases (such as Inflammatory bowel diseases including Crohn disease and Ulcerative colitis), a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis or a transplant dysfunctions.

The present invention also relates to a method of treatment of any condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells in a subject in need thereof comprising administering to said subject an effective amount of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

The present invention also relates to the use of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

in the manufacture of a medicament for the treatment of any condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

In an embodiment, the invention relates to:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

for use in the treatment of any condition susceptible of being improved or prevented by modifying macrophage polarization to pro-inflammatory macrophages.

Indeed, SIRPa acts as a checkpoint inhibitor and participates to macrophage polarization. In particular, blocking SIRPa induces a pro-inflammatory function of macrophages associated to type 1 macrophages (M1 pro-inflammatory = M (IFNg)) and inhibits the suppressive activity of macrophages in the tumor, since the pro-inflammatory profile of macrophages is obtained at the expense of type 2 macrophages (M2 type high phagocytic activity = M (IL4)). Thus, an antagonist of SIRPa is able to inhibit M2 phenotypic polarization of macrophages and/or favors pro-inflammatory M1-type macrophage function and can be used in therapeutic.

As defined herein, "a condition susceptible of being improved or prevented by modifying macrophage polarization to favor pro-inflammatory macrophages" corresponds for example to a solid cancer, a liquid cancer, an infectious disease, a trauma, an auto-immune disease, a vaccination, a brain injury, a nerve injury, a polycythemia, a hemochromatosis or a chronic inflammatory disease.

The present invention also relates to a method of treatment of any condition susceptible of being improved or prevented by modifying macrophage polarization to pro-inflammatory macrophages in a subject in need thereof comprising administering to said subject an effective amount of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

In an embodiment, the invention also relates to the use of :

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

in the manufacture of a medicament for the treatment of any condition susceptible of being improved or prevented by modifying macrophage polarization to pro-inflammatory macrophages.

Modifying the polarization of macrophages to favor pro-inflammatory cells can be useful in a number of pathologies or situations. As described above, this modification is particularly useful in the context of cancers, to restore an anti-tumor activity of macrophages and/or prevent the pro-tumoral activity of M2-type macrophages. Inappropriate immune responses due to an excess of M2-type macrophage polarization also occur in infectious diseases, fibrosis, vaccination, trauma and chronic inflammatory diseases.

Thus, according to a particular embodiment, anti-SIRPa antibody can be used to treat an individual who has a cancer selected from the group consisting of lung cancers, ovary cancers, liver cancers, bladder cancers, brain cancers, breast cancers, colon cancers, thymomas, gliomas, melanomas and hematologic cancers such as lymphomas (Hodgkin's lymphoma and non-Hodgkin's lymphoma), leukemias such as T and B Acute or Chronic Lymphoblastic Leukemia (ALL or CLL) or Acute or Chronic myeloid leukemia (AML or CML) and Myelomas.

In an embodiment, the invention relates to:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

for use in the treatment of a pathology selected from the group consisting of a cancer (in particular inflammatory cancers and cancers with infiltrated myeloid cells particularly with infiltrated MDSCs and/or TAM cells), an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis and a transplant dysfunction.

In an embodiment, the invention relates to a method of treatment of a pathology selected from the group consisting of a cancer (in particular inflammatory cancers and cancers with infiltrated myeloid cells particularly with infiltrated MDSCs and/or TAM cells), an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis and a transplant dysfunction in a subject in need thereof comprising administering to said subject an effective amount of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

In an embodiment, the invention relates to the use of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or

which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

in the manufacture of a medicament for the treatment of a pathology selected from the group consisting of a cancer (in particular inflammatory cancers and cancers with infiltrated myeloid cells particularly with infiltrated MDSCs and/or TAM cells), an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis and a transplant dysfunction.

In an embodiment, the invention relates to an anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, for its uses as defined above, wherein said anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention is administered to a patient presenting a SIRPa-positive tumor.

In an embodiment, the invention relates to:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

for use in vaccination.

In an embodiment, the invention relates to a method of vaccination of a subject comprising administering to said subject an effective amount of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

In an embodiment, the invention relates to the use of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

for the manufacture of a vaccine.

Suppressive myeloid cells limit the effectiveness of vaccination, especially in young children. Thus, an anti-SIRPa /g would limit the benefit provided by an anti-SIRPa on the vaccine response, preventing T lymphocytes from responding to vaccination.

The antibody or antigen-binding fragment thereof of the invention can be administered in a variety of suitable routes, *e.g.*, intravenously (IV), subcutaneously (SC), or, intramuscularly (IM) to the subject.

The antibody or antigen-binding fragment thereof can be administered alone or in combination with another therapeutic agent, *e.g.*, a second human monoclonal antibody or antigen binding fragment thereof. In another example, the antibody is administered together with another agent, for example, an immunosuppressive agent, an erythropoiesis-stimulating agent (ESA), in combination with therapeutic cell compositions, and the like.

In an embodiment, the invention relates to an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic for its use as defined above, wherein the anti-SIRPa antibody or antigen-binding fragment is combined with a second therapeutic agent.

In particular, anti-SIRPa antibodies of the present invention can be combined with some other potential strategies for overcoming tumor immune evasion mechanisms with agents in clinical development or already on the market (see table 1 from Antonia *et al.* Immuno-oncology combinations: a review of clinical experience and future prospects. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. *20*, 6258–6268, 2014):

1- Reversing the inhibition of adaptive immunity (blocking T-cell checkpoint pathways), for example by using an anti-CTLA4, an anti-PD1 or an anti-PD-L1 molecule;

2- Switching on adaptive immunity (promoting T-cell costimulatory receptor signaling using agonist molecules, in particular antibodies), for example by targeting CD137 (4-1BB) using agonist molecules including agonist anti-CD137 (anti-4-1BB) antibodies or CD137 (4-1BB) ligands ;

3- Improving the function of innate immune cells;

4- Activating the immune system (potentiating immune-cell effector function), for example through vaccine-based strategies.

The administration of the second agent can be simultaneous or not with the administration of the anti-SIRPa antibody. Depending on the nature of the second agent, a co-administration can be prepared in the form of a combination drug, also known as a "combo". A combo is a fixed-dose combination that includes two or more active pharmaceutical ingredients combined in a single dosage form, which is manufactured and distributed in fixed doses. But the dose regimen and/or the administration route can also differ.

In a preferred embodiment, this second therapeutic agent is selected from the group consisting of chemotherapeutic agents, radiotherapy agents, surgery agents, immunotherapeutic agents, cell therapy agents (such as CAR-T cells), antibiotics and probiotics.

In particular, immunotherapeutic agents useful in the context of the invention are selected from the group consisting of therapeutic vaccines (DNA, RNA or peptide vaccins), immune checkpoint blockers or activators or immunoconjugates such as antibody-drug conjugates.

As used herein, the term "immunotherapeutic agents" refers to agents that could take cancer vaccines from interesting biological phenomena to effective therapeutic agents including: T-cell growth factors to increase number and repertoire of naive T cells, growth factors to increase the number of dendritic cells (DCs), agonists to activate DCs and other antigen-pre senting cells (APCs), adjuvants to allow and augment cancer vaccines, agonists to activate and stimulate T cells, inhibitors of T-cell checkpoint blockade, T-cell growth factors to increase the growth and survival of immune T cells, agents to inhibit, block, or neutralize cancer cell and immune cell-derived immunosuppressive cytokine.

Numerous immune checkpoint blocker or activator are known in the art. In the context of the invention, examples of immune checkpoint blockers or activators that could be useful are anti-PDL1, anti-PD1, anti-CTLA4, anti-CD137, anti-Her2, anti-EGFR, anti-CD20, anti-CD19, anti-CD52, CD-137, CD2, CD28, CD40, HVEM, BTLA, CD160, TIGIT, TIM-1/3, LAG-3, 2B4 and OX40.

The antibody may be provided at an effective dose from about 1 ng/kg body weight to about 30 mg/kg body weight, or more. In specific embodiments, the dosage may range from 1 µg/kg to about 20 mg/kg, optionally from 10 µg/kg up to 10 mg/kg or from 100 µg/kg up to 5 mg/kg.

The term "effective dose" or "effective dosage" or "effective amount" is defined as an amount sufficient to achieve or at least partially achieve the desired effect. The term "effective dose" is

meant to encompass an amount sufficient to cure or at least partially arrest the disease and its complications in a patient already suffering from the disease. Amounts or doses effective for this use will depend on the condition to be treated, the delivered antibody construct, the therapeutic context and objectives, the severity of the disease, prior therapy, the patient's clinical history and response to the therapeutic agent, the route of administration, the size (body weight, body surface or organ size) and/or condition (the age and general health) of the patient, and the general state of the patient's own immune system. The proper dose can be adjusted such that it can be administered to the patient once or over a series of administrations, and in order to obtain the optimal therapeutic effect.

Dosing for such purposes may be repeated as required, *e.g.* daily, semi-weekly, weekly, semi-monthly, monthly, or as required during relapses.

In an aspect, the invention also relates to:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

for use in a diagnostic test, particularly in personalized medicine, more particularly in a companion diagnostic test.

In an embodiment, the invention relates to a method of diagnostic, particularly in personalized medicine, more particularly in a companion diagnostic test, using:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or
- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg.

In an embodiment, the invention relates to the use of:

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above, or

- an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and/or which does not bind specifically to T-cells and/or which does not inhibit the proliferation of T-cells and/or which does not inhibit the binding of CD47 to SIRPg,

in particular, which inhibits the binding of CD47 to SIRPa and which does not bind specifically to SIRPg and which does not bind specifically to T-cells and which does not inhibit the proliferation of T-cells and which does not inhibit the binding of CD47 to SIRPg,

in the manufacture of a medicament for a diagnostic test, particularly in personalized medicine, more particularly in a companion diagnostic test.

In an aspect, the invention also relates to the use of at least one anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention, as a means for determination of the expression and/or level of expression of SIRPa in a biological sample of an individual.

In an aspect, the invention also relates to an *in vitro* or *ex vivo* method to determine a SIRPa positive cells in a subject from a biological sample of said subject, comprising:

i) determining *in vitro* the expression and/or the level of expression of SIRPa, in a biological sample of said subject using the anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention.

In an aspect, the invention also relates to the use of at least one anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention in a method wherein SIRPa is used as a biomarker that is predictive for the response to a treatment in a subject, in particular in a cancer subject.

In an aspect, the invention also relates to an *in vitro* method of predicting the response of a cancer subject to a treatment, in particular with anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention, comprising:

- determining the expression level of SIRPa in a tumour sample of a subject, in particular with anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic of the invention, and

- comparing the expression level of SIRPa to a value representative of an expression level of SIRPa in a non-responding subject population,

wherein a higher expression level of SIRPa in the tumour sample of the subject is indicative for a subject who will respond to the treatment.

COMPOSITIONS

In another aspect, the invention relates to a pharmaceutical composition comprising an antibody or antigen-binding fragment thereof as defined above and a pharmaceutically acceptable carrier.

As used herein, a "pharmaceutical composition" is meant to encompass a composition suitable for administration to a subject or patient, such as a mammal, especially a human. In general, a "pharmaceutical composition" is sterile and is usually free of contaminants that are capable of eliciting an undesirable response within the subject (*e.g.*, the compound(s) in the pharmaceutical composition is pharmaceutical grade). Pharmaceutical compositions can be designed for administration to subjects or patients in need thereof via a number of different routes of administration including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, intracheal and the like.

As used herein, a "pharmaceutically acceptable carrier" is meant to encompass an excipient, diluent, carrier, and adjuvant that are useful in preparing a pharmaceutical composition that are generally safe, non-toxic and neither biologically nor otherwise undesirable, and include an excipient, diluent, carrier, and adjuvant that are acceptable for veterinary use as well as human pharmaceutical use. "A pharmaceutically acceptable carrier" as used herein includes both one and more than one such excipient, diluent, carrier, and adjuvant.

COMBINATION PRODUCTS

In another aspect, the invention relates to a combination product comprising an anti-SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined above and a second therapeutic agent for simultaneous, separate or sequential use a medicament.

In an embodiment, the invention relates to a combination product as defined above, wherein the second therapeutic agent is selected from the group consisting of chemotherapeutic agents, radiotherapy agents, surgery agents, cell therapy agents, immunotherapeutic agents, antibiotics and probiotics.

In an embodiment, the invention relates to a combination product as defined above, wherein said immunotherapeutic agent is selected from the group consisting of therapeutic vaccines, immune checkpoint blockers or activators or antibody-drug conjugates.

In an embodiment, the invention relates to a combination product as defined above, said immune checkpoint blocker or activator is selected from the group consisting of anti-PDL1, anti-PD1, anti-CTLA4, anti-CD137, anti-Her2, anti-EGFR, anti-CD20, anti-CD19, anti-CD52, CD-137, CD2, CD28, CD40, HVEM, BTLA, CD160, TIGIT, TIM-1/3, LAG-3, 2B4, and OX40.

In an aspect, the invention relates to a combination product as defined above, for simultaneous, separate or sequential use in the treatment of any condition susceptible of being improved or

prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

In an embodiment, the invention relates to a method of treatment of any condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells in a subject in need thereof comprising administering simultaneously, separately or sequentially to said subject an effective amount of a combination product as defined above.

In an embodiment, the invention relates to the use of a combination product as defined above in the manufacture of a medicament for the treatment any condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

In an aspect, the invention relates to a combination product as defined above, for simultaneous, separate or sequential use in the treatment of any condition susceptible of being improved or prevented by modifying macrophage polarization to pro-inflammatory macrophages.

In an embodiment, the invention relates to a method of treatment of any condition susceptible of being improved or prevented by modifying macrophage polarization to pro-inflammatory macrophages in a subject in need thereof comprising administering simultaneously, separately or sequentially to said subject an effective amount of a combination product as defined above.

In an embodiment, the invention relates to the use of a combination product as defined above in the manufacture of a medicament for the treatment any condition susceptible of being improved or prevented by modifying macrophage polarization to pro-inflammatory macrophages.

In an aspect, the invention relates to a combination product as defined above, for simultaneous, separate or sequential use in the treatment of a pathology selected from the group consisting of a cancer, an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis and a transplant dysfunction or for use in vaccination.

In an embodiment, the invention relates to a method of treatment of a pathology selected from the group consisting of a cancer, an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis and a transplant dysfunction of a subject in need thereof comprising administering simultaneously, separately or sequentially to said subject an effective amount of a combination product as defined above.

In an embodiment, the invention relates to the use of a combination product as defined above, in the manufacture of a medicament for the treatment of a pathology selected from the group consisting of a cancer, an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (such as with Pseudomonas or CMV), fibrosis, atherosclerosis and a transplant dysfunction or for use in vaccination.

NUCLEIC ACIDS

In another aspect, the invention relates to an isolated nucleic acid molecule encoding an antibody or antigen-binding fragment thereof as defined above.

As used herein, a nucleic acid molecule can be double stranded and single stranded, linear and circular. It is preferably comprised in a vector which is preferably comprised in a host cell.

In particular, the invention relates to a nucleic acid molecule comprising or consisting of a sequence selected from SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 and SEQ ID NO: 55.

Nucleic acid sequence coding the heavy chain variable domain of the wild-type antibody **(18D5)**	CAGGTGCAGCTGCAGCAGCCAGGAGCTGAGCTGGTGAG GCCTGGCTCCAGCGTGAAGCTGTCCTGCAAGGCTAGCGG CTACACCTTCACAAGCTATTGGGTGCACTGGGTGAAGCA GCGGCCAATCCAGGGCCTGGAGTGGATCGGCAACATCG ACCCCAGCGACTCTGATACCCATTACAATCAGAAGTTTAA GGACAAGGCCTCTCTGACCGTGGATAAGTCTTCCAGCAC AGCTTATATGCAGCTGTCTTCCCTGACATTCGAGGATTCC GCCGTGTACTATTGCGTGAGGGGAGGAACCGGAACAAT GGCTTGGTTTGCTTACTGGGGCCAGGGCACCCTGGTGAC AGTGTCTGCT	SEQ ID NO: 46
Nucleic acid sequence coding the heavy chain variable domain of humanized variant **(HA)**	GAGGTGCAGCTGGTGCAGAGCGGAGCTGAGGTGAAGA AGCCAGGCGAGTCTCTGAGGATCTCCTGCAAGGCTAGCG GCTACACCTTCACATCTTATTGGGTGCACTGGGTGCGGC AGATGCCAGGCAAGGGCCTGGAGTGGATCGGCAACATC GACCCTAGCGACTCTGATACCCACTACAATCAGAAGTTTA AGGACCATGTGACCCTGTCTGTGGATAAGTCCATCAGCA CAGCCTATCTGCAGCTGTCCAGCCTGAAGGCCTCCGATAC AGCTATGTACTATTGCGTGAGGGGAGGAACCGGAACAA TGGCTTGGTTCGCTTACTGGGGCCAGGGCACCCTGGTGA CAGTGTCTTCC	SEQ ID NO: 47
Nucleic acid sequence coding the heavy chain variable domain of humanized variant **(HB)**	GAGGTGCAGCTGGTGCAGTCCGGAGCTGAGGTGAAGAA GCCAGGCGAGTCTCTGAGGATCTCCTGCAAGGCTTCTGG CTACTCCTTCACCAGCTATTGGGTGCACTGGGTGCGGCA GATGCCAGGCAAGGGCCTGGAGTGGATGGGCAACATCG ACCCTAGCGACTCTGATACACACTACAATCAGAAGTTTAA GGACCATGTGACCCTGAGCGTGGATAAGTCCATCAGCAC AGCCTATCTGCAGCTGTCCAGCCTGAAGGCCTCTGATACC GCTATGTACTATTGCGTGAGGGGAGGAACCGGAACAAT GGCTTGGTTCGCTTACTGGGGCCAGGGCACCCTGGTGAC	SEQ ID NO: 48

	AGTGTCTTCC	
Nucleic acid sequence coding the heavy chain variable domain of humanized variant **(HC)**	GAGGTGCAGCTGGTGCAGTCTGGCGCCGAGGTGAAGAA GCCAGGCGAGAGCCTGAGGATCTCTTGCAAGGCTAGCG GCTACTCTTTCACCTCCTATTGGGTGCACTGGGTGCGGCA GATGCCAGGCAAGGGCCTGGAGTGGATGGGCAACATCG ACCCCAGCGACTCTGATACACACTACTCCCCTAGCTTTCA GGGCCATGTGACCCTGTCCGTGGACAAGTCTATCTCCAC AGCCTATCTGCAGCTGTCCAGCCTGAAGGCCAGCGATAC CGCTATGTACTATTGCGTGAGGGGAGGAACCGGAACAAT GGCTTGGTTCGCTTACTGGGGCCAGGGCACCCTGGTGAC AGTGTCTTCC	SEQ ID NO: 49
Nucleic acid sequence coding the heavy chain variable domain of humanized variant **(HE)**	GAGGTGCAGCTGGTGCAGTCTGGCGCCGAGGTGAAGAA GCCAGGCGAGAGCCTGAGGATCTCTTGCAAGGCTAGCG GCTACTCTTTCACCTCCTATTGGGTGCACTGGGTGCGGCA GATGCCAGGCAAGGGCCTGGAGTGGATGGGCAACATCG ACCCCAGCGACTCTGATACACACTACTCCCCTAGCTTTCA GGGCCATGTGACCCTGTCCGTGGACAAGTCTATCTCCAC AGCCTATCTGCAGCTGTCCAGCCTGAAGGCCAGCGATAC CGCTATGTACTATTGCGTGAGGGGAGGAACCGGCACACT GGCTTGGTTCGCTTACTGGGGCCAGGGCACCCTGGTGAC AGTGTCTTCC	SEQ ID NO: 50
Nucleic acid sequence coding the heavy chain variable domain of humanized variant **(HF)**	GAGGTGCAGCTGGTGCAGTCTGGCGCCGAGGTGAAGAA GCCAGGCGAGAGCCTGAGGATCTCTTGCAAGGCTAGCG GCTACTCTTTCACCTCCTATTGGGTGCACTGGGTGCGGCA GATGCCAGGCAAGGGCCTGGAGTGGATGGGCAACATCG ACCCCAGCGACTCTGATACACACTACTCCCCTAGCTTTCA GGGCCATGTGACCCTGTCCGTGGACAAGTCTATCTCCAC AGCCTATCTGCAGCTGTCCAGCCTGAAGGCCAGCGATAC CGCTATGTACTATTGCGTGAGGGGAGGAACCGGAACAAT GGCTTACTTCGCTTATTGGGGCCAGGGCACCCTGGTGAC AGTGTCTTCC	SEQ ID NO: 51
Nucleic acid sequence coding the heavy chain variable domain of humanized variant **(HEF)**	GAGGTGCAGCTGGTGCAGTCTGGCGCCGAGGTGAAGAA GCCAGGCGAGAGCCTGAGGATCTCTTGCAAGGCTAGCG GCTACTCTTTCACCTCCTATTGGGTGCACTGGGTGCGGCA GATGCCAGGCAAGGGCCTGGAGTGGATGGGCAACATCG ACCCCAGCGACTCTGATACACACTACTCCCCTAGCTTTCA GGGCCATGTGACCCTGTCCGTGGACAAGTCTATCTCCAC AGCCTATCTGCAGCTGTCCAGCCTGAAGGCCAGCGATAC CGCTATGTACTATTGCGTGAGGGGAGGAACCGGCACACT GGCTTACTTCGCTTATTGGGGCCAGGGCACCCTGGTGAC AGTGTCTTCC	SEQ ID NO: 52
Nucleic acid sequence coding the light chain of the wild-type antibody **(18D5)**	GACGTGGTCATGACCCAGACACCACTGAGCCTGCCCGTG TCCCTGGGCGATCAGGCCTCTATCTCCTGCAGGTCCAGCC AGTCCCTGGTGCACAGCTATGGCAACACATACCTGTATTG GTACCTGCAGAAGCCAGGCCAGTCCCCCAAGCTGCTGAT CTACAGGGTGTCTAATCGGTTCTCCGGCGTGCCTGACAG GTTCTCCGGCTCTGGCTCCGGCACCGATTTCACACTGAAG ATCAGCAGGGTGGAGGCTGAGGACCTGGGCGTGTATTT CTGTTTTCAGGGCACCCATGTGCCATACACATTTGGCTCT GGCACCAAGCTGGAGATCAAG	SEQ ID NO: 53
Nucleic acid sequence	GACGTGGTCATGACACAGAGCCCACTGTCTCTGCCTGTG	SEQ ID NO: 54

coding the light chain variable domain of humanized variant A **(LA)**	ACCCTGGGACAGCCAGCCTCTATCTCCTGCAGGTCCAGCC AGTCCCTGGTGCACAGCTATGGCAACACATACCTGTATTG GTACCAGCAGAGGCCCGGACAGAGCCCAAGGCTGCTGA TCTACAGGGTGTCTAATCGGTTCTCCGGCGTGCCTGACA GGTTTAGCGGCTCTGGCTCCGGCACCGATTTCACACTGA AGATCTCTAGAGTGGAGGCTGAGGATGTGGGCGTGTAT TTCTGTTTTCAGGGCACCCATGTGCCATACACATTTGGCG GCGGCACCAAGGTGGAGATCAAG	
Nucleic acid sequence coding the light chain variable domain of humanized variant **(LB)**	GACGTGGTCATGACACAGAGCCCACTGTCTCTGCCTGTG ACCCTGGGACAGCCAGCCTCTATCTCCTGCAGGTCCAGCC AGTCCCTGGTGCACAGCTACGGCAACACATACCTGTATT GGTTCCAGCAGAGGCCCGGACAGAGCCCAAGGCTGCTG ATCTATAGGGTGTCTAATCGGTTCTCCGGCGTGCCTGACA GGTTTAGCGGATCTGGATCCGGAACCGACTTCACCCTGA AGATCTCTAGAGTGGAGGCTGAGGATGTGGGCGTGTAC TATTGTTTCCAGGGCACCCATGTGCCATACACATTTGGCG GCGGCACCAAGGTGGAGATCAAG	SEQ ID NO: 55

Table 5. Sequences coding the heavy chain variable domains and the light chain variable domains of antibodies according to the invention.

VECTORS

In another aspect, the invention relates to a vector comprising the nucleic acid molecule as defined above.

As used herein, a "vector" is a nucleic acid molecule used as a vehicle to transfer genetic material into a cell. The term "vector" encompasses plasmids, viruses, cosmids and artificial chromosomes. In general, engineered vectors comprise an origin of replication, a multicloning site and a selectable marker. The vector itself is generally a nucleotide sequence, commonly a DNA sequence, that comprises an insert (transgene) and a larger sequence that serves as the "backbone" of the vector. Modern vectors may encompass additional features besides the transgene insert and a backbone: promoter, genetic marker, antibiotic resistance, reporter gene, targeting sequence, protein purification tag. Vectors called expression vectors (expression constructs) specifically are for the expression of the transgene in the target cell, and generally have control sequences.

HOST CELLS

In another aspect, the invention relates to an isolated host cell comprising a vector as defined above.

As used herein, the term "host cell" is intended to include any individual cell or cell culture that can be or has been recipient of vectors, exogenous nucleic acid molecules, and polynucleotides encoding the antibody construct of the present invention; and/or recipients of the antibody construct itself. The introduction of the respective material into the cell can be carried out by way of transformation, transfection and the like. The term "host cell" is also intended to include progeny or potential

progeny of a single cell. Suitable host cells include prokaryotic or eukaryotic cells, and also include but are not limited to bacteria, yeast cells, fungi cells, plant cells, and animal cells such as insect cells and mammalian cells, *e.g.*, murine, rat, rabbit, macaque or human.

KITS

In another aspect, the invention relates to a kit comprising:

- an antibody or antigen-binding fragment thereof as defined above,
- a nucleic acid molecule coding said antibody or antigen-binding,
- a vector comprising said nucleic acid molecule, and/or
- a cell comprising said vector.

As a matter of convenience, the antibody of the present invention can be provided in a kit, i.e., a packaged combination of reagents.

In the context of the present invention, the term "kit" means two or more components (one of which corresponding to the antibody or antigen-binding thereof, the nucleic acid molecule, the vector or the cell of the invention) packaged together in a container, recipient or otherwise. A kit can hence be described as a set of products and/or utensils that are sufficient to achieve a certain goal, which can be marketed as a single unit.

The kit may comprise one or more recipients (such as vials, ampoules, containers, syringes, bottles, bags) of any appropriate shape, size and material containing the antibody construct of the present invention in an appropriate dosage for administration. The kit may additionally contain directions for use (e.g. in the form of a leaflet or instruction manual), means for administering the antibody construct of the present invention such as a syringe, pump, infuser or the like, means for reconstituting the antibody construct of the invention and/or means for diluting the antibody construct of the invention.

In an embodiment, the invention relates to a kit as defined above for a single-dose administration unit. The kit of the invention may also contain a first recipient comprising a dried/lyophilized antibody construct and a second recipient comprising an aqueous formulation. In certain embodiments of this invention, kits containing single-chambered and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes) are provided.

ANTIGENS

In an aspect, the invention relates to an antigen comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E).

In an embodiment, the invention relates to an antigen comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E) and at least one epitope sequence of human SIRPa consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH),

SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) or SEQ ID NO: 6 (YPQRLQLTWLE).

In an aspect, the invention relates to an antigen comprising or consisting of the epitope sequences of human SIRPa consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE).

METHOD OF MANUFACTURING AN ANTIBODY

In an aspect, the invention also relates to a method of manufacturing an antibody comprising immunizing a non-human animal against an antigen as defined above, and in particular collecting the resulting serum from said immunised non-human animal to obtain antibodies directed against said antigen.

In particular, the invention also relates to a method of manufacturing an antibody comprising immunizing a non-human animal against an antigen comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), and in particular collecting the resulting serum from said immunised non-human animal to obtain antibodies directed against said antigen.

In particular, the invention also relates to a method of manufacturing an antibody comprising immunizing a non-human animal against an antigen comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E) and at least one epitope sequence of human SIRPa selected from the group consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) or SEQ ID NO: 6 (YPQRLQLTWLE), and in particular collecting the resulting serum from said immunised non-human animal to obtain antibodies directed against said antigen.

In particular, the invention also relates to a method of manufacturing an antibody comprising immunizing a non-human animal against an antigen comprising or consisting of the epitope sequences of human SIRPa consisting of SEQ ID NO: 1 (SLIPVGP), SEQ ID NO: 2 (G/ARELIYNQKEGH), SEQ ID NO: 3 (KFRKGSPD[DV]/[T]E), SEQ ID NO: 4 (QHTVSFTCESHGFSPRDITLKWF), SEQ ID NO: 5 (ICEVAHVTLQG) and SEQ ID NO: 6 (YPQRLQLTWLE), and in particular collecting the resulting serum from said immunised non-human animal to obtain antibodies directed against said antigen.

METHOD OF SELECTING AN ANTIBODY

In an aspect, the invention relates to a method of selecting an antibody of the invention, an antigen-binding fragment or mimetic of such an antibody, comprising or consisting of at least one of the following steps:

a. testing (e.g. according to a method describing in the Examples 1, 2 and 3) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to bind to SIRPa, in particular to an antigen as defined above,

b. testing (e.g. according to a method describing in the Examples 7 and 8) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to bind to SIRPb,

c. testing (e.g. according to a method describing in the Examples 9 and 10) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to bind to SIRPg,

d. testing (e.g. according to a method describing in the Examples 4 and 5) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to inhibit the binding of human CD47 to human SIRPa;

e. testing (e.g. according to a method describing in the Example 12) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to bind to T cells;

f. testing (e.g. according to a method describing in the Example 13) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to inhibit the T cells proliferation;

g. testing (e.g. according to a method describing in the Example 11) the ability of an antibody, an antigen-binding fragment or mimetic of such an antibody to inhibit the binding of human CD47 to human SIRPg;

and optionally comprising the following step:

- selecting an antibody, an antigen-binding fragment or mimetic of such an antibody which specifically binds to SIRPa, in particular to an antigen as defined above, and which significantly inhibits the binding of CD47 to SIRPa, and which does not bind specifically to human SIRPg, and/or which does not bind specifically to human T-cells, and/or which does not significantly inhibit the proliferation of human T-cells, and/or which does not significantly inhibit the binding of human CD47 to human SIRPg; more particularly which specifically binds to SIRPa, in particular to an antigen as defined above, and which significantly inhibits the binding of CD47 to SIRPa, and which does not bind specifically to human SIRPg, and which does not bind specifically to human T-cells, and which does not significantly inhibit the proliferation of human T-cells, and which does not significantly inhibit the binding of human CD47 to human SIRPg.

The method of selecting an antibody of the invention can advantageously being performed further to the method of manufacturing an antibody according to the invention.

The following Figures and Examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. While the

present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

FIGURE LEGENDS

Figure 1. Binding analyses of anti-SIRPa antibodies by ELISA assay (human SIRPa-His coating and anti-human kappa detection).

Assessment by ELISA on immobilized SIRPa-His of chimeric (♦), HALA (□), HFLA (*), HFLB (+), HEFLA (▲), HEFLB (■), SIRP29 (Δ), Kwar23 (o) on **Figure A**; of HCLA (•), HCLB (x), HELA (◊), HELB (-) on **Figure B**; of HALB (-), HBLA (_), HBLB (▩) on **Figure C**. Revelation was performed with a donkey anti-human antibody and revealed by colorimetry at 450nm using TMB substrate. ED50 is the concentration of the indicated antibody to reach 50% of the signal in this assay.

Figure 2. Affinity analysis by Biacore of anti-SIRP antibodies on human SIRPa recombinant protein.

SIRPa-His recombinant protein was immobilized onto a CM5 chip at 5µg/ml (500RU) and the indicated antibodies were added at different concentration. Values were measured after an association period (ka) of 3min followed by a dissociation period of 10 min (kd) to determine affinity constant (KD).

Figure 3. Binding analyses of anti-SIRPa antibodies on human monocytes (homozygote for SIRPa variant 1 (v1/v1)).

Assessment by cytofluorometry on human monocytes v1/v1 (previously stained with human Fc Receptor Binding Inhibitor antibody) of chimeric (♦), HALA (□), HFLA (*), HFLB (+), HEFLA (▲), HEFLB (■), SIRP29 (Δ), Kwar23 (o). Revelation was performed with a PE labeled mouse anti-human Fc mAb on Cantoll cytometer, values corresponding to percentage of stained monocytes. ED50 is the concentration of the indicated antibody to reach 50% of the signal in this assay. **Figure A** corresponds to the percentage of monocytes v1/v1 stained. **Figure B** corresponds to the mean of fluorescence intensity (MFI) of monocytes v1/v1.

Figure 4. Competition of anti-SIRPa antibodies with CD47 on SIRPa.

Assessment by ELISA on immobilized SIRPa-His of chimeric (♦), HFLA (*), HFLB (+), HEFLA (▲), HEFLB (■), SIRP29 (Δ), Kwar23 (o) at different concentrations incubated with constant concentration of biotinylated CD47-Fc (6µg/ml). Revelation was performed with streptavidin peroxidase to detect CD47 molecule and revealed by colorimetry at 450nm using TMB substrate. The results of a second experiment are given with the IC50 values. IC50 is the concentration of the indicated antibody to inhibit 50% of the signal in this assay.

Figure 5. Competition of anti-SIRPa antibodies with CD47 on human monocytes.
Assessment by cytometry on human monocytes (v1/v1) of chimeric (♦), HFLA (*), HFLB (+), HEFLA (▲), HEFLB (■) at different concentrations incubated with constant concentration of biotinylated CD47-Fc (10 µg/ml). Revelation was performed with PhycoErythrin-streptavidin to detect CD47 molecule and revealed by Cantoll cytometer. IC50 is the concentration of the indicated antibody to inhibit 50% of the signal in this assay. **Figure A** corresponds to the percentage of positive cells. **Figure B** corresponds to the mean of fluorescence intensity.

Figure 6. (A) Affinity analysis by Blitz of anti-SIRP antibody on human SIRPa recombinant protein pre-incubated or not with SP-D ligand. SIRPa-His recombinant protein was immobilized onto a NINTA biosensor at 10 µg/ml and the SP-D ligand was added at 100 µg/ml (saturating concentration). Then anti-SIRPa antibody was added at 20µg/ml and affinity values were deduced after an association period (ka) of 120sec followed by a dissociation period of 120sec (kd) to determine affinity constant (KD). **(B)** Affinity analysis by Blitz of anti-SIRP antibody on human SIRPa recombinant protein pre-incubated with mouse 18D5 antibody. SIRPa-His recombinant protein was immobilized onto a NINTA biosensor at 10 µg/ml and the anti-SIRPa antibody was added at 20µg/ml (saturating concentration). Then SP-D ligand was added at 100 µg/ml and affinity values were deduced after an association period (ka) of 120sec followed by a dissociation period of 120sec (kd) to determine affinity constant (KD).

Figure 7. (A) Affinity analysis by Blitz of anti-SIRP antibodies on human SIRPb recombinant protein. SIRPb-His recombinant protein was immobilized onto a NINTA biosensor and the indicated antibodies were added at 20µg/ml. Values were deduced after an association period (ka) of 120sec followed by a dissociation period of 120sec (kd) to determine affinity constant (KD). **(B)** Binding analysis of anti-SIRP antibodies (human SIRPb-His coating and anti-human kappa detection). Assessment by ELISA on immobilized SIRPb-His of HEFLB (■), SIRP29 (Δ), Kwar23 (o), B4B6 (♦) and IgG4 Ab control (■). Revelation was performed with a donkey anti-human antibody with the exception of B4B6 revealed with a mouse antibody and revealed by colorimetry at 450nm using TMB substrate.

Figure 8. (A) Affinity analysis by Blitz of anti-SIRP antibodies on human SIRPg recombinant protein. SIRPg-His recombinant protein was immobilized onto a NINTA biosensor and the indicated antibodies were added at 10 µg/ml. Values were deduced after an association period (ka) of 120sec followed by a dissociation period of 120sec (kd) to determine affinity constant (KD). **(B)** Binding analysis by ELISA assay of anti-SIRP antibodies on SIRPg (human SIRPg-His coating and anti-human kappa detection). Assessment by ELISA on immobilized SIRPg-His of HEFLB (■), SIRP29 (Δ), Kwar23 (o), LSB2-20 (●) and

IgG4 Ab control (■). Revelation was performed with a donkey anti-human antibody and revealed by colorimetry at 450nm using TMB substrate.

Figure 9. Affinity analysis by Blitz of CD47 on human SIRPg recombinant protein pre-incubated with anti-SIRP antibodies. SIRPg-His recombinant protein was immobilized onto a NINTA biosensor at 10 µg/ml and the indicated antibodies were added at 20µg/ml (saturating concentration). Then, CD47Fc was added at 100 µg/ml and affinity values were deduced after an association period (ka) of 120sec followed by a dissociation period of 120sec (kd) to determine affinity constant (KD).

Figure 10. Geometric mean fluorescence intensity measured by flow cytometry on **(A)** peripheral human CD3+ T cells, **(B)** red blood cells or **(C)** platelets after staining with different monoclonal antibodies and revealing with secondary anti-IgG fluorescent antibody.

Figure 11. Percentage of positive peripheral human CD3+ T cells after staining with different monoclonal antibodies. The table indicates the value of % of positive cells in duplicate experiments.

Figure 12. Human T cells isolated from peripheral blood mononuclear cells from healthy volunteers were stimulated with **(A)** anti-CD3+ anti-CD28 beads at a 1:1 ratio for 3 days or **(B)** with allogeneic dendritic cells (DC) at a 5 T cell : 1 DC ratio for 5 days. Antibodies were added at day 0 of the culture. Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Figure 13. Mouse CD8+ T cells were isolated from splenocytes of naive mice. CD8 T cells were stimulated with anti-CD3+ anti-CD28 beads at a 1:1 ratio for 3 days. Antibodies were added at day 0 of the culture. Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Figure 14. Human T cells isolated from peripheral blood mononuclear cells from healthy volunteers were stimulated with allogeneic dendritic cells (DC) at a 5 T cell : 1 DC ratio for 5 days. Antibodies were added at day 0 of the culture. Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Figure 15. (A) Anti-tumor effect of anti-SIRPa (P84 clone) i.p. administration three times a week for 4 weeks (300µg/injection) in combination or not with two injection (day 4 & 8) of anti-41-BB mAb (3H3 clone, 100 µg/injection) in an orthotopic model of murine hepatoma (2.5.10^6 of Hepa 1.6 cells

injected through the portal vein on day 0). Mice were considered cured when they survived three times longer than the time necessary to all control mice died. **(B)** Mice previously cured in the hepatoma model by anti-SIRPa + anti-4-1BB injection or SIRPa mutant mice treated with anti-4-1BB were rechallenged by Hepa 1.6 cells injection in the spleen (2.5.10^6 cells/mouse). Naive mice were injected in parallel in the same route in order to compare tumor development with rechallenged mice. Mice were then left untreated. **(C)** Mice previously cured in the hepatoma model by anti-SIRPa + anti-4-1BB were rechallenged by Hepa 1.6 cells injection in the spleen (2.5.10^6 cells/mouse) and their spleen was harvested 30 days after rechallenge. Splenocytes and T-cell splenocytes were isolated. Naive mice were injected intravenously with vehicle, whole splenocytes (10.10^6/mouse) or T-cell purified from splenocytes (2.5.10^6/mouse) and all reveived Hepa1.6 cell injection in the spleen (2.5.10^6 cells/mouse). Mice were then left untreated and considered cured when they survived three times longer than the time necessary to all control mice died.

Figure 16. (A) Anti-tumor effect of anti-SIRPa (P84 clone) i.p. administration three times a week for 4 weeks (300µg/injection) in combination or not with twice a week i.p. injection for 4 weeks of anti-PDL1 mAb (10F-9G2 clone, 200µg/injection) in an orthotopic model of murine hepatoma (2.5.10^6 of Hepa 1.6 cells injected through the portal vein on day 0). Mice were considered cured when they survived three times longer than the time necessary to all control mice died. **(B)** Mice previously cured in the hepatoma model by anti-SIRPa + anti-PDL1 injection were rechallenged by Hepa 1.6 cells injection in the spleen (2.5.10^6 cells/mouse). Naive mice were injected in parallel in the same route in order to compare tumor development with rechallenged mice. Mice were then left untreated.

Figure 17. Anti-tumor effect of anti-SIRPa (P84 clone) i.p. administration three times a week for 4 weeks (200µg/injection) in an orthotopic model of murine mammary carcinoma (0.25.10^6 of 4T1 cells injected in the mammary gland). The tumor development was evaluated by measuring the diameter of the tumor and calculated according to the formula : $=(0,52*(d^2))^{1,5}$. Mice were euthanized when tumor development was nearly 1000 mm^3 according to the ethical guidelines.

Figure 18. Immune cells phenotype analysis in spleen, tumor and lymph nodes of mice treated with anti-SIRPa (P84 clone) or ctrl mAb i.p. three times a week for two weeks (200µg/injection) in an orthotropic model of murine mammary carcinoma (0.25.10^6 of 4T1 cells injected in the mammary gland). Immune cell analysis were performed two weeks after tumor inoculation.

Figure 19. Anti-SIRPa (P84 clone), anti-CD47 (MIAP410 clone) and irrelevant isotype control were administered intraperitoneally at day 0 and day 2 at 12mg/kg in C57Bl/6 mice. Blood samples were

collected at day 0 and day 3 in EDTA containing tubes and blood count was performed with a XS-800i hematology analyzer (Sysmex). The level of hemoglobin (left) and the percentage of hematocrit (right) were evaluated at day 3. The dotted-lines represent normal range values in the C57Bl/6 mice for each parameter.

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EXAMPLES

In the following Examples, the antibody "18D5" corresponds to the mouse antibody 18D5, the "chimeric" antibody corresponds to the chimeric mouse/human 18D5 antibody, and the antibodies "HALA, HALB, HBLA, HBLB, HCLA, HCLB, HELA, HELB, HFLA, HFLB, HEFLA and HEFLB" correspond to the humanized 18D5 variants.

Example 1. Binding analyses of the anti-SIRPa antibodies on SIRPa by ELISA

Method: The binding activity of the anti-SIRPa antibodies was assessed by ELISA. For the ELISA assay, a recombinant hSIRPa (Sino Biologicals, Beijing, China; reference 11612-H08H) was immobilized on plastic at 0.5µg/ml in carbonate buffer (pH9.2) and the purified antibody was added to measure binding. After incubation and washing, peroxidase-labeled donkey anti-human IgG (Jackson Immunoresearch; USA; reference 709-035-149) was added and revealed by conventional methods.

Results: As shown in **Figure 1**, the binding activity of the different anti-SIRPa antibodies on SIRPa as measured by ELISA showed effective concentrations (EC50) of 2.9 ng/ml for the chimeric antibody, 3.9 ng/ml for HALA, 5.1 ng/ml for HFLA, 4.0 ng/ml for HFLB, 7.1 ng/ml for HEFLA, 4.4 ng/ml HEFLB in a first experiment, 4.06 ng/ml for the chimeric antibody, 5.60 ng/ml for HCLA, 5.59 ng/ml for HCLB, 4.61 ng/ml for HELA, 4.13 ng/ml for HELB in a second experiment, and 2.74 ng/ml for the chimeric antibody, 2.53 ng/ml for HALB, 2.68 ng/ml for HBLA, 2.95 ng/ml for HBLB in a third experiment. Those results indicate that the antibodies of the invention tested are good SIRPa binders by ELISA as compared to other known anti-SIRPa antibodies SIRP29 (3.7 ng/ml) and Kwar23 (3.3 ng/ml). Those results indicate that the epitope recognized by all the antibodies of the invention is accessible when SIRPa is coated on a plastic well.

Example 2. Biosensor affinity measurement of the anti-SIRPa antibodies for SIRPa

Method: Recombinant hSIRPa (Sino Biologicals, Beijing, China; reference 11612-H08H) was immobilized into a CM5 sensor chip (GeHealthcare; France) at 5µg/ml (500RU) and antibodies were applied at different concentrations with a flow rate of 40µl/min. Analysis was performed with a BIAcore 3000 (Biacore, GeHealthcare). Values were measured after an association period (ka) of 3min followed by a dissociation period of 10 min (kd) to determine affinity constant (KD).

Results: As shown in **Figure 2**, the antibodies of the invention have a strong affinity (KD) for SIRPa (from 1.67e-10 M to 3.67e-10 M), which is equivalent to the affinity of the known anti-SIRPa antibodies SIRP29 and Kwar23 and better than the affinity of the commercial anti-SIRPa antibodies SE7C2 and SE5A5.

Example 3. SIRPa binding assay on human monocytes by cytofluorometry

Method: To measure the binding of the anti-SIRPa antibodies on human monocytes, human Fc Receptor Binding Inhibitor (BD pharmingen; USA; reference 564220) was first added for 30min at room-temperature to block human Fc receptors on human monocytes to reduce background. Then, an antibody was incubated for 30min at 4°C, and washed before stained 30min at 4°C with PE-labelled anti-human IgG Fc (Biolegend; USA; reference 409303). Samples were analyzed on BD LSRII or Canto II cytofluorometer.

Results: As shown in **Figure 3**, the results indicate a strong binding of the anti-SIRPa antibodies of the invention on human monocytes.

Example 4. Competitive analysis between CD47 and the anti-SIRPa antibodies by antagonist ELISA assay

Method: For competitive ELISA assay, recombinant hSIRPa (Sino Biologicals, Beijing, China; reference 11612-H08H) was immobilized on plastic at 0.5µg/ml in carbonate buffer (pH9.2). Purified antibody (at different concentrations) was mixed with 6µg/ml final (fix concentration) of biotinylated Human CD47Fc (AcroBiosystems interchim; France; reference: #CD7-H82F6) to measure competitive binding for 2h at 37°C. After incubation and washing, peroxidase-labeled streptavidin (Vector laboratoring; USA; reference SA-5004) was added to detect Biotin-CD47Fc binding and revealed by conventional methods.

Results: As shown in **Figure 4**, the antibodies of the invention have an antagonist activity on the SIRPa-CD47 interaction. In particular, it is observed that the chimeric antibody, HFLA, HFLB, HEFLA and HEFLB have a better antagonist activity as compared to the antagonist activity of SIRP29.

Example 5. Competitive analysis between CD47 and the humanized anti-SIRPa antibodies on human monocytes by antagonist cytofluorometry assay

Method: To measure the competition between CD47 and the humanized anti-SIRPa on human monocytes, a purified antibody was added on monocytes for 15min at 4°C, then mixed with 5µg/ml final of biotinylated Human CD47Fc (AcroBiosystems interchim; France; reference: #CD7-H82F6) and incubated for 30min at 4°C to measure competitive binding antibody. After incubation and washing, PE-labelled streptavidin (BDBiosciences; USA; reference 554061) was added for 15min at 4°C to detect Biotin-CD47Fc binding and analyzed on BD LSRII or Canto II cytofluorometer.

Results: As shown in **Figure 5**, the antibodies of the invention have an antagonist activity on SIRPa-CD47 interaction on human monocytes.

Example 6. Blitz method competition with SP-D

Method: This method was performed with a Blitz (Forté Bio; USA; reference C22-2 No 61010-1). Condition A: SIRPa + Anti-SIRPa antibody + Surfactant Protein D (SP-D). In a first step, SIRPa (His) recombinant protein (Sino Biologicals, Beijing, China; reference 11612-H08H) was immobilized at 10 µg/ml by histidine tail into a Ni-NTA biosensor (Forté Bio; USA; reference 18-0029) for 30 seconds. In a second step, anti-SIRPa antibodies were added at 20µg/mL (saturating concentration) for 120 seconds. Then, human SP-D (R et D Systems; USA; reference 1920-SP-050) was associated at 100 µg/mL, in competition with anti-SIRPa antibodies, for 120 seconds. The dissociation of SP-D was made in kinetics buffer for 120 seconds. Analysis of data was made with the Blitz pro 1.2 software, which calculated association constant (ka) and dissociation constant (kd) and determined the affinity constant KD (ka/kd).

Condition B: SIRPa + Surfactant Protein D (SP-D) + Anti-SIRPa antibody. In a first step, Sirp-a (His) recombinant protein (Sino Biologicals, Beijing, China; reference 11612-H08H) was immobilized at 10 µg/ml by histidine tail into a Ni-NTA biosensor (Forté Bio; USA; reference 18-0029) for 30 seconds. In a second step, human SP-D (R et D Systems; USA; reference 1920-SP-050) was added at 100 µg/mL for 120 seconds. Then, anti-SIRPa antibodies were associated at 20µg/mL (saturating concentration) for 120 seconds. The dissociation of anti-SIRPa antibody was made in kinetics buffer for 120 seconds. Analysis data was made with the Blitz pro 1.2 software, which calculated association constant (ka) and dissociation constant (kd) and determined the affinity constant KD (ka/kd).

Results: As shown in **Figure 6**, the binding of the anti-SIRPa antibody 18D5 does not block the binding of SP-D to SIRPa. Thus, the antibody of the invention does not inhibit the interaction between SIRPa and SP-D.

Example 7. Affinity of the anti-SIRPa antibodies for SIRPb by Blitz method

Method: This method was performed with a Blitz (Forté Bio; USA; reference C22-2 No 61010-1). Recombinant hSIRPb-His (Antibodies-online; USA; reference ABIN3077231) was immobilized at 10 µg/ml by histidine tail into a Ni-NTA biosensor (Forté Bio; USA; reference 18-0029) for 30 seconds. Then, an anti-SIRPa antibody was associated at 20µg/mL for 120 seconds. The dissociation of anti-SIRPa antibody was made in kinetics buffer for 120 seconds. Analysis of data was made with the Blitz pro 1.2 software, which calculated association constant (ka) and dissociation constant (kd) and determined the affinity constant KD (ka/kd).

Results: As shown in **Figure 7A**, the antibodies of the invention have a lower affinity for SIRPb as compared to SIRPa. In particular, it is noted that the chimeric antibody, HFLA, HFLB, HEFLA, HEFLB have a reduced affinity for SIRPb as compared to SIRP29 and Kwar23.

Example 8. ELISA binding of anti-SIRP antibodies on SIRPb

Method: For activity ELISA assay, recombinant hSIRPb-His (Antibodies-online; USA; reference ABIN1466557) was immobilized on plastic at 1µg/ml in carbonate buffer (pH9.2) and a purified antibody was added to measure binding. After incubation and washing, peroxidase-labeled donkey anti-human IgG (Jackson Immunoresearch; USA; reference 709-035-149) was added and revealed by conventional methods.

Results: As shown in **Figure 7B**, the anti-SIRPa antibodies have a low affinity for SIRPb. It must be indicated that the revelation was performed with a donkey anti-human antibody for all antibodies except for B4B6 (revealed with a mouse antibody), which may explain that the signal obtained for the anti-SIRPb antibody B4B6 is lower than the signal obtained for the anti-SIRPa antibodies.

Example 9. Affinity analysis of the anti-SIRPa antibodies for SIRPg by Blitz method

Method: This method was performed with a Blitz (Forté Bio; USA; reference C22-2 No 61010-1). Recombinant hSIRPg-His (Sino Biologicals, Beijing, China; reference 11828-H08H) was immobilized at 10 µg/ml by histidine tail into a Ni-NTA biosensor (Forté Bio; USA; reference 18-0029) for 30 seconds. Then, an anti-SIRPa antibody was associated at 20µg/mL for 120 seconds. The dissociation of anti-SIRPa antibody was made in kinetics buffer for 120 seconds. Analysis of data was made with the Blitz pro 1.2 software, which calculated association constant (ka) and dissociation constant (kd) and determined the affinity constant KD (ka/kd).

Results: As shown in **Figure 8A**, the anti-SIRPa antibodies of the invention have a low affinity for SIRPg. This affinity is slightly weaker than the affinity of the known anti-SIRPa antibodies SIRP29 and Kwar23. In particular, HFLB has the lowest affinity for SIRPg with a KD value of 1.036e-7 M that equals to a 2-log difference as compared to the KD values of SIRP29 and Kwar23.

Example 10. ELISA binding of the anti-SIRP antibodies on SIRPg

Method: For activity ELISA assay, hSIRPg-His (Sino Biologicals, Beijing, China; reference 11828-H08H) was immobilized on plastic at 1µg/ml in carbonate buffer (pH9.2) and purified antibody were added to measure binding. After incubation and washing, peroxidase-labeled donkey anti-human IgG (Jackson Immunoresearch; USA; reference 709-035-149) was added and revealed by conventional methods.

Results: As shown in **Figure 8B**, the anti-SIRPa antibody HEFLB does not bind SIRPg while the known anti-SIRPa antibodies SIRP29 and Kwar23 show a significant binding to SIRPg.

Example 11. Blitz method competition with CD47 for SIRPg: SIRPg + anti-SIRPa antibody + CD47

Method: This method was performed with a Blitz (Forté Bio; USA; reference C22-2 No 61010-1). In a first step, hSIRPg-His (Sino Biologicals, Beijing, China; reference 11828-H08H) was immobilized at 10

µg/ml by histidine tail into a Ni-NTA biosensor (Forté Bio; USA; reference 18-0029) for 30 seconds. In a second step, an anti-SIRPa antibody was added at 20µg/mL (saturating concentration) for 120 seconds. Then, human CD47Fc ((Sino Biologicals, Beijing, China; reference 12283-H02H) was associated at 100 µg/mL, in competition with anti-SIRPa antibodies, for 120 seconds. The dissociation of CD47Fc was made in kinetics buffer for 120 seconds. Analysis data was made with the Blitz pro 1.2 software, which calculated association constant (ka) and dissociation constant (kd) and determined the affinity constant KD (ka/kd).

Results: As shown in **Figure 9**, the anti-SIRPa HEFLB of the invention does not compete with the binding of CD47 to SIRPg. At the opposite, the other known antibodies SIRP29 and, in particular, kwar23 compete with the binding of CD47 to SIRPg.

Example 12. Binding to blood cells by flow cytometry

Method: The experiment was realized to analyze the binding of the anti-SIRPa antibodies on human blood cells. CD3-positive T lymphocytes, red blood cells and platelets were extracted from purified blood from healthy volunteers. Cells were then stained for 30min at 4°C with 10 micrograms/ml of each tested antibody, washed and then stained with a secondary fluorescent anti-IgG antibody for another 30min at 4°C. After washes, cells were analyzed on a CANTO II (BD Bioscience) flow cytometer.

Results: As shown in **Figure 10**, the T cells, the red blood cells and the platelets are positive for CD47, which is expressed ubiquitously, and they were stained with the B6H12 antibody. The SIRP29 and the Kwar23 antibodies, like LSB2.20 (specific anti-SIRPγ antibody), bind to T cells that are known to express SIRPγ. However, the anti-SIRPa humanized 18D5 antibody does not bind to the T cells (same results obtained with four different 18D5 humanized variants tested). Red blood cells and platelets do not express SIRPa and, thus, they were not revealed with the humanized 18D5 antibody and the other anti-SIRPa antibodies. This result shows the specificity of the humanized 18D5 antibody for SIRPa on live cells as compared to the known anti-SIRPa antibodies.

As shown in **Figure 11**, the T-cells are not stained by the humanized 18D5 antibody (same results obtained with four different 18D5 humanized variants tested) whereas more than 70% of T cells are stained by SIRP29 and Kwar23.

Example 13. Human CD3+ T cell proliferation

Method: hPBMC were isolated from buffy coat of healthy volunteers. CD4 or CD8 T cells were selected by positive selection using an AutoMACS (Miltenyi) and plated in 96-round well plate (50 000 cells/well). The proliferative signals were provided by either anti-CD3/anti-CD28 coated microbeads (LifeTechnologies) at a 1 bead for 1 T cell ratio during three days, or allogeneic mature

dendritic cells generated in vitro at a 5 T cell for 1 mDC during 5 days. Antibodies targeting the SIRPa/CD47 pathway were added from the beginning of the proliferation test at a saturating concentration (10 µg/mL). Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Results: As shown in **Figure 12**, the anti-SIRPa antibody HALA variant does not inhibit the T cell proliferation when T cells are stimulated with anti-CD3+ anti-CD28 beads **(A)** or with allogenic dendritic cells **(B)**, whereas the anti-SIRPa Kwar23 inhibits T cell proliferation when T cells are stimulated with allogenic dendritic cells. As expected, the anti-CD47 antibodies are inhibitors of the T cell proliferation.

Example 14. Mouse CD8+ T cell proliferation

Method: Splenocytes were isolated from naive mice. CD8 T cells were selected by positive selection using an AutoMACS (Miltenyi) and plated in 96-round well plate (50 000 cells/well). The proliferative signals were provided by anti-CD3/anti-CD28 coated microbeads (LifeTechnologies) at a 1 bead for 1 T cell ratio during three days. A mouse anti-SIRPa antibody (P84) and an anti-CD47 antibody (MIAP310) targeting the SIRPa/CD47 pathway were added from the beginning of the proliferation test at a saturating concentration (10 µg/mL). Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Results: As shown in **Figure 13**, there is no inhibition of the anti-SIRPa or anti-CD47 antibody on the proliferation of mouse T cells. This result is explained by the fact that mice does not express the SIRPg gene. Thus, mice can be used as a model to predict the *in vivo* effects of a specific anti-SIRPa antibody that does not bind SIRPg. In contrast, anti-CD47 antibody *in vivo* preclinical efficacy, in particular on adaptive immunity and generation of memory T lymphocytes, is not predictive of human situation.

Example 15. Human T cell proliferation

Method: hPBMC were isolated from buffy coat of healthy volunteers. CD4 or CD8 T cells were selected by positive selection using an AutoMACS (Miltenyi) and plated in 96-round well plate (50 000 cells/well). The proliferative signals were provided by either anti-CD3/anti-CD28 coated microbeads (LifeTechnologies) at a 1 bead for 1 T cell ratio during three days, or allogeneic mature dendritic cells generated in vitro at a 5 T cell for 1 mDC during 5 days. Antibodies were added from the beginning of the proliferation test at a saturating concentration (5µg/mL for anti-CD47 and anti-SIRPa antibodies and 2,5µg/mL for the anti-PD-1/PD-L1 antibodies and the recombinant 4-1BBL). Proliferation was measured by incorporation of H^3-thymidine during the last 12h of culture.

Results: As shown in **Figure 14**, the anti-PD-1/PD-L1 antibodies and the recombinant 4-1BBL have a boost effect on the proliferation of human T cells, while anti-CD47 has a negative effect on the proliferation of human T cells and thus prevents the efficacy of anti-PD-1/PD-L1 or 4-1BB agonist agents. The anti-SIRPa HEFLB has no significant effect on the proliferation of T cells.

Example 16. Anti-tumor effects in mice

Method: Mice were anesthetized with a cocktail of xylazine/ketamine. After a laparotomy, tumoral Hepa 1.6 cells were injected through the portal vein ($2,5.10^6$ cells/100 µL) in PBS. The treatment was started 4 days after tumor injection. The agonistic anti-4-1BB monoclonal antibody (3H3) was injected two times at d4 and d8 after Hepa 1.6 cells injection intraperitoneally in PBS (100 µg/injection). The antagonistic anti-SIRPa antibody (P84) was injected three time a week during four weeks intraperitoneally in PBS (300µg/injection).

Results: As shown in **Figure 15A**, the anti-SIRPa antibody highly increases the positive effects of the anti-4-1BB antibody on the survival of mice.

Example 17. Anti-tumor effects in mice previously cured

Method: Mice previously cured in the hepatoma model by anti-SIRPa + anti-4-1BB injection or SIRPa mutant mice treated with anti-4-1BB were rechallenged by Hepa 1.6 cells injection in the spleen (2.5.10^6 cells/mouse). Mice were anesthetized with 3% of isoflurane in the air. After incision on the flank of the mice and isolation of the spleen, tumoral Hepa 1.6 cells were injected into the spleen ($2,5.10^6$ cells/50µL) in PBS. Naive mice were injected in parallel in the same route in order to compare tumor development with rechallenged mice.

Results: As shown in **Figure 15B**, all the cured mice survived when rechallenged and, at the opposite, all naïve mice died. This result suggests that memory T cells are still present in cured mice.

Example 18. Anti-tumor effects of T-cell splenocytes or whole splenocytes collected from mice previously cured

Method: Cured anti-SIRPa + anti-4-1BB rechallenged mice were euthanized and the spleen was collected. After red blood cell lysis, splenocytes were extracted and CD3 positive T cells were isolated from a part of splenocytes with an AutoMACS. After anesthesia, mice were injected with either T-cell splenocytes ($2,5.10^6$ cells/100 µL) or whole splenocytes (10.10^6 cells/100 µL) or excipient alone (PBS) intravenously. All mice received Hepa 1.6 cells through the portal vein as described previously ($2,5.10^6$ cells/100 µL).

Results: As shown in **Figure 15B**, the splenocytes and isolated T lymphocytes collected from cured mices has a high positive effect on the survival of mice. This results indicate that memory T cells are present in the splenocytes of the cured mice after treatment of the hepatoma.

Example 19. Anti-tumor effects in mice

Method: Mice were anesthetized with a cocktail of xylazine/ketamine. After a laparotomy, tumoral Hepa 1.6 cells were injected through the portal vein ($2,5.10^6$ cells/100 µL) in PBS. The anti-SIRPa (P84 clone) was administereded three times a week for 4 weeks in combination or not with twice a week i.p. injection for 4 weeks of anti-PDL1 mAb.

Results: As shown in **Figure 16A**, the anti-SIRPa antibody and the anti-PDL1 antibody show high positive effects when used simultaneously on the survival of mice.

Example 20. Anti-tumor effects in mice previously cured

Method: Mice previously cured in the hepatoma model by anti-SIRPa + anti-PDL-1 injection were rechallenged by Hepa 1.6 cells injection in the spleen (2.5.10^6 cells/mouse). Mice were anesthetized with 3% of isoflurane in the air. After incision on the flank of the mice and isolation of the spleen, tumoral Hepa 1.6 cells were injected into the spleen ($2,5.10^6$ cells/50µL) in PBS. Naive mice were injected in parallel in the same route in order to compare tumor development with rechallenged mice.

Results: As shown in **Figure 16B**, all the cured mice survived when rechallenged and, at the opposite, all naïve mice died. This result suggests that memory T cells are still present in cured mice.

Example 21. Effects of the growth of a tumor in a mammary carcinoma model

Method: Mice were anesthetized with 3% of isoflurane in the air. Mice were shaved on the abdomen and 4T1 cells were injected in the mammary gland with an insulinic syringe (30 Gauges) in 50µL of PBS. The antagonistic anti-SIRPa antibody (P84) or a control antibody was injected three time a week during four weeks intraperitoneally in PBS (200µg/injection).

Results: As shown in **Figure 17**, the anti-SIRPa antibody significantly ($p<0.01$) reduces the growth of the tumor in the mammary carcinoma model as compared to a control antibody.

Figure 18 shows the immune cell analysis two weeks after inoculation. Anti-SIRPa has a positive effect on myeloid and non-myeloid cells (T and NK cells) both in tumor and in periphery (spleen).

Example 22. Effects of SIRPa antibodies on the concentration of hemoglobin and on the hematocrit

Method: Anti-SIRPa (P84 clone), anti-CD47 (MIAP410 clone) and irrelevant isotype control were administered intraperitoneally at day 0 and day 2 at 12mg/kg in C57Bl/6 mice. Blood samples were

collected at day 0 and day 3 in EDTA containing tubes and blood count was performed with a XS-800i haematology analyzer (Sysmex). The level of hemoglobin (left) and the percentage of hematocrit (right) were evaluated at day 3.

Results: As shown in **Figure 19**, the anti-SIRPa antibody has no toxic effect on the concentration of hemoglobin and on the hematocrit. At the opposite, the anti-CD47 antibody induces a decrease of the concentration of hemoglobin and of the hematocrit.

CLAIMS

1. An anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic that specifically binds to at least one peptide of amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6 within human SIRPa,

in particular, that specifically binds to the peptide of amino acid sequence set forth in SEQ ID NO: 3 within human SIRPa and to at least another peptide of amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6 within human SIRPa.

2. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to claim 1, that specifically binds to the peptides of amino acid sequence SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6 within human SIRPa.

3. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to claim 1 or 2, which is an anti-SIRPa antagonist antibody, which inhibits the binding of human CD47 to human SIRPa.

4. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 3, which does not specifically bind to human SIRPg.

5. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 4, which does not specifically bind to human T-cells, in particular CD3+ T-cells.

6. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 5, which does not inhibit the proliferation of human T-cells.

7. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 6, which does not inhibit the binding of human CD47 to human SIRPg.

8. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 7, which comprises:

a) a heavy chain variable domain comprising HCDR1, HCDR2 and HCDR3, and/or

b) a light chain variable domain comprising LCDR1, LCDR2 and LCDR3,

wherein:

- HCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 14,

- HCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 15 or SEQ ID NO: 16,

- HCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 17 SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20,

- LCDR1 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 21,

- LCDR2 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 22 and

- LCDR3 comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 23.

9. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 8, which comprises:

- a heavy chain variable domain comprising or consisting of the amino acid sequence 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 and SEQ ID NO: 30, and/or

- a light chain variable domain comprising or consisting of the amino acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32 and SEQ ID NO: 33,

in particular, which comprises:

- a light chain variable domain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 33, and

- a heavy chain variable domain comprising or consisting of the amino acid sequence 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 and SEQ ID NO: 30, in particular SEQ ID NO: 29, SEQ ID NO: 30, and more particularly SEQ ID NO: 30.

10. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 9, which comprises:

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 24, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 31,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 25, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 25, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 26, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 26, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 27, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 27, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 28, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 28, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 29, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 29, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 30, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 32,

or

- a heavy chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 30, and

- a light chain variable domain comprising or consisting of amino acid sequence set forth in SEQ ID NO: 33.

11. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 10, wherein said antibody is a humanized monoclonal antibody, in particular wherein the antibody light chain constant domain is derived from a human kappa light chain constant domain, more particularly wherein the light chain constant domain consists of the sequence of SEQ ID NO: 35, and wherein the antibody heavy chain constant domain is derived from a human IgG1, IgG2, IgG3, or IgG4 heavy chain constant domain, in particular

from a human IgG4 heavy chain constant domain, more particularly wherein the antibody heavy chain constant domain consists of the sequence with SEQ ID NO: 34.

12. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 11, or

an anti-human SIRPa antagonist antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of human CD47 to human SIRPa, and which does not bind specifically to human SIRPg, and/or which does not bind specifically to human T-cells, and/or which does not inhibit the proliferation of human T-cells, and/or which does not inhibit the binding of human CD47 to human SIRPg,

for use as a medicament.

13. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 11, or

an anti-human SIRPa antagonist antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic, which inhibits the binding of human CD47 to human SIRPa, and which does not bind specifically to human SIRPg, and/or which does not bind specifically to human T-cells, and/or which does not inhibit the proliferation of human T-cells, and/or which does not inhibit the binding of human CD47 to human SIRPg,

for use in the prevention or treatment of a disease selected from the group consisting of a cancer (in particular inflammatory cancer and cancer with infiltrated myeloid cells, particularly with infiltrated MDSCs and/or TAM cells), an infectious disease, a chronic inflammatory disease, an auto-immune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a sceptic shock, a chronic infectious disease (in particular Pseudomonas and CMV), fibrosis, atherosclerosis and a transplant dysfunction, or for use in vaccination.

14. The anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 13, for its use according to claim 13, wherein said anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic is administered to a patient presenting a SIRPa-positive tumor.

15. A pharmaceutical composition comprising at least one anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 13, and a pharmaceutically acceptable carrier.

16. A combination product comprising:

- at least one anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 13, and

- at least one second therapeutic agent selected from the group consisting of chemotherapeutic agents, radiotherapy agents, surgery agents, immunotherapeutic agents, antibiotics and probiotics;

for simultaneous, separate or sequential use as a medicament.

17. An isolated nucleic acid molecule encoding an antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic according to any one of claims 1 to 11.

18. A vector comprising a nucleic acid molecule according to claim 17.

19. An isolated host cell comprising a nucleic acid molecule according to claim 17 and/or a vector according to claim 18.

20. An antigen comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3,

in particular an antigen comprising or consisting of the epitope sequence of human SIRPa consisting of SEQ ID NO: 3 and at least one epitope sequence of human SIRPa selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6, more particularly an antigen comprising or consisting of the epitope sequences of human SIRPa consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.

21. A method of manufacturing an antibody as defined in any one of claims 1 to 11 comprising immunizing a non-human animal against an antigen as defined in claim 20, and collecting the resulting serum from said immunised non-human animal to obtain antibodies directed against said antigen.

22. An *in vitro* or *ex vivo* method to determine a SIRPa positive cells in a subject from a biological sample of said subject, comprising:

i) determining *in vitro* the expression and/or the level of expression of SIRPa, in a biological sample of said subject using the anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 13.

23. Use of at least one anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 13 in a method wherein SIRPa is used as a biomarker that is predictive for the response to a treatment in a subject, in particular in a cancer subject.

24. An *in vitro* method of predicting the response of a cancer subject to a treatment, in particular with anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 13, comprising:

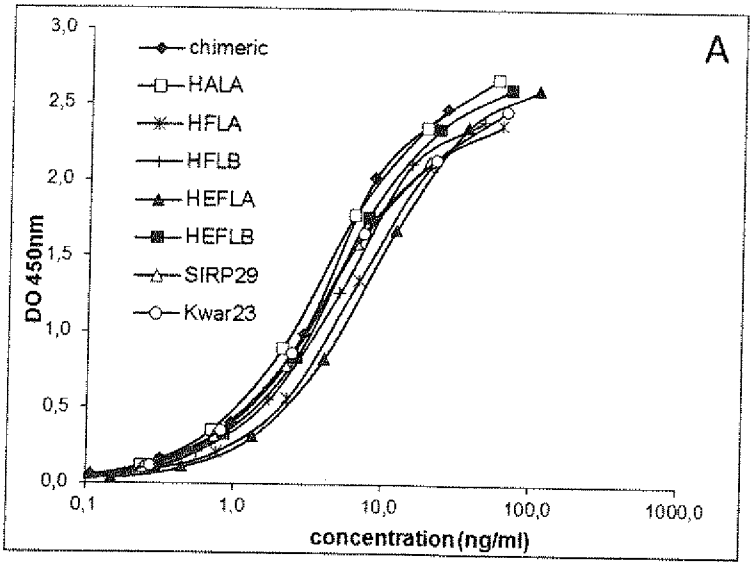
- determining the expression level of SIRPa in a tumor sample of a subject, in particular with anti-human SIRPa antibody or antigen-binding fragment thereof or antigen-binding antibody mimetic as defined in any one of claims 1 to 13, and
- comparing the expression level of SIRPa to a value representative of an expression level of SIRPa in a non-responding subject population,

wherein a higher expression level of SIRPa in the tumor sample of the subject is indicative for a patient who will respond to the treatment.

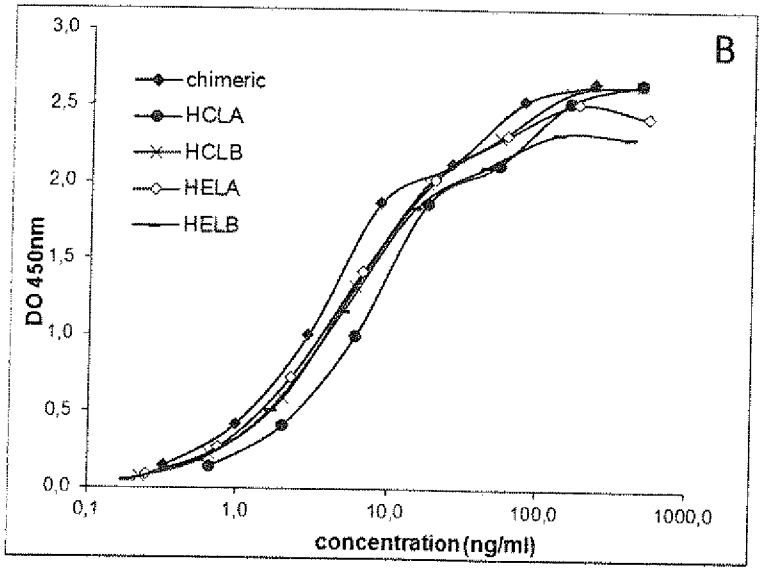
ABSTRACT

The present invention provides new anti-SIRPa antibodies able to specifically antagonize the interaction between SIRPa and CD47, without affecting the interaction between SIRPg and CD47, and their uses.

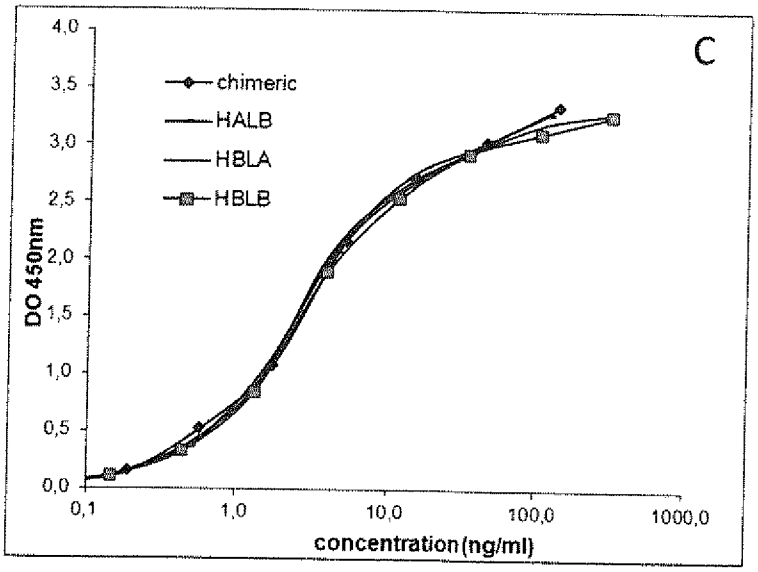
(No figure)



	EC50 (ng/ml)
chimeric	2,9
HALA	3,9
HFLA	5,1
HFLB	4,0
HEFLA	7,1
HEFLB	4,4
SIRP29	3,7
Kwar23	3,3



	EC50 (ng/ml)
chimeric	4,06
HCLA	5,60
HCLB	5,59
HELA	4,61
HELB	4,13

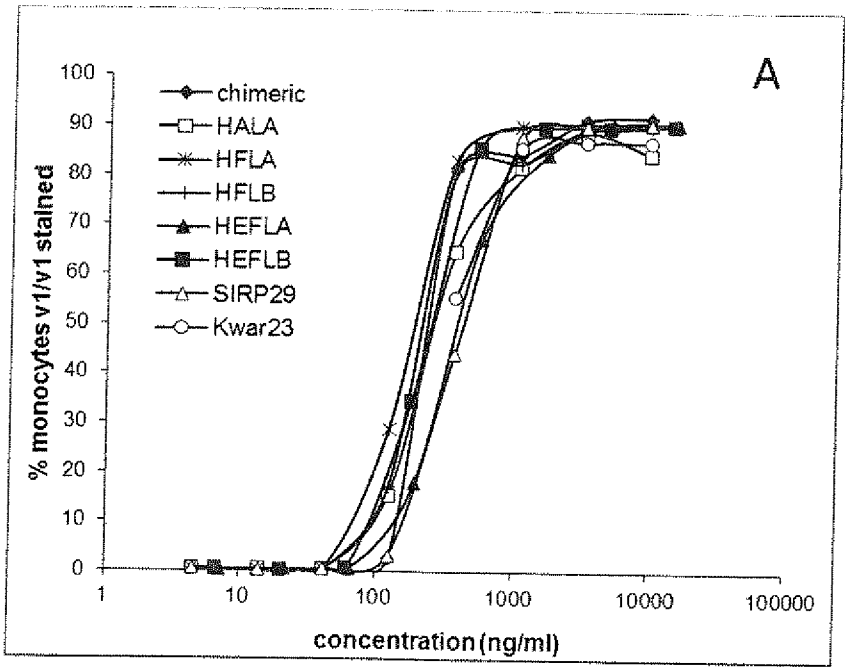


	EC50 (ng/ml)
chimeric	2,74
HALB	2,53
HBLA	2,68
HBLB	2,95

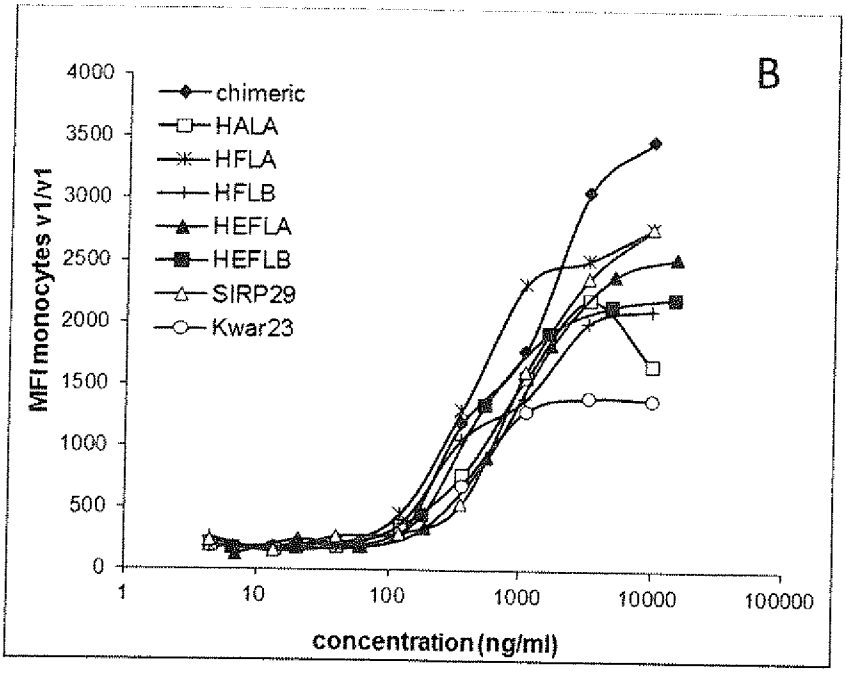
FIGURE 1

	Association (ka) (1/Ms)	Dissociation (kd) (1/s)	Affinity (KD) (M)
mouse 18D5	6,23e4	1,20e-5	**1,93e-10**
chimeric	1,49e5	5,14e-5	**3,44e-10**
HFLA	8,57e5	1,86e-4	**2,17e-10**
HFLB	7,91e5	2,49e-4	**3,15e-10**
HEFLA	5,59e5	1,48e-4	**2,64e-10**
HEFLB	6,54e5	1,89e-4	**2,89e-10**
SIRP29	4,15e4	1,01e-5	**2,43e-10**
Kwar23	2,44e5	8,95e-5	**3,67e-10**
SE7C2	5,98e3	0,01	**1,67e-6**
SE5A5	2,45e4	9,49e-4	**3,87e-8**

FIGURE 2



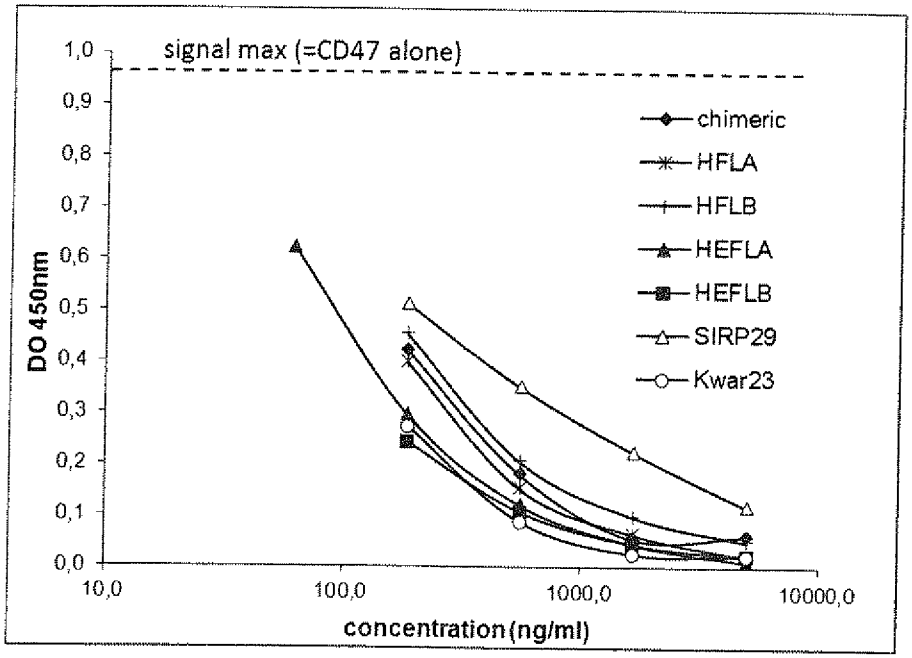
% stained	EC50 (ng/ml)
chimeric	225
HALA	242
HFLA	174
HFLB	201
HEFLA	356
HEFLB	208
SIRP29	376
Kwar23	260



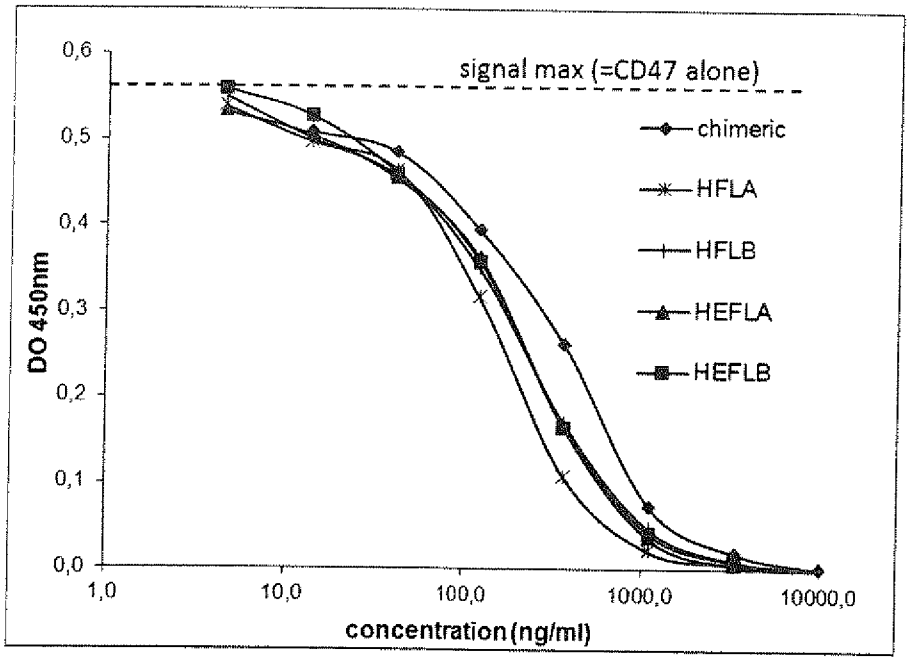
MFI	EC50 (ng/ml)
chimeric	780
HALA	590
HFLA	328
HFLB	467
HEFLA	858
HEFLB	449
SIRP29	960
Kwar23	378

FIGURE 3

First experiment



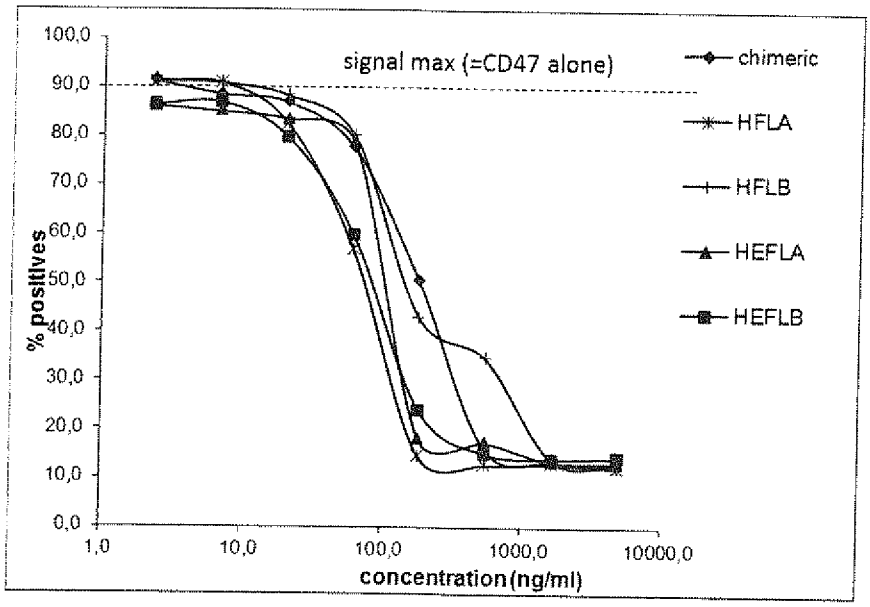
Second experiment



	IC50 (ng/ml)
chimeric	303,8
HFLA	146,2
HFLB	182,7
HEFLA	190,6
HEFLB	186,3

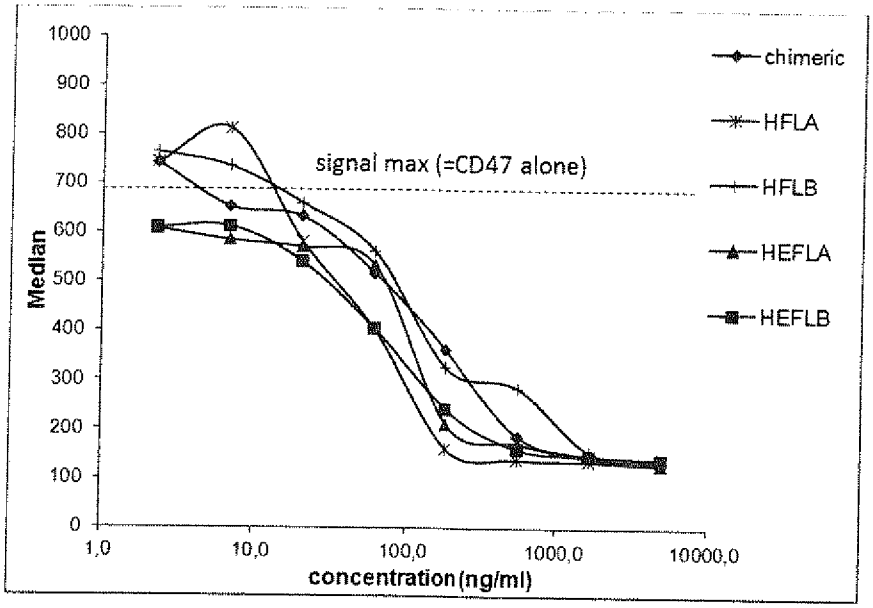
FIGURE 4

A



	IC50 (ng/ml)
chimeric	171,83
HFLA	64,09
HFLB	149,73
HEFLA	104,56
HEFLB	73,49

B



	IC50 (ng/ml)
chimeric	199,9
HFLA	75,1
HFLB	169,9
HEFLA	117,6
HEFLB	90,1

FIGURE 5

A	Association (ka) (1/Ms)	Dissociation (kd) (1/s)	Affinity (KD) (M)
mouse 18D5	1,58e4	1,76e-3	1,11e-7
mouse 18D5 with SP-D	3,797e5	1,088e-2	2,865e-8

B	Association (ka) (1/Ms)	Dissociation (kd) (1/s)	Affinity (KD) (M)
SP-D	2,465e3	1,078e-2	4,372e-6
SPD with mouse 18D5	3,272e3	2,405e-3	7,35e-7

FIGURE 6

A

	Association (ka) (1/Ms)	Dissociation (kd) (1/s)	Affinity (KD) (M)
Chimeric	8,837e4	1,385e-3	1,568e-8
HALA	1,328e5	8,504e-4	6,403e-9
HFLA	7,447e4	1,919e-3	2,576e-8
HFLB	6,694e4	1,738e-3	2,596e-8
HEFLA	9,827e4	1,221e-3	1,243e-8
HEFLB	1,939e4	1,711e-3	8,69e-8
SIRP29	1,078e5	8,114e-4	7,531e-9
kwar23	2,863e5	7,935e-4	2,772e-9

B

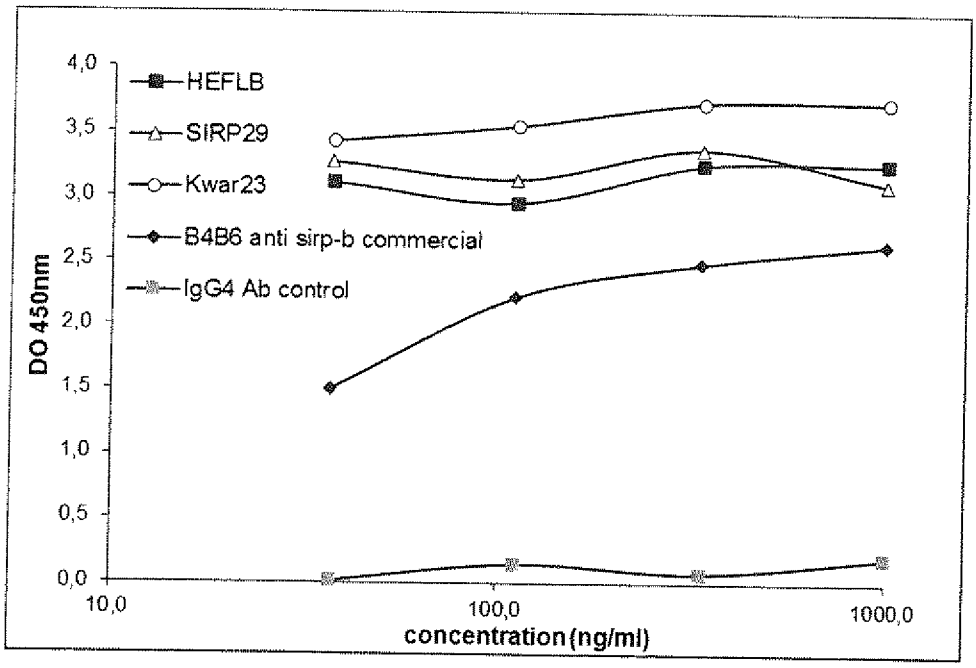


FIGURE 7

A

	Association (ka) (1/Ms)	Dissociation (kd) (1/s)	Affinity (KD) (M)
Chimeric	4,93e4	3,194e-3	6,479e-8
HALA	1,539e5	7,093e-3	4,609e-8
HFLA	2,477e5	1,18e-2	4,764e-8
HFLB	1,191e5	1,234e-2	1,036e-7
HEFLA	2,173e5	1,14e-2	5,244e-8
HEFLB	1,92e5	1,193e-2	6,215e-8
SIRP29	1,36e5	7,2e-4	5,296e-9
kwar23	3,57e5	7,648e-4	2,142e-9

B

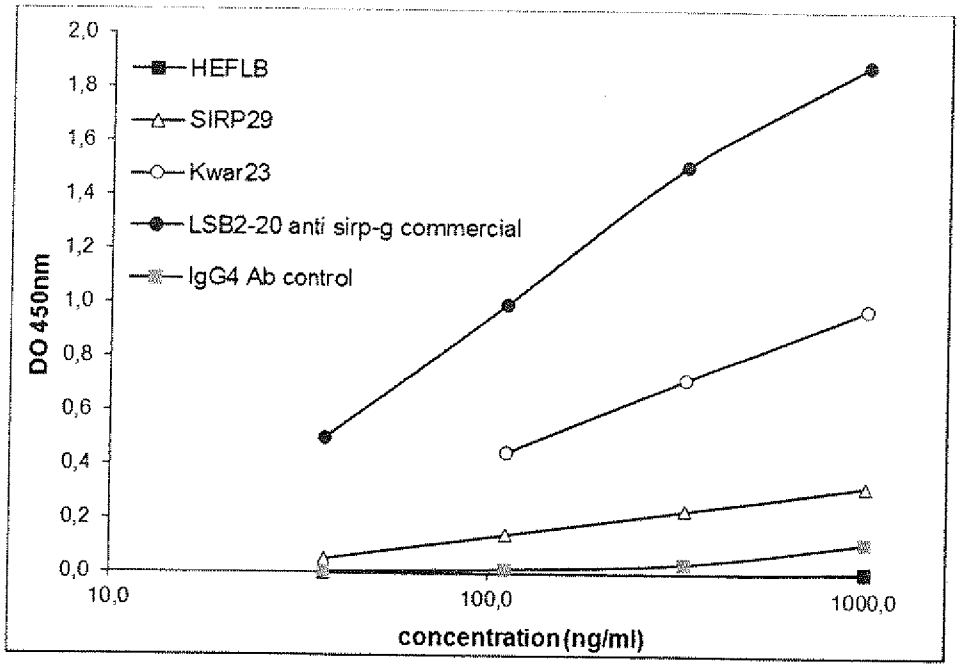


FIGURE 8

	Association (ka) (1/Ms)	Dissociation (kd) (1/s)	Affinity (KD) (M)
CD47 alone	**2,193e4**	**1,309e-2**	**5,969e-7**
HEFLB +CD47	**5,372e4**	**5,334e-2**	**9,928e-7**
SIRP29 +CD47	**2,118e4**	**5,525e-2**	**2,572e-6**
Kwar23 +CD47	4,369e2	1,259e-1	2,88e-4

FIGURE 9

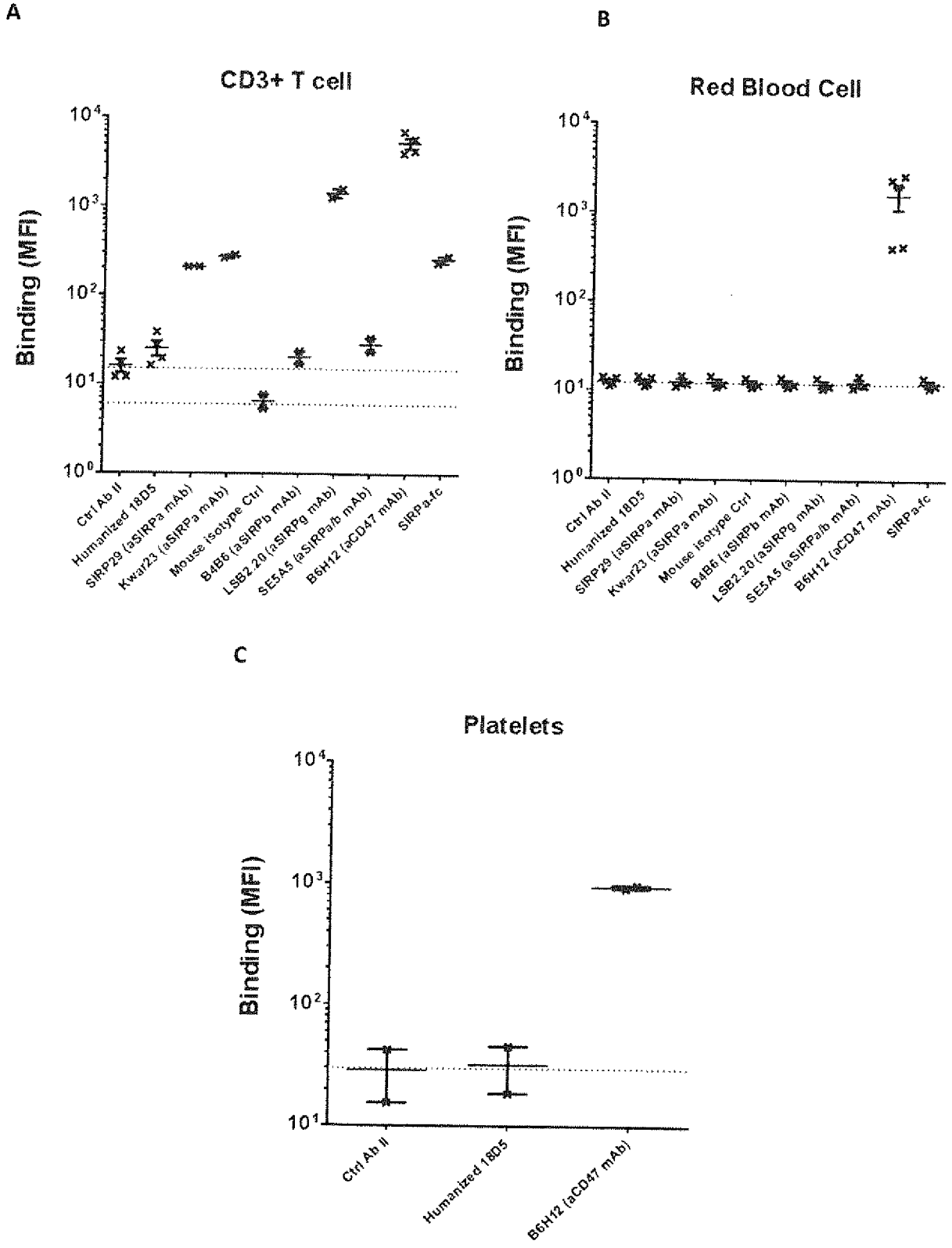
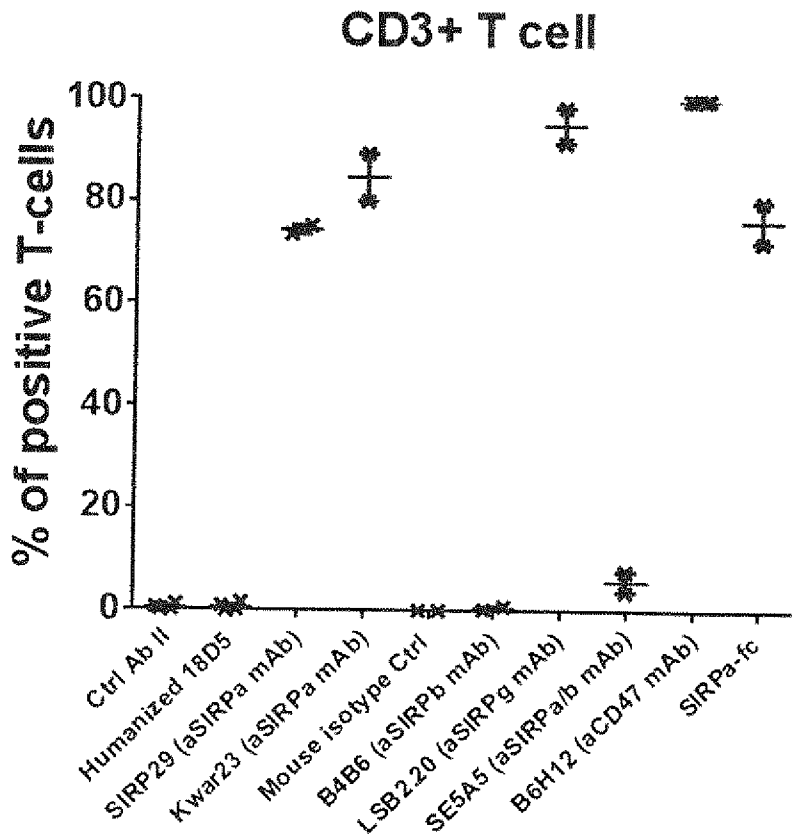

A
CD3+ T cell
Binding (MFI)
10^4
10^3
10^2
10^1
10^0
Ctrl Ab II
Humanized 18D5
SIRP29 (aSIRPa mAb)
Kwar23 (aSIRPa mAb)
Mouse isotype Ctrl
B4B6 (aSIRPb mAb)
LSB2.20 (aSIRPg mAb)
SE5A5 (aSIRPa/b mAb)
B6H12 (aCD47 mAb)
SIRPa-fc
B
Red Blood Cell
Binding (MFI)
10^4
10^3
10^2
10^1
10^0
Ctrl Ab II
Humanized 18D5
SIRP29 (aSIRPa mAb)
Kwar23 (aSIRPa mAb)
Mouse isotype Ctrl
B4B6 (aSIRPb mAb)
LSB2.20 (aSIRPg mAb)
SE5A5 (aSIRPa/b mAb)
B6H12 (aCD47 mAb)
SIRPa-fc
C
Platelets
Binding (MFI)
10^4
10^3
10^2
10^1
Ctrl Ab II
Humanized 18D5
B6H12 (aCD47 mAb)

FIGURE 10



	Ctrl Ab II	Humanized 18D5	SIRP29 (aSIRPa mAb)	Kwar23 (aSIRPa mAb)	Mouse isotype Ctrl	B4B6 (aSIRPb mAb)	LSB2.20 (aSIRPg mAb)	SE5A5 (aSIRPa/b mAb)	B6H12 (aCD47 mAb)	SIRPa-fc
Exp 1 HV1	0,45	1,54	73,5	80	0	0,87	91,4	3,72	100	72,2
Exp 1 HV2	1,12	0,84	75,2	89,3	0,016	0,25	98,4	7,69	100	80,1
Exp 2 HV1	0,057	0,016							99,9	
Exp 2 HV2	0,076	0,058							99,9	

FIGURE 11

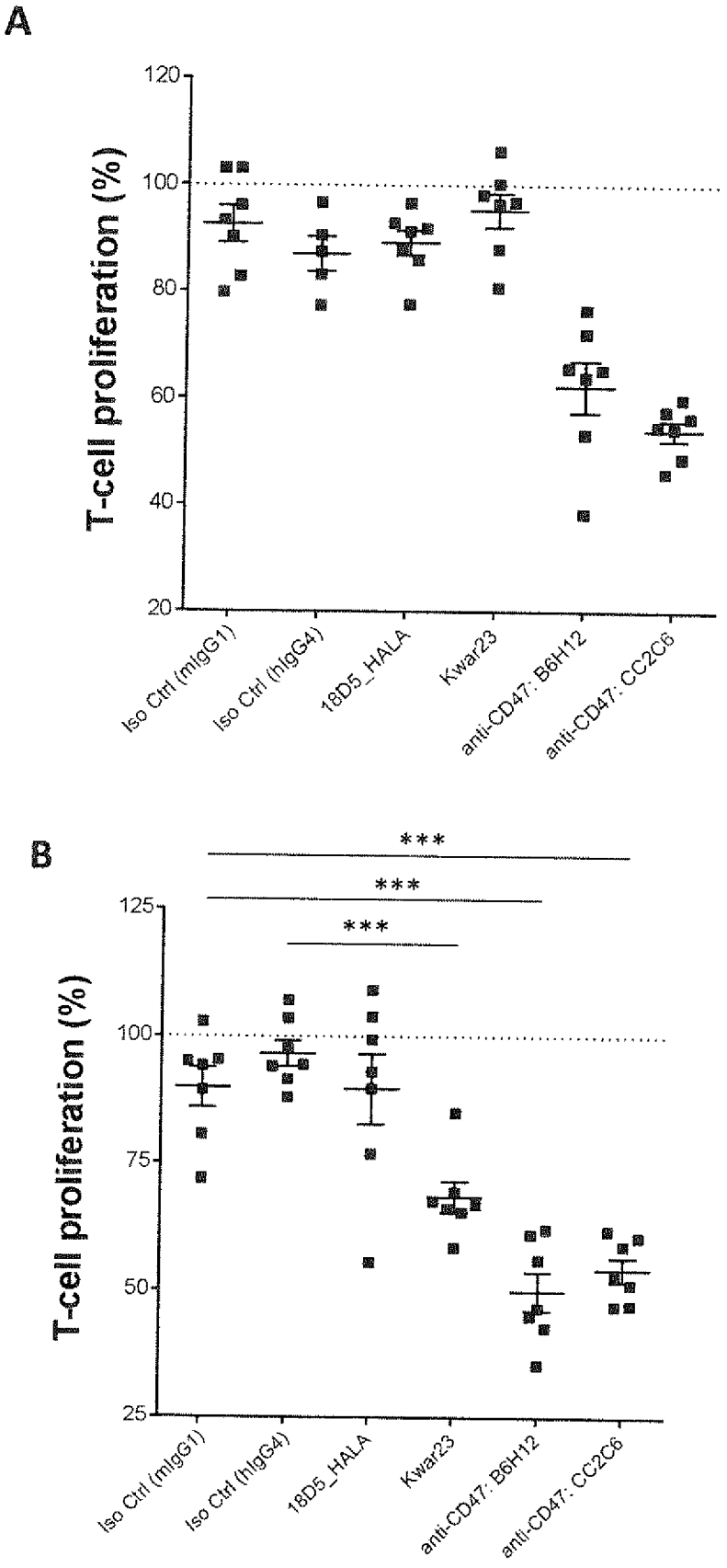

A
T-cell proliferation (%)
120
100
80
60
40
20
Iso Ctrl (mIgG1)
Iso Ctrl (hIgG4)
18D5_HALA
Kwar23
anti-CD47: B6H12
anti-CD47: CC2C6
B
***
***
***
T-cell proliferation (%)
125
100
75
50
25
Iso Ctrl (mIgG1)
Iso Ctrl (hIgG4)
18D5_HALA
Kwar23
anti-CD47: B6H12
anti-CD47: CC2C6

FIGURE 12

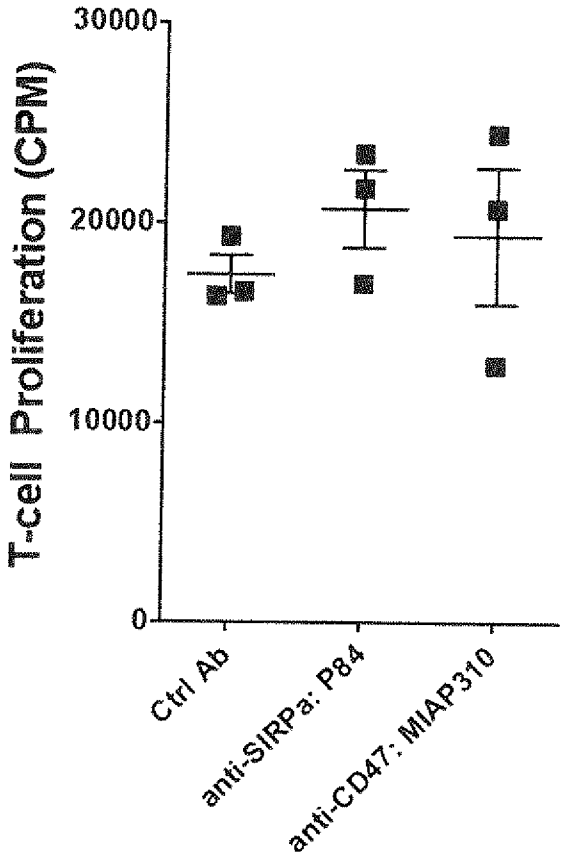
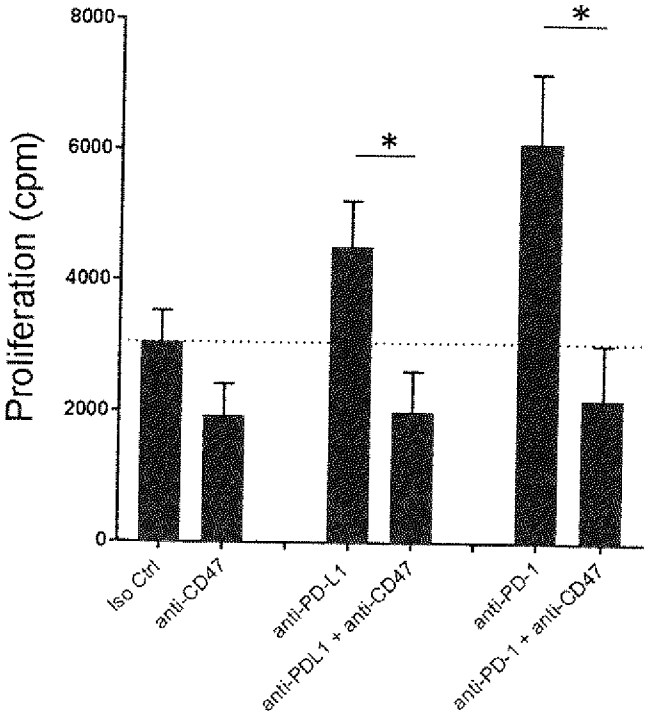

30000
20000
10000
0
T-cell Proliferation (CPM)
Ctrl Ab
anti-SIRPa: P84
anti-CD47: MIAP310

FIGURE 13


8000
6000
4000
2000
0
Proliferation (cpm)
*
*
Iso Ctrl
anti-CD47
anti-PD-L1
anti-PDL1 + anti-CD47
anti-PD-1
anti-PD-1 + anti-CD47

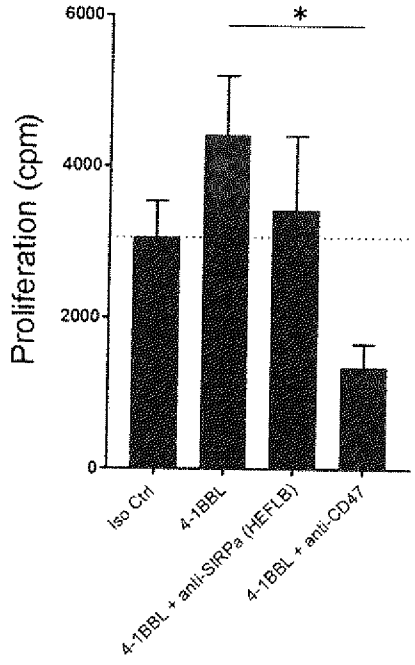

6000
4000
2000
0
Proliferation (cpm)
*
Iso Ctrl
4-1BBL
4-1BBL + anti-SIRPa (HEFLB)
4-1BBL + anti-CD47

FIGURE 14

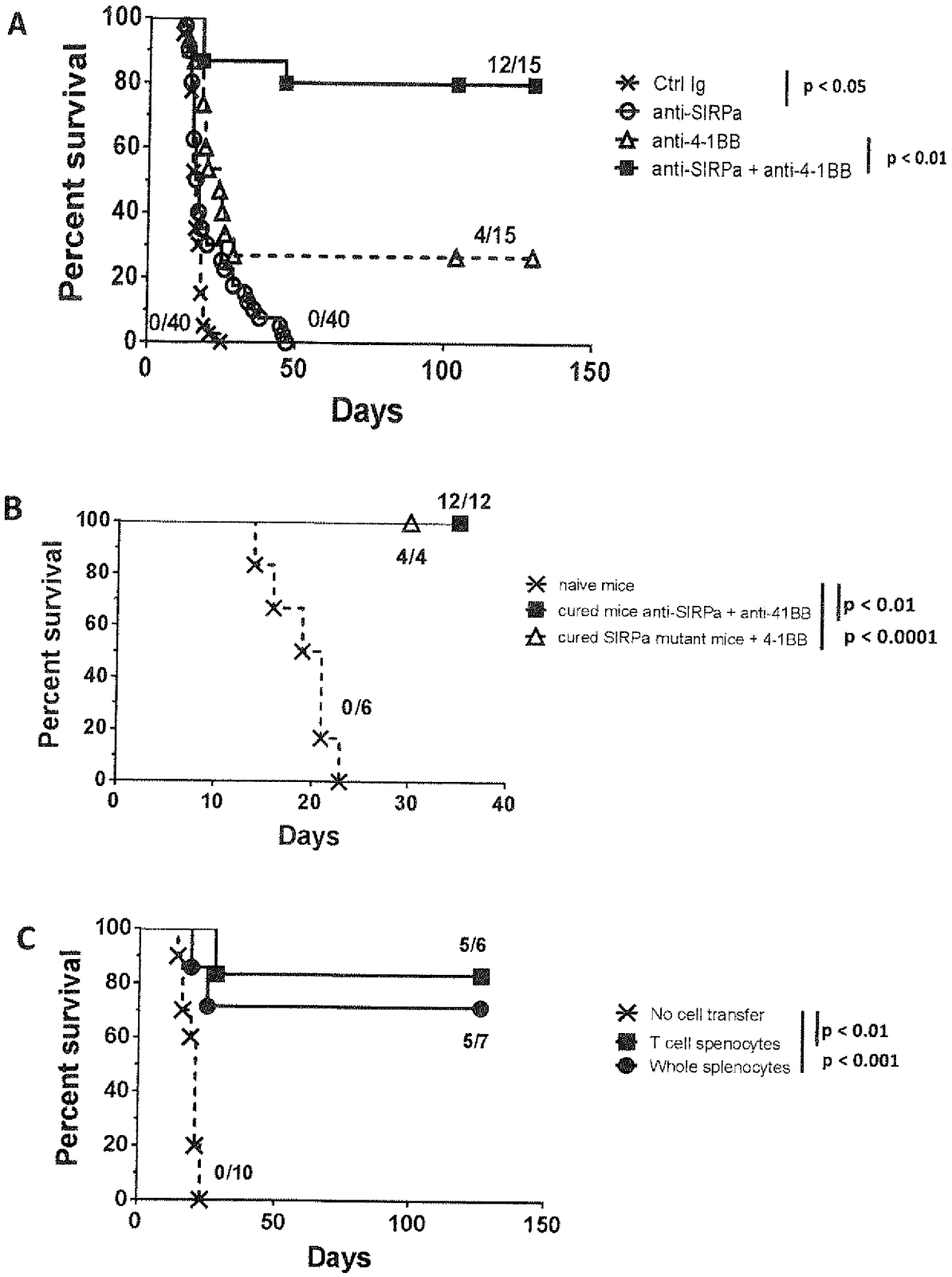
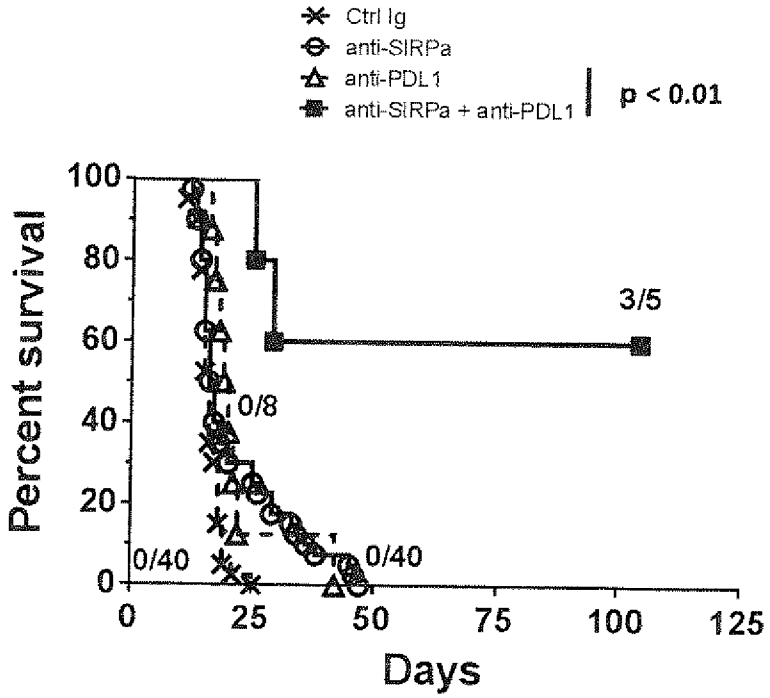

A
Percent survival
Days
12/15
4/15
0/40
0/40
Ctrl Ig
anti-SIRPa
anti-4-1BB
anti-SIRPa + anti-4-1BB
p < 0.05
p < 0.01
B
Percent survival
Days
12/12
4/4
0/6
naive mice
cured mice anti-SIRPa + anti-41BB
cured SIRPa mutant mice + 4-1BB
p < 0.01
p < 0.0001
C
Percent survival
Days
5/6
5/7
0/10
No cell transfer
T cell spenocytes
Whole splenocytes
p < 0.01
p < 0.001

FIGURE 15

A


Ctrl Ig
anti-SIRPa
anti-PDL1
anti-SIRPa + anti-PDL1
p < 0.01
Percent survival
100
80
60
40
20
0
0/40
0/8
0/40
3/5
0
25
50
75
100
125
Days

B

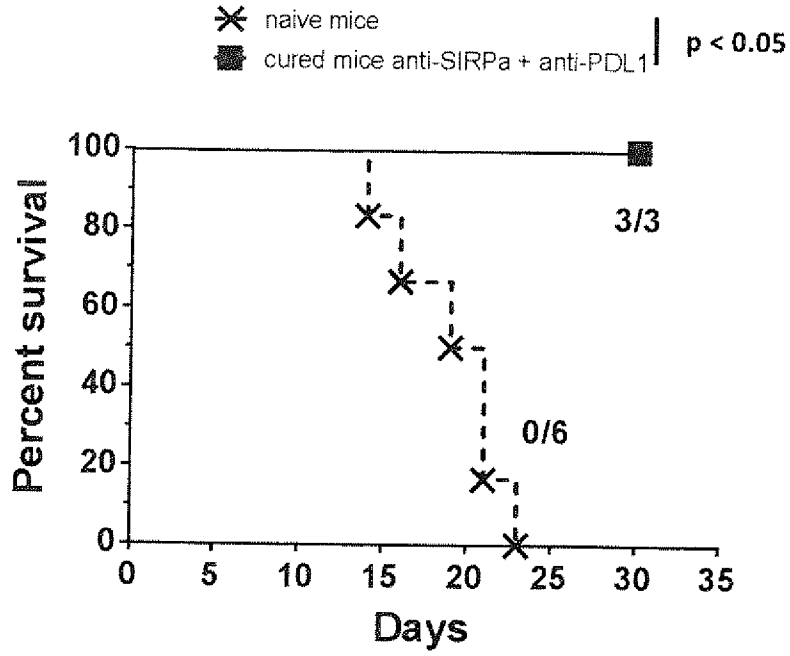

naive mice
cured mice anti-SIRPa + anti-PDL1
p < 0.05
Percent survival
100
80
60
40
20
0
3/3
0/6
0
5
10
15
20
25
30
35
Days

FIGURE 16

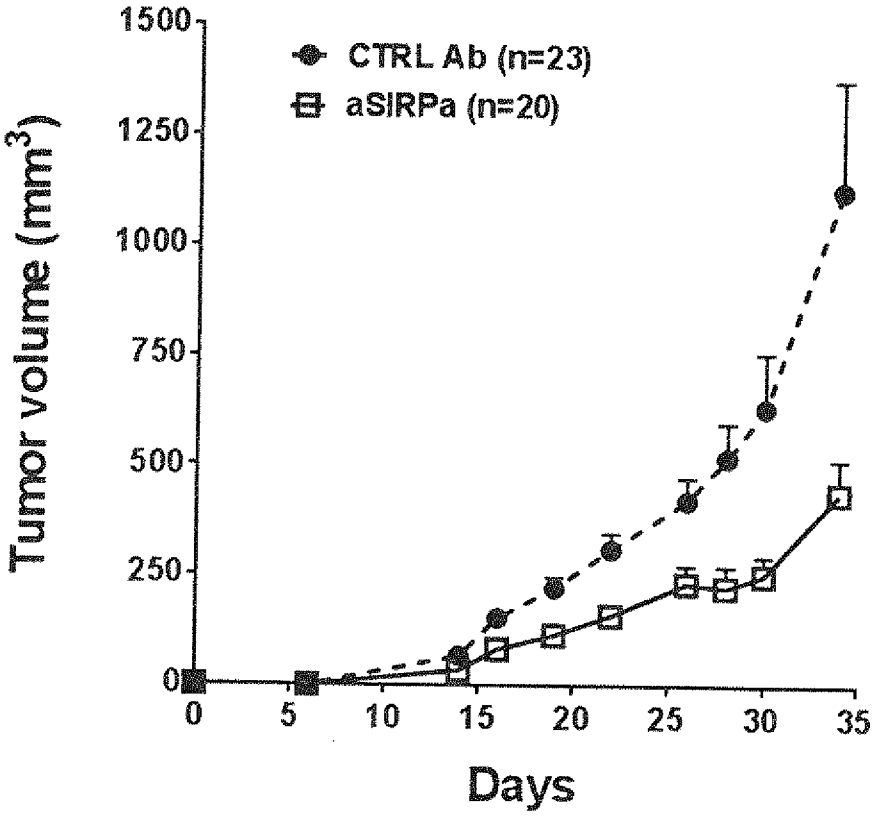

CTRL Ab (n=23)
aSIRPa (n=20)
Tumor volume (mm3)
1500
1250
1000
750
500
250
0
0
5
10
15
20
25
30
35
Days

FIGURE 17

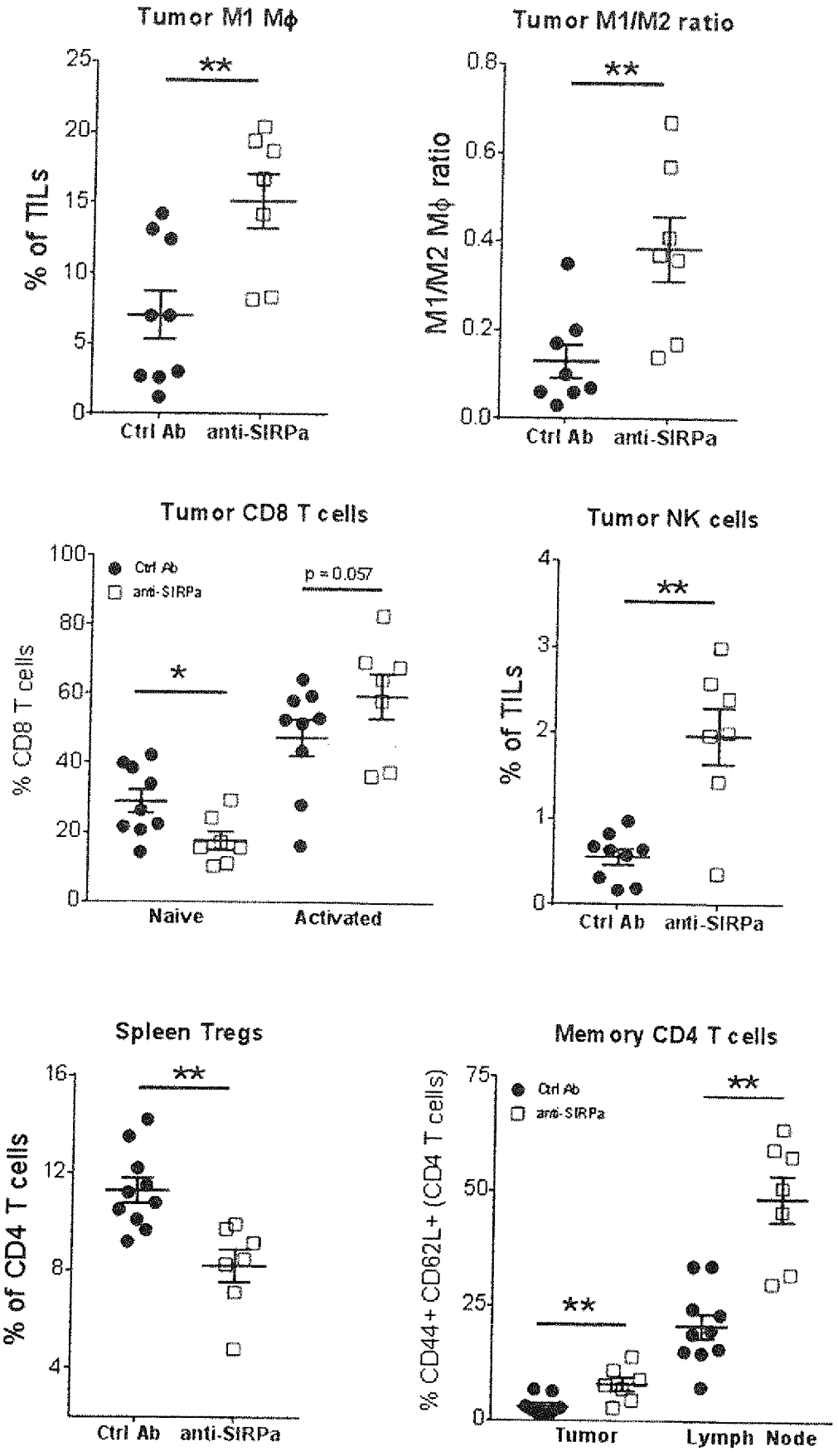

Tumor M1 Mϕ
% of TILs
25
20
15
10
5
0
**
Ctrl Ab
anti-SIRPa
Tumor M1/M2 ratio
M1/M2 Mϕ ratio
0.8
0.6
0.4
0.2
0.0
**
Ctrl Ab
anti-SIRPa
Tumor CD8 T cells
% CD8 T cells
100
80
60
40
20
0
Ctrl Ab
anti-SIRPa
p = 0.057
*
Naive
Activated
Tumor NK cells
% of TILs
4
3
2
1
0
**
Ctrl Ab
anti-SIRPa
Spleen Tregs
% of CD4 T cells
16
12
8
4
**
Ctrl Ab
anti-SIRPa
Memory CD4 T cells
% CD44+ CD62L+ (CD4 T cells)
75
50
25
0
Ctrl Ab
anti-SIRPa
**
**
Tumor
Lymph Node

FIGURE 18

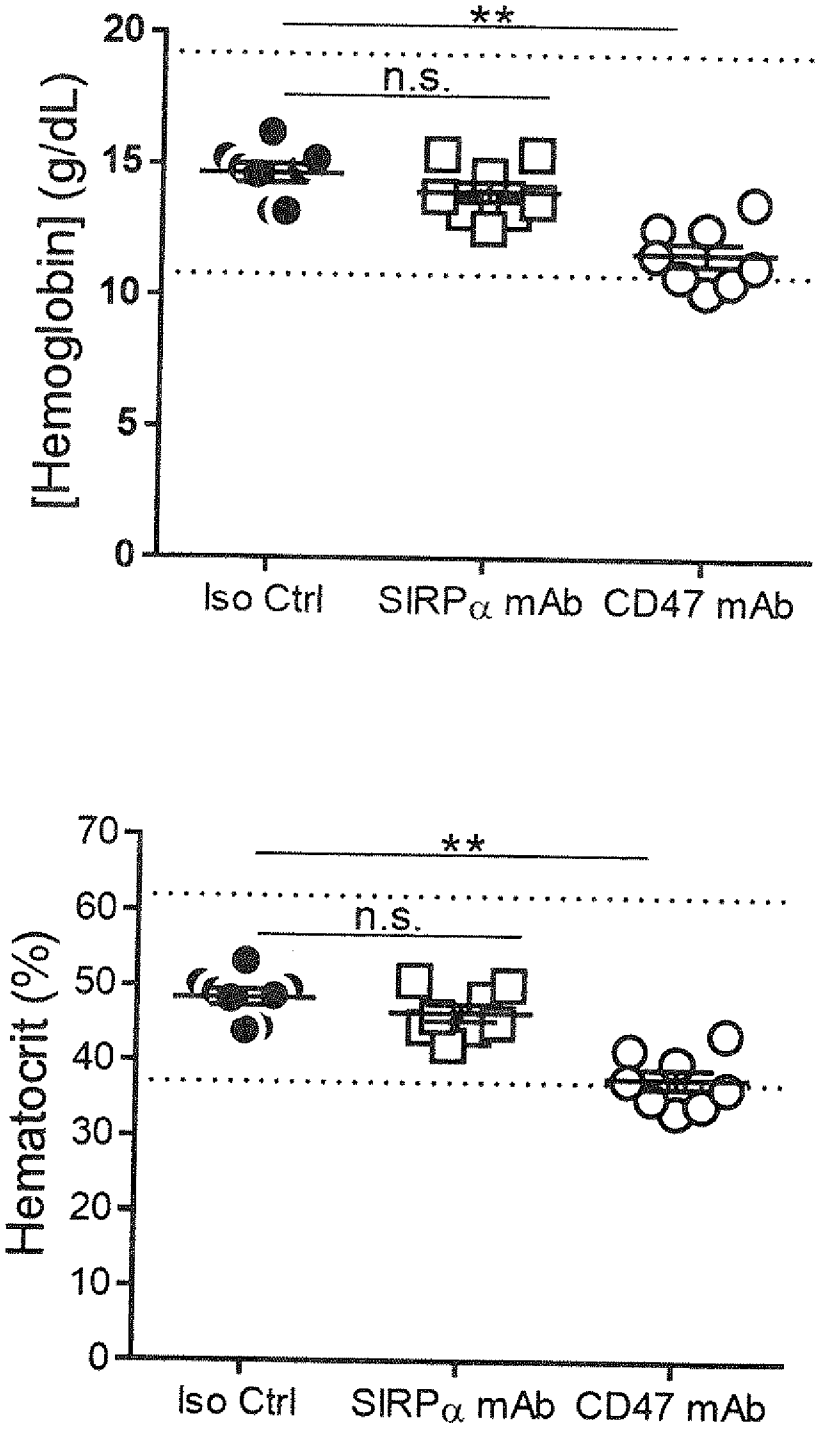

**
n.s.
20
15
10
5
0
[Hemoglobin] (g/dL)
Iso Ctrl
SIRPα mAb
CD47 mAb
**
n.s.
70
60
50
40
30
20
10
0
Hematocrit (%)
Iso Ctrl
SIRPα mAb
CD47 mAb

FIGURE 19

SEQUENCE LISTING

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<120> NEW ANTI-SIRPa ANTIBODIES AND THEIR THERAPEUTIC APPLICATIONS

<130> F2173/8EP

<160> 55

<170> PatentIn version 3.5

<210> 1
<211> 7
<212> PRT
<213> Homo sapiens

<400> 1

Ser Leu Ile Pro Val Gly Pro
1 5

<210> 2
<211> 12
<212> PRT
<213> Homo sapiens

<220>
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<222> (1)..(1)
<223> Xaa = G or A

<400> 2

Xaa Arg Glu Leu Ile Tyr Asn Gln Lys Glu Gly His
1 5 10

<210> 3
<211> 10
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Xaa = DV or T

<220>
<221> misc_feature
<222> (9)..(9)
<223> Xaa can be any naturally occurring amino acid

<400> 3

Lys Phe Arg Lys Gly Ser Pro Asp Xaa Glu
1 5 10

<210> 4
<211> 23

<212> PRT
<213> Homo sapiens

<400> 4

Gln His Thr Val Ser Phe Thr Cys Glu Ser His Gly Phe Ser Pro Arg
1 5 10 15

Asp Ile Thr Leu Lys Trp Phe
20

<210> 5
<211> 11
<212> PRT
<213> Homo sapiens

<400> 5

Ile Cys Glu Val Ala His Val Thr Leu Gln Gly
1 5 10

<210> 6
<211> 11
<212> PRT
<213> Homo sapiens

<400> 6

Tyr Pro Gln Arg Leu Gln Leu Thr Trp Leu Glu
1 5 10

<210> 7
<211> 12
<212> PRT
<213> Homo sapiens

<400> 7

Gly Arg Glu Leu Ile Tyr Asn Gln Lys Glu Gly His
1 5 10

<210> 8
<211> 11
<212> PRT
<213> Homo sapiens

<400> 8

Lys Phe Arg Lys Gly Ser Pro Asp Asp Val Glu
1 5 10

<210> 9
<211> 12
<212> PRT
<213> Homo sapiens

<400> 9

Ala Arg Glu Leu Ile Tyr Asn Gln Lys Glu Gly His
1 5 10

<210> 10
<211> 10
<212> PRT
<213> Homo sapiens

<400> 10

Lys Phe Arg Lys Gly Ser Pro Asp Thr Glu
1 5 10

<210> 11
<211> 7
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<213> Homo sapiens

<400> 11

Gly Arg Glu Leu Ile Tyr Asn
1 5

<210> 12
<211> 22
<212> PRT
<213> Homo sapiens

<400> 12

His Thr Val Ser Phe Thr Cys Glu Ser His Gly Phe Ser Pro Arg Asp
1 5 10 15

Ile Thr Leu Lys Trp Phe
20

<210> 13
<211> 7
<212> PRT
<213> Homo sapiens

<400> 13

Ala Arg Glu Leu Ile Tyr Asn
1 5

<210> 14
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain CDR1

<400> 14

Ser Tyr Trp Val His
1 5

<210> 15
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<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain CDR2

<400> 15

Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Asn Gln Lys Phe Lys
1 5 10 15

Asp

<210> 16
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain CDR2

<400> 16

Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Ser Pro Ser Phe Gln
1 5 10 15

Gly

<210> 17
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain CDR3

<400> 17

Gly Gly Thr Gly Thr Met Ala Trp Phe Ala Tyr
1 5 10

<210> 18
<211> 11
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<213> Artificial Sequence

<220>
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<400> 18

Gly Gly Thr Gly Thr Leu Ala Trp Phe Ala Tyr
1 5 10

<210> 19
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<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain CDR3

<400> 19

Gly Gly Thr Gly Thr Met Ala Tyr Phe Ala Tyr
1 5 10

<210> 20
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain CDR3

<400> 20

Gly Gly Thr Gly Thr Leu Ala Tyr Phe Ala Tyr
1 5 10

<210> 21
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Light chain CDR1

<400> 21

Arg Ser Ser Gln Ser Leu Val His Ser Tyr Gly Asn Thr Tyr Leu Tyr
1 5 10 15

<210> 22
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Light chain CDR2

<400> 22

Arg Val Ser Asn Arg Phe Ser
1 5

<210> 23
<211> 9

<212> PRT
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<400> 23

Phe Gln Gly Thr His Val Pro Tyr Thr
1 5

<210> 24
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<212> PRT
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<220>
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<400> 24

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Arg Pro Gly Ser
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Trp Val His Trp Val Lys Gln Arg Pro Ile Gln Gly Leu Glu Trp Ile
35 40 45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Lys Ala Ser Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Phe Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Val Arg Gly Gly Thr Gly Thr Met Ala Trp Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ala
115 120

<210> 25
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
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<400> 25

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Arg Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Trp Val His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Asp His Val Thr Leu Ser Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Leu Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Met Ala Trp Phe Ala Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 26
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain variable domain

<400> 26

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
            20                  25                  30

Trp Val His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Asp His Val Thr Leu Ser Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80
```

```
Leu Gln Leu Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Met Ala Trp Phe Ala Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 27
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain variable domain

<400> 27

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
            20                  25                  30

Trp Val His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Ser Pro Ser Phe
    50                  55                  60

Gln Gly His Val Thr Leu Ser Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Leu Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Met Ala Trp Phe Ala Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 28
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain variable domain

<400> 28

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
            20                  25                  30

Trp Val His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Ser Pro Ser Phe
    50                  55                  60

Gln Gly His Val Thr Leu Ser Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Leu Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Leu Ala Trp Phe Ala Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 29
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain variable domain

<400> 29

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
            20                  25                  30

Trp Val His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Ser Pro Ser Phe
    50                  55                  60

Gln Gly His Val Thr Leu Ser Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80
```

```
Leu Gln Leu Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Met Ala Tyr Phe Ala Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 30
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<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain variable domain

<400> 30

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
            20                  25                  30

Trp Val His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Ser Pro Ser Phe
    50                  55                  60

Gln Gly His Val Thr Leu Ser Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Leu Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Leu Ala Tyr Phe Ala Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 31
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Light chain variable domain

<400> 31

```
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1               5                  10                  15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
            20                  25                  30

Tyr Gly Asn Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Lys Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Phe Gln Gly
                85                  90                  95

Thr His Val Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
```

<210> 32
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Light chain variable domain

<400> 32

```
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1               5                  10                  15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
            20                  25                  30

Tyr Gly Asn Thr Tyr Leu Tyr Trp Tyr Gln Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Arg Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Phe Gln Gly
                85                  90                  95
```

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 33
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Light chain variable domain

<400> 33

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Tyr Gly Asn Thr Tyr Leu Tyr Trp Phe Gln Gln Arg Pro Gly Gln Ser
35 40 45

Pro Arg Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 34
<211> 327
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain constant domain-IgG4m-S228P

<400> 34

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

```
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
            100                 105                 110

Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
        115                 120                 125

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
    130                 135                 140

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145                 150                 155                 160

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
                165                 170                 175

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
            180                 185                 190

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
        195                 200                 205

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
    210                 215                 220

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225                 230                 235                 240

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
                245                 250                 255

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
            260                 265                 270

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
        275                 280                 285

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
    290                 295                 300
```

```
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305                 310                 315                 320

Leu Ser Leu Ser Pro Gly Lys
                325
```

<210> 35
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Light chain constant domain-CLkappa

<400> 35

```
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1               5                   10                  15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
            20                  25                  30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
        35                  40                  45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
    50                  55                  60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65                  70                  75                  80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
                85                  90                  95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105
```

<210> 36
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain sequence 18D5

<400> 36

```
Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Arg Pro Gly Ser
1               5                   10                  15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
```

```
            20                  25                  30

Trp Val His Trp Val Lys Gln Arg Pro Ile Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Asp Lys Ala Ser Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Phe Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Met Ala Trp Phe Ala Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
    210                 215                 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
            260                 265                 270
```

```
Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445


<210>  37
<211>  447
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Heavy chain sequence HA

<400>  37

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Arg Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Trp Val His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Ile
```

```
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Asp His Val Thr Leu Ser Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Leu Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Met Ala Trp Phe Ala Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
    210                 215                 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285
```

```
Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 38
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain sequence HB

<400> 38

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
            20                  25                  30

Trp Val His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Asn Gln Lys Phe
```

```
    50                  55                  60

Lys Asp His Val Thr Leu Ser Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Leu Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Met Ala Trp Phe Ala Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
    210                 215                 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300
```

```
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 39
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain sequence HC

<400> 39

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
            20                  25                  30

Trp Val His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Ser Pro Ser Phe
    50                  55                  60

Gln Gly His Val Thr Leu Ser Val Asp Lys Ser Ile Ser Thr Ala Tyr
```

```
65                  70                  75                  80

Leu Gln Leu Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Met Ala Trp Phe Ala Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
    210                 215                 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320
```

```
Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 40
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain sequence HE

<400> 40

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
            20                  25                  30

Trp Val His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Ser Pro Ser Phe
    50                  55                  60

Gln Gly His Val Thr Leu Ser Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Leu Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
```

```
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Leu Ala Trp Phe Ala Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
    210                 215                 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335
```

```
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 41
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain sequence HF

<400> 41

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
            20                  25                  30

Trp Val His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Ser Pro Ser Phe
    50                  55                  60

Gln Gly His Val Thr Leu Ser Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Leu Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Met Ala Tyr Phe Ala Tyr Trp Gly Gln
```

```
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
    210                 215                 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350
```

```
Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 42
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<223> Heavy chain sequence HEF

<400> 42

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1               5                   10                  15

Ser Leu Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser Tyr
            20                  25                  30

Trp Val His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Asn Ile Asp Pro Ser Asp Ser Asp Thr His Tyr Ser Pro Ser Phe
    50                  55                  60

Gln Gly His Val Thr Leu Ser Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Leu Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Val Arg Gly Gly Thr Gly Thr Leu Ala Tyr Phe Ala Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
```

```
        115                 120                 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
    210                 215                 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
            260                 265                 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365
```

```
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 43
<211> 219
<212> PRT
<213> Artificial Sequence

<220>
<223> Light chain sequence 18D5

<400> 43

```
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1               5                   10                  15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
            20                  25                  30

Tyr Gly Asn Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Lys Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Phe Gln Gly
                85                  90                  95

Thr His Val Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
```

```
    130                 135                 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165                 170                 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215


<210>  44
<211>  219
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Light chain sequence LA

<400>  44

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1               5                   10                  15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
            20                  25                  30

Tyr Gly Asn Thr Tyr Leu Tyr Trp Tyr Gln Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Arg Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Phe Gln Gly
                85                  90                  95

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125
```

```
Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130                 135                 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165                 170                 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215


<210>  45
<211>  219
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Light chain sequence LB

<400>  45

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1               5                   10                  15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
            20                  25                  30

Tyr Gly Asn Thr Tyr Leu Tyr Trp Phe Gln Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Arg Leu Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
                85                  90                  95

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110
```

```
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130                 135                 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165                 170                 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 46
<211> 360
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleic acid sequence coding the heavy chain variable domain 18D5

<400> 46
caggtgcagc tgcagcagcc aggagctgag ctggtgaggc ctggctccag cgtgaagctg 60

tcctgcaagg ctagcggcta caccttcaca agctattggg tgcactgggt gaagcagcgg 120

ccaatccagg gcctggagtg gatcggcaac atcgacccca gcgactctga tacccattac 180

aatcagaagt ttaaggacaa ggcctctctg accgtggata agtcttccag cacagcttat 240

atgcagctgt cttccctgac attcgaggat tccgccgtgt actattgcgt gaggggagga 300

accggaacaa tggcttggtt tgcttactgg ggccagggca ccctggtgac agtgtctgct 360

<210> 47
<211> 360
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleic acid sequence coding the heavy chain variable domain HA

<400> 47
gaggtgcagc tggtgcagag cggagctgag gtgaagaagc caggcgagtc tctgaggatc 60

tcctgcaagg ctagcggcta caccttcaca tcttattggg tgcactgggt gcggcagatg 120

ccaggcaagg gcctggagtg gatcggcaac atcgacccta gcgactctga tacccactac 180

aatcagaagt ttaaggacca tgtgaccctg tctgtggata agtccatcag cacagcctat 240

ctgcagctgt ccagcctgaa ggcctccgat acagctatgt actattgcgt gaggggagga 300

accggaacaa tggcttggtt cgcttactgg ggccagggca ccctggtgac agtgtcttcc 360

<210> 48
<211> 360
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleic acid sequence coding the heavy chain variable domain HB

<400> 48
gaggtgcagc tggtgcagtc cggagctgag gtgaagaagc caggcgagtc tctgaggatc 60

tcctgcaagg cttctggcta ctccttcacc agctattggg tgcactgggt gcggcagatg 120

ccaggcaagg gcctggagtg gatgggcaac atcgacccta gcgactctga tacacactac 180

aatcagaagt ttaaggacca tgtgaccctg agcgtggata agtccatcag cacagcctat 240

ctgcagctgt ccagcctgaa ggcctctgat accgctatgt actattgcgt gaggggagga 300

accggaacaa tggcttggtt cgcttactgg ggccagggca ccctggtgac agtgtcttcc 360

<210> 49
<211> 360
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleic acid sequence coding the heavy chain variable domain HC

<400> 49
gaggtgcagc tggtgcagtc tggcgccgag gtgaagaagc caggcgagag cctgaggatc 60

tcttgcaagg ctagcggcta ctctttcacc tcctattggg tgcactgggt gcggcagatg 120

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PA 7631754

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APPLICATION NUMBER: *62/322,707*
FILING DATE: *April 14, 2016*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US62/322,707*

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EFS ID:	25498265
Application Number:	62322707
International Application Number:	
Confirmation Number:	1419
Title of Invention:	Human SIRPA Antagonist Antibodies and their Therapeutic Applications
First Named Inventor/Applicant Name:	Nicolas Poirier
Customer Number:	9629
Filer:	Robert John Smyth
Filer Authorized By:	
Attorney Docket Number:	045636-5291
Receipt Date:	14-APR-2016
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RAM confirmation Number	6046
Deposit Account	500310
Authorized User	SMYTH, ROBERT J.

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Paris, May 4, 2017

International Procedure N° **PCT/EP2017/059071 filed on April 14, 2017**
In the name of : OSE IMMUNOTHERAPEUTICS.
Title: «NEW ANTI-SIRPa ANTIBODIES AND THEIR THERAPEUTIC APPLICATIONS»
Invention : POIRIER Nicolas, MARY Caroline, VANHOVE Bernard, GAUTTIER Vanessa, THEPENIER Virginie, PENGAM Sabrina

Y/Ref. : PCT/EP2017/059071 *O/Ref. ERBI/CLBE/safl - F217300008/WO/PCT*

Dear Sirs,

Please find herein enclosed:

The United States Priority Document **No. 62/322,707**
filed on **April 14, 2017**

for the regularization of formal requirements regarding the above-referred application.

Yours faithfully,

C. BERNSTEIN

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Provisional Cover Sheet (SB16)	045636-5291coversheet041416.pdf	84700 6d1f69c913e57c2da5d2abae938ac6cd249db0e8	no	2
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Information:					
2		045636-5291app041416.pdf	427851 7f53de0c6e7b14cae50ac05c7e1385651da06934	yes	50

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Specification	1	45
Claims	46	50

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3	Drawings-only black and white line drawings	045636-5291figs041416.pdf	292941 39bcdcb238e3a0de0ffc54bfc47f5a3fe3c92e25	no	4
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4	Application Data Sheet	045636-5291ads041416.pdf	114533 265459865009961a37cfd8a3ecd5b13ddf72cf6f2	no	7
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5	Fee Worksheet (SB06)	fee-info.pdf	29514 5f80284ba8e5c3b68d1093478d0f5f86bea9822b	no	2
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Doc Code: **TR.PROV**
Document Description: Provisional Cover Sheet (SB16)

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Provisional Application for Patent Cover Sheet

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

Inventor(s)

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Vanessa		Gauttier	Nantes		FR

All Inventors Must Be Listed – Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. — Add

Title of Invention	Human SIRPA Antagonist Antibodies and their Therapeutic Applications
Attorney Docket Number (if applicable)	045636-5291

Correspondence Address

Direct all correspondence to (select one):

◉ The address corresponding to Customer Number	○ Firm or Individual Name
Customer Number	09629

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

- ◉ No.
- ○ Yes, the invention was made by an agency of the United States Government. The U.S. Government agency name is:
- ○ Yes, the invention was under a contract with an agency of the United States Government. The name of the U.S. Government agency and Government contract number are:

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Entity Status
Applicant asserts small entity status under 37 CFR 1.27 or applicant certifies micro entity status under 37 CFR 1.29

- ○ Applicant asserts small entity status under 37 CFR 1.27
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- ◉ No

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Signature

Please see 37 CFR 1.4(d) for the form of the signature.

Signature	/robert smyth/			Date (YYYY-MM-DD)	2016-04-14
First Name	Robert	Last Name	Smyth	Registration Number (If appropriate)	50801

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NEW HUMAN SIRPA ANTAGONIST ANTIBODIES AND THEIR THERAPEUTIC APPLICATIONS

The invention pertains to the field of immunotherapy. The present invention provides new anti-SIRPa antibodies able to antagonize the interaction between SIRPa and CD47. These antibodies are (i) able to induce the differentiation of monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells and (ii) able to inhibit M2 phenotypic polarization of macrophages and/or favors pro-inflammatory M1-type macrophage function. The invention also concerns the use of the antibodies of the invention for the treatment of diseases wherein immune modulation is involved such as cancer, infectious disease, vaccination, trauma, autoimmune diseases, chronic inflammatory diseases and transplantation.

BACKGROUND

Myeloid derived suppressor cells (MDSC)

Myeloid-derived suppressor cells (MDSC) are a group of heterogeneous cells of immature hematopoietic myeloid-cell progenitors, exerting immunosuppressive functions and therefore negatively regulating immune responses (Gabrilovich and Nagaraj, 2009; Talmadge and Gabrilovich, 2013). So far, three main MDSC populations have been phenotypically and functionally characterized: pro-myelocytic MDSC, monocytic MDSC (M-MDSC or Mo-MDSC) and polymorphonuclear (also called granulocytic) MDSC (PMN-MDSC or G-MDSC). Mo-MDSC, characterized by the $CD11b^+$ $Ly6C^+$ $Ly6G^-$ phenotype in mice and $CD11b^+$ $CD33^+$ $HLA\text{-}DR^{-/low}$ $CD14^+$ phenotype in humans, are the most potent immunosuppressive MDSC population, function at least by expressing nitrite oxide synthase (iNOS) and arginase (ARG1) enzymes, and are abundant in the tumor microenvironment. Pro-myelocytic MDSC resemble Mo-MDSC but do not express the CD14 marker, suggesting a more immature state as compared to Mo-MDSC (Diaz-Montero et al., 2014). PMN-MDSC, characterized by the $CD11b^+$ $Ly6C^{low}$ $Ly6G^+$ phenotype in mice and $CD11b^+$ $CD33^+$ $HLA\text{-}DR^-$ $CD15^+$ phenotype in humans, are more predominant in the periphery and lymphoid organs, and function mainly by producing reactive oxygen species (ROS) (Solito et al., 2014).

MDSC are now considered as key cells expanding in pathological situations and preventing adequate immune responses and are associated with significant morbidities and co-morbidities in a large number of diseases. In particular, the levels of MDSC have been correlated with cancer stages severity, metastasis in numerous types of cancers. Further, MDSC accumulation has also been documented in non-cancer settings, especially in a variety of infectious diseases. They are for example involved in the evasion of host immunity employed by pathogens to establish persistence

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and chronic infection strikingly parallels mechanisms of tumor escape. MDSC have also been shown to play a significant deleterious role in vaccination, both against pathogens or tumor antigen.

Based on these numerous clinical associations and key role played by MDSC in these diverse pathologies independently of their etiology and physiology, pharmaceutical approaches modulating MDSC in order to prevent or promote their accumulation now represent a growing field of interest. However, to date, there is no therapeutic approach allowing efficient MDSC modulation without adverse secondary effects. In fact, contrary to the majority of other cellular populations, MDSC in humans do not express specific markers allowing simply direct and specific targeting of these cells.

It was recently found out that chemotherapeutical drugs such as gemcitabine, 5-fluorouracil, docetaxel or 2-hydroxy acetophenone glycinate can actually induce apoptosis of MDSC. However, their efficacy to eliminate MDSC appears limited and such drugs also induce undesired apoptosis of other cells and are therefore associated with known toxicities. Furthermore, this approach faces the problem that inflammation confers MDSC resistance to apoptosis (Hu et al., 2013; Ostrand-Rosenderg et al. 2012).

In this context, the inventors have shown that MDSC could differentiate into a novel and unexpected population of non-suppressive cells having a cytotoxic NK cell phenotype. Interestingly, they also identified that the signal regulatory protein alpha (SIRPa) tightly controls this previously unidentified MDSC road of differentiation (data not yet published). Thus, an antagonist of SIRPa induces the differentiation of myeloid-derived suppressor cells into non suppressive cells and can be used in therapeutic.

Macrophages

Macrophages are cells that have the highest plasticity of the hematopoietic system. They are involved both in innate immunity (phagocytosis capacity) and in adaptive immunity (cell polarization), but also in ontogeny, in homeostasis and in tissue repair (Mantavani et al., 2013; WYNN et al., 2013). Macrophages are present in all tissues. They have a large phenotypic and functional diversity. During ontogeny, these cells also exhibit a diversity of origins which persists into adulthood. In the tissues, monocytes-macrophages respond to environmental stimuli (product from microbial infection, damaged cells, activated lymphocytes) and acquire distinct phenotypes.

Depending on stimuli monocyte-macrophage received, they reprogram their transcriptome, resulting in distinct functional and phenotypic spectra. Macrophages are categorized simplistically into 2 sub-populations or states of polarization (or activation): classical activation phenotype M1 and the alternative activation phenotype M2 (Gabrilovich et al. 2012). The M1 classification is associated

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in vitro with the use of IFNg factor alone or in combination with microbial factors such as LPS or inflammatory cytokines such as TNF and GM-CSF. The polarization M2 is rather associated with the IL4 or IL13 (Stein et al., 1992). Other cytokines are also identified as inducing M2 type polarization such as IL33, which induces overexpression of Arg1 (arginase 1), CCL24 or CCL17 playing a role in inflammation. The IL21 and more commonly CSF1 are major players in the polarization of macrophages. Macrophages may also acquire the status of "M2-like", sharing common characteristics of M2. In fact, a large number of stimuli such as immune complexes associated with LPS, IL-1, glucocorticoids, TGF beta, Wnt5a and IL10 result in a functional phenotype of type "M2-like".

Similarly, *in vivo* studies have shown the existence of M1, M2 and M2-like macrophages. These subtypes represent only the extremes on a continuum of functional states that must be integrated in an environmental complex system.

Generally, M1 macrophages present $IL12^{high}$, $IL23^{high}$ and $IL10^{low}$ phenotype and produce molecular effectors such as reactive oxygen species (ROS) and intermediates of Nitric Oxide (NO) and inflammatory cytokines (IL1b, TNFa, IL-6). M1 macrophages participate in Th1 responses, play a role in resistance against intracellular parasites and are key effectors in the elimination of tumor cells. In contrast, M2 macrophages have an $IL12^{low}$, $IL\text{-}23^{low}$ and $IL10^{high}$ phenotype with a variability in the production of inflammatory cytokines according to stimuli present in the environment. M2 cells display on their surface a strong expression of scavenger, mannose and galactose-type receptors.

M1 and M2 also have distinct expression profiles of chemokines. M1 macrophages express CXCL9 and CXCL10 chemokines which are known for attracting Th1, while M2 macrophages express CCL17, CCL22 and CCL24. Chemokines such as CCL2 and CXCL4 can also polarize macrophages to an M2-like phenotype.

Macrophages are found in large numbers in tumors where they participate to the tumor initiation and progression as well as to the metastatic process. During the tumor process, macrophages secrete pro-inflammatory cytokines such as IFNγ, TNFα, IL12 and IL6, attracting other immune cells creating chronic inflammation and causing the initiation and tumor progression. However, once the tumor installed, tumor macrophages (TAMs) adopt an immunosuppressive cellular profile and are less active, allowing tumor growth and transition to malignancy. TAMs are responsible for migration, extravasation and invasion of tumor cells (metastasis) and are involved in tumor angiogenesis (Qian and Pollard, 2010; DeNardo et al. 2010; Hanahan and Coussens, 2012).

In this context, the inventors have previously demonstrated that SIRPa acts as a new checkpoint inhibitor and participates to macrophage polarization. They have in particular shown that blocking SIRPa induces a pro-inflammatory function of macrophages associated to type 1

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macrophages (M1 pro-inflammatory = M (IFNg)) and inhibits the suppressive activity of macrophages in the tumor, since the pro-inflammatory profile of macrophages is obtained at the expense of type 2 macrophages (M2 type high phagocytic activity = M (IL4)). This effect is obtained by targeting SIRPa rather than CD47, which is involved in phagocytosis function. Thus, an antagonist of SIRPa is able to inhibit M2 phenotypic polarization of macrophages and/or favors pro-inflammatory M1-type macrophage function and can be used in therapeutic.

SIRPa

Signal regulatory protein alpha, or SIRPa (also termed CD172a or SHPS-1) was first identified as a membrane protein present mainly on macrophages and myeloid cells that was associated with the Src homology region 2 (SH2) domain–containing phosphatases—SHP-1 and SHP-2. SIRPa is the prototypic member of the SIRP paired receptor family of closely related SIRP proteins.

The gene coding for human SIRPa is a polymorphic gene. Several variants were described by Takenaka et al. in Human population. The most common protein variants are Sirpa v1 and v2 (accession numbers NP_542970 (P78324) and CAA71403) (Takenaka et al., 2007). The polymorphisms in human SIRP lead to changes in surface-exposed amino acids, but this does not affect binding to CD47 (Hatherley et al., 2014).

Engagement of SIRPa by CD47 provides a downregulatory signal that inhibits host cell phagocytosis, and CD47 therefore functions as a "don't-eat-me" signal.

SIRPa is expressed on monocytes, most subpopulations of tissue macrophages, granulocytes, subsets of dendritic cells (DCs) in (lymphoid) tissues, some bone marrow progenitor cells, and to varying levels on neurons, with a notably high expression in synapse-rich areas of the brain, such as the granular layer of the cerebellum and the hippocampus (Seiffert et al, 1994; Adams et al, 1998; Milling et al, 2010).

The SIRPa interaction with CD47 is largely described and provides a downregulatory signal that inhibits host cell phagocytosis (see review Barclay et al, Annu. Rev. Immunol., 2014). Both CD47 and SIRPa also engage in other interactions. Investigators have suggested that the lung surfactant proteins SP-A and SP-D control inflammatory responses in the lung through interactions with SIRPa (Janssen et al, 2008).

One of the best characterized physiological functions of CD47-SIRPa interactions is their role in the homeostasis of hematopoietic cells, in particular red blood cells and platelets. Because CD47 serves as a don't-eat-me signal and, as such, is an important determinant of host cell phagocytosis by macrophages, the potential contribution of CD47-SIRPa interactions in cancer cell clearance has been intensely investigated in recent years.

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The SIRPa/CD47 pathway is nowadays also subject to different pharmaceutical developments to enhance macrophages phagocytosis. In fact, like infected cells, cancer cells carry aberrant cargo such as unfamiliar proteins or normal proteins at abnormal levels, yet these cells frequently subvert innate immune control mechanisms by concurrently over-expressing immunoregulatory molecules. It is becoming increasingly clear that one such mechanism involves CD47 (Barclay and Van den Berg, 2014), a protein of "self" expressed by normal cells. CD47 interacts with SIRPa. This leads to the transmission of a "don't eat me" signal to phagocytic macrophages, which then leave target cells unaffected (Oldenborg et al., 2000). Over-expression of CD47 by cancer cells renders them resistant to macrophages, even when the cancer cells are coated with therapeutic antibodies (Zhao et al., 2011), and correlates with poor clinical outcomes in numerous solid and hematological cancers (Majeti et al., 2009; Willingham et al., 2012). In experimental models, in particular human tumor-xenograft models in immunodeficient mice, blockade of the CD47/SIRPa pathway was very effective to promote tumor elimination by macrophages and to decrease cancer cell dissemination and metastasis formation (Chao et al., 2011; Edris et al., 2012; Uluçkan et al., 2009; Wang et al., 2013). In these studies, MDSC function or phenotype has not been studied. Blockade of the CD47/SIRPa pathway, by enhancing antibody-dependent phagocytosis by macrophages, has been described to synergize with depleting therapeutic anticancer antibodies (Weiskopf et al., 2013) such as Trastuzumab (anti-Her2), Cetuximab (anti-EGFR), Rituximab (anti-CD20) and Alemtuzumab (anti-CD52).

SIRPa antagonist antibodies and their uses

A few prior art documents disclose anti-SIRPa antibodies able to antagonize the interaction between SIPRa and CD47 and their use in the treatment of particular diseases. For example, the patent application WO2013056352 relates to the use of anti-SIRPa antibodies to treat hematological cancer. These antibodies were screened for their binding to both variants v1 and v2 of SIRPa and are antagonists of SIRPa-CD47 interaction. The authors showed that the anti-SIRPa antibodies of the invention are able to inhibit human primary AML (Acute Myeloid Leukemia) into NSG mouse. Another patent application number WO2015138600 describes anti-SIRPa antibodies and bi-specific macrophage enhancing antibodies. These antibodies bind to human SIRPa and block the interaction of CD47 expressed on a target cell of interest with SIRPs expressed on a phagocytic cell. However, none of these documents discloses an anti-SIRPa antibody able to modify macrophage polarization to favor pro-inflammatory macrophages. Furthermore, none of them describes an anti-SIRPa antibody able to induce the differentiation of MDSC into non suppressive cells.

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There is still a need for anti-SIRPa antibodies able to modulate the SIRPa-CD47 pathway, in particular able to inhibit this interaction for modifying the behavior of macrophages and/or MDSC in pathological conditions.

Here, the inventors provide new anti-SIRPa antibodies, in particular humanized antibodies, which have been selected on their ability to modify macrophage polarization especially in favor of M1 pro-inflammatory macrophages and/or to induce the differentiation of MDSC into non-suppressive cells. These antibodies selected on the basis of these new characteristics are particularly promising for numerous therapeutic applications. They should be for example useful in the treatment of cancer, infectious diseases, vaccination, trauma, autoimmune diseases, chronic inflammatory diseases and transplantation.

SUMMARY OF THE INVENTION

In a first aspect, the invention concerns an anti-SIRPa antibody or antigen-binding fragment thereof. In a particular embodiment, said antibody is defined by the sequences of its CDR, or the sequences of both its CDR and its FR regions.

In another particular embodiment, said antibody is defined by its functional characteristics. In a preferred embodiment, said antibody is defined by its ability to inhibit M2 phenotypic polarization of macrophages and/or to favor pro-inflammatory M1-type macrophage function. In another preferred embodiment, said antibody is defined by its ability to induce the differentiation of MDSC into non suppressive cells, and more preferably of Mo-MDSC into non suppressive cells.

In another aspect, the invention concerns a composition comprising an anti-SIRPa antibodies or functional fragments thereof. In a particular embodiment, the antibodies or functional fragments thereof of the composition are a homogeneous population of antibodies or functional fragments thereof or are monoclonal antibodies or functional fragments thereof. In another particular embodiment, the composition is pharmaceutical composition comprising an antibody or an antigen-binding fragment thereof of the invention, which may further contain an additional therapeutic agent. In yet another embodiment, the additional agent is an immunomodulatory agent, in particular an immune checkpoint blocker or activator.

In a further aspect, the invention concerns the therapeutic use of the anti-SIRPa antibodies of the invention, especially for inducing the differentiation of Mo-MDSC into non suppressive cells and/or for modulating the macrophage polarization, in view of treating diseases wherein immune response is involved.

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DETAILLED DESCRIPTION OF THE INVENTION

The invention relates to new anti-SIRPa antibodies able to antagonize the interaction between SIRPa and CD47.

As used herein, the term "antibody" comprises polyclonal antibodies, monoclonal antibodies or recombinant antibodies.

As used herein, a "monoclonal antibody" is intended to refer to a preparation of antibody molecules, antibodies which share a common heavy chain and common light chain amino acid sequence, in contrast with "polyclonal" antibody preparations which contain a mixture of antibodies of different amino acid sequence. Monoclonal antibodies can be generated by several known technologies like phage, bacteria, yeast or ribosomal display, as well as by classical methods exemplified by hybridoma-derived antibodies. Thus, the term "monoclonal" is used to refer to all antibodies derived from one nucleic acid clone.

The monoclonal antibodies of the present invention include recombinant antibodies. As used herein, the term "recombinant antibody" refers to antibodies which are produced, expressed, generated or isolated by recombinant means, such as antibodies which are expressed using a recombinant expression vector transfected into a host cell; antibodies isolated from a recombinant combinatorial antibody library; antibodies isolated from an animal (e.g. a mouse) which is transgenic due to human immunoglobulin genes (see, for example, (Taylor et al., 1992)); or antibodies which are produced, expressed, generated or isolated in any other way in which particular immunoglobulin gene sequences (such as human immunoglobulin gene sequences) are assembled with other DNA sequences. Recombinant antibodies include, for example, chimeric and humanized antibodies.

As used herein, an "antigen-binding fragment of an antibody" means a part of an antibody, i.e. a molecule corresponding to a portion of the structure of the antibody of the invention that exhibits antigen-binding capacity for SIRPa, possibly in its native form; such fragment especially exhibits the same or substantially the same antigen-binding specificity for said antigen compared to the antigen-binding specificity of the corresponding four-chain antibody. Advantageously, the antigen-binding fragments have a similar binding affinity as the corresponding 4-chain antibodies. However, antigen-binding fragment that have a reduced antigen-binding affinity with respect to corresponding 4-chain antibodies are also encompassed within the invention. The antigen-binding capacity can be determined by measuring the affinity between the antibody and the target fragment. These antigen-binding fragments may also be designated as functional fragments of antibodies. Antigen-binding fragments of antibodies are fragments which comprise their hypervariable domains

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designated CDRs (Complementary Determining Regions) or part(s) thereof encompassing the recognition site for the antigen, i.e. the extracellular domain of SIRPa, thereby defining antigen recognition specificity. Each Light and Heavy chain (respectively VL and VH) of a four-chain immunoglobulin has three CDRs, designated VL-CDR1, VL-CDR2, VL-CDR3 and VH-CDR1, VH-CDR2, VH-CDR3, respectively.

The skilled person is able to determine the location of the various regions/domains of antibodies by reference to the standard definitions in this respect set forth, including a reference numbering system [Martin, A.C.R. (2001) Protein Sequence and Structure Analysis of Antibody Variable Domains. In: Antibody Engineering Lab Manual, ed.: Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg] or by reference to the numbering system of Kabat (Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of
Health and Human Services, NIH, 1987) or by application of the IMGT "collier de perle" algorithm (http://www.imgt.org/IMGTindex/Colliers.html). In this respect, for the definition of the sequences of the invention, it is noted that the delimitation of the regions/domains may vary from one reference system to another. Accordingly, the regions/domains as defined in the present invention encompass sequences showing variations in length or localization of the concerned sequences within the full-length sequence of the variable domains of the antibodies, of approximately +/- 10%.

Based on the structure of four-chain immunoglobulins, antigen-binding fragments can thus be defined by comparison with sequences of antibodies in the available databases and prior art (Martin A.C.R. et al), and especially by comparison of the location of the functional domains in these sequences, noting that the positions of the framework and constant domains are well defined for various classes of antibodies, especially for IgGs, in particular for mammalian IgGs. Such comparison also involves data relating to 3-dimensional structures of antibodies.

For illustration purpose of specific embodiments of the invention, antigen binding fragments of an antibody that contain the variable domains comprising the CDRs of said antibody encompass Fv, dsFv, scFv, Fab, Fab', F(ab')2 which are well defined with reference to Kabat (NIH 1987), Martin A.C.R. et al and also Roitt I. et al (Fundamental and Applied Immunology MEDSI/McGraw-Hill). Fv fragments consist of the VL and VH domains of an antibody associated together by hydrophobic interactions; in dsFv fragments, the VH:VL heterodimer is stabilised by a disulphide bond; in scFv fragments, the VL and VH domains are connected to one another via a flexible peptide linker thus forming a single-chain protein. Fab fragments are monomeric fragments obtainable by papain digestion of an antibody; they comprise the entire L chain, and a VH-CH1 fragment of the H chain, bound together through a disulfide bond. The F(ab')2 fragment can be produced by pepsin digestion of an antibody below the hinge disulfide; it comprises two Fab' fragments, and additionally a portion

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of the hinge region of the immunoglobulin molecule. The Fab' fragments are obtainable from F(ab')2 fragments by cutting a disulfide bond in the hinge region. F(ab')2 fragments are divalent, i.e. they comprise two antigen binding sites, like the native immunoglobulin molecule; on the other hand, Fv (a VHVL dimmer constituting the variable part of Fab), dsFv, scFv, Fab, and Fab' fragments are monovalent, i.e. they comprise a single antigen-binding site (For review see Chan and Carter, 2010). These basic antigen-binding fragments of the invention can be combined together to obtain multivalent antigen-binding fragments, such as diabodies, tribodies or tetrabodies. These multivalent antigen-binding fragments are also part of the present invention.

As used herein, the term "bispecific" antibodies (also known as bifunctional antibodies) refers to antibodies that recognize two different antigens by virtue of possessing at least one region (e.g., derived from a variable region of a first antibody) that is specific for a first antigen, and at least a second region (e.g., derived from a variable region of a second antibody) that is specific for a second antigen. A bispecific antibody specifically binds to two target antigens and is thus one type of multispecific antibody. Multispecific antibodies, which recognize two or more different antigens, can be produced by recombinant DNA methods or include, but are not limited to, antibodies produced chemically by any convenient method. Bispecific antibodies include all antibodies or conjugates of antibodies, or polymeric forms of antibodies which are capable of recognizing two different antigens. Bispecific antibodies include antibodies that have been reduced and reformed so as to retain their bivalent characteristics and to antibodies that have been chemically coupled so that they can have several antigen recognition sites for each antigen such as BiME (Bispecific Macrophage Enhancing antibodies), BiTE (bispecific T cell engager), DART (Dual affinity retargeting); DNL (dock-and-lock), DVD-Ig (dual variable domain immunoglobulins), HAS (human serum albumin), kih (knobs into holes).

Accordingly, bispecific antibodies of the invention are directed against SIRPa and a second antigen. Exemplary bispecific antibodies include those targeting a combination of SIRPa and a cancer cell marker, such as CD19, CD20, CD22, CD24, CD25, CD30, CD33, CD38, CD44, CD52, CD56, CD70, CD96, CD97, CD99, CD123, CD279 (PD-1), EGFR, HER2, CD117, C-Met, PTHR2 and HAVCR2 (TIM3).

Several researches to develop therapeutic antibodies had led to engineer the Fc regions to optimize antibody properties allowing the generation of molecules that are better suited to the pharmacology activity required of them (Strohl, 2009). The Fc region of an antibody mediates its serum half-life and effector functions, such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP). Several mutations located at the interface between the CH2 and CH3 domains, such as T250Q/M428L (Hinton et al., 2004) and M252Y/S254T/T256E + H433K/N434F (Hinton et al., 2004), have been shown to increase the binding affinity to FcRn and the half-life of IgG1 in vivo. However, there is not

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always a direct relationship between increased FcRn binding and improved half-life (Datta-Mannan et al., 2007). One approach to improve the efficacy of a therapeutic antibody is to increase its serum persistence, thereby allowing higher circulating levels, less frequent administration and reduced doses. Engineering Fc regions may be desired to either reduce or increase the effector function of the antibody. For antibodies that target cell-surface molecules, especially those on immune cells, abrogating effector functions is required. Conversely, for antibodies intended for oncology use, increasing effector functions may improve the therapeutic activity. The four human IgG isotypes bind the activating Fcγ receptors (FcγRI, FcγRIIa, FcγRIIIa), the inhibitory FcγRIIb receptor, and the first component of complement (C1q) with different affinities, yielding very different effector functions. Binding of IgG to the FcγRs or C1q depends on residues located in the hinge region and the CH2 domain. Two regions of the CH2 domain are critical for FcγRs and C1q binding, and have unique sequences in IgG2 and IgG4 (Armour et al., 1999) (Shields et al., 2001) (Idusogie et al., 2000) (Steurer et al., 1995) (Lazar et al., 2006) (Ryan et al., 2007) (Richards et al., 2008) (Labrijn et al., 2009).

Methods for preparing recombinant antigen-binding fragments, or chimeric antibodies by combining the variable regions of an antibody with appropriate linkers, or with the constant regions of another antibody, are well known in the art. The antibodies of the invention are said to be monoclonal antibodies, meaning that a composition of these antibodies is homogeneous, especially identical, in terms of antigen-binding specificity and accordingly in terms of variable region composition.

As used herein, a "chimeric molecule" corresponds to a molecule comprising an antibody according or an antigen-binding fragment thereof, wherein said monoclonal antibody or functional fragment thereof is associated with a functionally different molecule. A chimeric molecule of the invention may be either a fusion chimeric protein or a conjugate resulting from any suitable form of attachment including covalent attachment, grafting, chemical bonding with a chemical or biological group or with a molecule, such as a PEG polymer or another protective group or molecule suitable for protection against proteases cleavage *in vivo*, for improvement of stability and/or half-life of the antibody or functional fragment. With similar techniques, especially by chemical coupling or grafting, a chimeric molecule can be prepared with a biologically active molecule said active molecule being for example chosen among toxins, in particular Pseudomonas exotoxin A (Risberg et al, PLoS One. 2011;6(9):e24012), the A-chain of plant toxin ricin (Van Oosterhout et al, Int J Pharm. 2001 Jun 19;221(1-2):175-86.) or saporin toxin (Flavell et al, Br J Haematol. 2006 Jul;134(2):157-70)), especially a therapeutic active ingredient, a vector (including especially a protein vector) suitable for targeting the antibody or functional fragment to specific cells or tissues of the human body, or it may be associated with a label or with a linker, especially when fragments of the antibody are used.

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PEGylation of the antibody or functional fragments thereof is a particular interesting embodiment as it improves the delivery conditions of the active substance to the host, especially for a therapeutic application. PEGylation can be site specific to prevent interference with the recognition sites of the antibodies or functional fragments, and can be performed with high molecular weight PEG. PEGylation can be achieved through free cysteine residues present in the sequence of the antibody or functional fragment or through added free Cysteine residues in the amino sequence of the antibody or functional fragment.

A first object of the invention consists in an anti-SIRPa antibody or antigen-binding fragment thereof comprising a heavy chain which comprises a heavy chain comprising CDR1, CDR2 and CDR3, and a light chain comprising 3 CDRs chosen among CDR4, CDR5, CDR6, CDR7, CDR8 and CDR9, wherein said CDRs are defined as follows:

- CDR1 comprises the amino acid sequence SYX$_1$VH wherein X$_1$ is W, Y or F (SEQ ID NO. 81);
- CDR2 comprises the amino acid sequence NIDPSDSDTHYX$_2$X$_3$X$_4$FX$_5$X$_6$ where X$_2$ is N, A or S; X$_3$ is Q or P; X$_4$ is K or S; X$_5$ is K or Q and X$_6$ is D or G (SEQ ID NO. 82);
- CDR3 comprises the amino acid sequence GGTGTX$_7$A X$_8$FAY where X$_7$ is M, L, F or I and X$_8$ is W or Y or F or L (SEQ ID NO. 83);
- CDR4 comprises the amino acid sequence RSSQSLVHSYGNX$_9$YLX$_{10}$ wherein X$_9$ is T or S and X$_{10}$ is Y or N (SEQ ID NO. 84);
- CDR5 comprises the amino acid sequence X$_{11}$VS X$_{12}$ X$_{13}$ X$_{14}$ X$_{15}$, wherein X$_{11}$ is R, K, A or D; X$_{12}$ is N or S; X$_{13}$ is R or L; X$_{14}$ is F, D, Q or A and X$_{15}$ is S or T (SEQ ID NO. 85);
- CDR6 comprises the amino acid sequence X$_{16}$Q X$_{17}$ X$_{18}$ X$_{19}$ X$_{20}$P X$_{21}$T, wherein X$_{16}$ is F or Q; X$_{17}$ is G, S or R; X$_{18}$ is T, Y or S; X$_{19}$ is H, S or N, X$_{20}$ is V or T and X$_{21}$ is Y or L (SEQ ID NO. 86);
- CDR7 comprises the amino acid sequence X$_{22}$ASQX$_{23}$ X$_{24}$ X$_{25}$ X$_{26}$ X$_{27}$ X$_{28}$A wherein X$_{22}$ is K or R; X$_{23}$ is Y, G or S; X$_{24}$ is V or I; X$_{25}$ G or S; X$_{26}$ is T, N or S, X$_{27}$ is Y or N and X$_{28}$ is V or L (SEQ ID NO. 87);
- CDR8 comprises the amino acid sequence X$_{29}$ASX$_{30}$ X$_{31}$ X$_{32}$ X$_{33}$, wherein X$_{29}$ is S, A, D or G, X$_{30}$ is Y, S, N or T; X$_{31}$ is R or L; X$_{32}$ is H, Q or A and X$_{33}$ is T or S (SEQ ID NO. 88);
- CDR9 comprises the amino acid sequence QQX$_{34}$SSX$_{35}$PYT wherein X$_{34}$ is Y or R and X$_{35}$ is S or Y (SEQ ID NO. 89).

In a particular embodiment, the CDRs of the light chain correspond whether to CDR4, CDR5 and CDR6 or to CDR7, CDR8 and CDR9. Accordingly, a preferred antibody of the invention contains 6 CDRs which are CDR1, CDR2, CDR3, CDR4, CDR5, CDR6 or CDR1, CDR2, CDR3, CDR7, CDR8, CDR9.

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In a certain embodiment, the CDRs of the heavy chain present particular sequences which may be combined as described:

a) A CDR1 sequence corresponding to SYWVH (SEQ ID NO.1); a CDR2 sequence corresponding to NIDPSDSDTHYNQKFKD (SEQ ID NO.2) and a CDR3 sequence corresponding to GGTGTMAWFAY (SEQ ID NO.3), or

b) A CDR1 sequence corresponding to SYYVH (SEQ ID NO.10); a CDR2 sequence corresponding to NIDPSDSDTHYAQKFQG (SEQ ID NO.11) and a CDR3 sequence corresponding to GGTGTLAYFAY (SEQ ID NO.12), or

c) A CDR1 sequence corresponding to SYFVH (SEQ ID NO.13); a CDR2 sequence corresponding to NIDPSDSDTHYSPSFQG (SEQ ID NO.14) and a CDR3 sequence corresponding to GGTGTFAFFAY (SEQ ID NO.15).

In another embodiment, the CDRs of the light chain present particular sequences which may be combined as described:

a) A CDR4 sequence corresponding to RSSQSLVHSYGNTYLY (SEQ ID NO.4); a CDR5 sequence corresponding to RVSNRFS (SEQ ID NO.5) and a CDR6 sequence corresponding to FQGTHVPYT (SEQ ID NO.6), or

b) A CDR4 sequence corresponding to RSSQSLVHSYGNTYLN (SEQ ID NO.17); a CDR5 sequence corresponding to KVSNRDS (SEQ ID NO.18) and a CDR6 sequence corresponding to QQSYSTPYT (SEQ ID NO.19), or

c) A CDR4 sequence corresponding to RSSQSLVHSYGNSYLY (SEQ ID NO.20); a CDR5 sequence corresponding to AVSSLQS (SEQ ID NO.21) and a CDR6 sequence corresponding to QQRSNVPLT (SEQ ID NO.22), or

d) A CDR7 sequence corresponding to KASQYVGTYVA (SEQ ID NO.7); a CDR8 sequence corresponding to SASYRHT (SEQ ID NO.8) and a CDR9 sequence corresponding to QQYSSSPYT (SEQ ID NO.9), or

e) A CDR7 sequence corresponding to RASQGISNYLA (SEQ ID NO.24); a CDR8 sequence corresponding to AASSLQS (SEQ ID NO.25) and a CDR9 sequence corresponding to QQYSSYPYT (SEQ ID NO.26), or

f) A CDR7 sequence corresponding to RASQSVSSYLA (SEQ ID NO.27); a CDR8 sequence corresponding to DASNRAT (SEQ ID NO.28) and a CDR9 sequence corresponding to QQRSSSPYT (SEQ ID NO.29).

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Sequences SEQ ID NO.1 to SEQ ID NO.9 correspond to wild-type sequences from murine antibodies whereas sequences SEQ ID NO.10 to SEQ ID NO.29 correspond to variants obtained by humanization techniques as described below. The complete list of the CDR sequences are described in Example 2, Table 4.

In addition, the framework (FR) sequences of the antibodies have been humanized. In a particular embodiment of the invention, the 4 FR regions of the light chain of anti-SIRPa antibodies comprise the FR1, FR2, FR3 and FR4 regions which are defined as follows:
The FR1 region comprises the amino acid sequence $DX_1VMTQX_2X_3X_4X_5X_6X_7X_8SX_9GDX_{10}X_{11}SX_{12}X_{13}C$ wherein X_1 is V or I; X_2 is T or S; X_3 is P or Q; X_4 is L, S, A or N; X_5 is S, T or F; X_6 is L or M; X_7 is P or S; X_8 is V, A, L or T; X_9 is L, V or P; X_{10} is Q, P or R; X_{11} is V or A; X_{12} is I, L or V; and X_{13} is S or T (SEQ ID NO.90).

The FR2 region comprises the amino acid sequence $WYX_1QKPGQSPKX_2LIY$ wherein X_1 is L or Q; and X_2 is L or A (SEQ ID NO.91).

The FR3 region comprises the amino acid sequence $GVPDRFX_1GSGSGTDFTLX_2ISX_3VX_4X_5EDLX_6X_7YFC$ wherein X_1 is S or T; X_2 is K or T; X_3 is R, S or N; X_4 is E or Q; X_5 is A, P or S; X_6 is G or A; X_7 is V, T or D (SEQ ID NO.92).

The FR4 region comprises the amino acid sequence $FGX_1GTKLEIK$ wherein X_1 is a S, G or Q (SEQ ID NO.93).

In a particular embodiment, the FR sequences of the wild-type antibodies correspond to FR1 to FR12 defined respectively by the following sequences: SEQ ID NO.32, SEQ ID NO.33, SEQ ID NO.34, SEQ ID NO.80 (for the heavy chain), SEQ ID NO. 44, SEQ ID NO. 45, SEQ ID NO.46, SEQ ID NO. 47, SEQ ID NO.61, SEQ ID NO.62, SEQ ID NO.64 and SEQ ID NO.64 (for the light chain). Both the wild-type sequences and the humanized variants of FR regions are described in Example 2, Table 5.

In a particular embodiment, the antibodies of the invention comprise the combination of CDRs as described above, in particular of CDRs listed in Table 4 with FR regions as listed in Table 5. Examples of heavy chains resulting from these combinations correspond to sequences described in Example 2, Table 6. Examples of light chains resulting from these combinations correspond to sequences described in Example 2, Table 7.

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Antibodies resulting from combinations of a heavy chain and a light chain as described above are of particular interest.

Antibodies of the invention thus result from the combination of a heavy chain with a light chain, as described above.

As described above, the antibodies of the invention encompass wild-type antibodies but also variants which result from application of optimization techniques such as humanization or de-immunisation approach. Different methods exist.

The approach of humanization by CDR grafting aims to remove the foreign framework regions (FR) in the variable domains that can evoke an immune response. The solution is to graft the complementary determining regions (CDRs) of the murine or rodent origin antibody onto a human acceptor framework (Jones et al., 1986). However, CDR-grafting alone can lead to a significant reduction or complete loss of binding affinity, as a set of supporting framework residues in the Vernier zone are important for maintaining the conformation of the CDRs. This problem can be solved by reintroducing murine or rodent residues into the human framework (Queen et al., 1989); such substitutions are commonly called back-mutations.

Resurfacing of the variable domains (Padlan, 1991) is an alternative solution to humanization based on the observation that antigenicity is often associated with solvent exposed residues, especially those protruding out from the surface. By substituting exposed foreign residues for common and appropriate human replacements "surface veneering" removes potential B cell epitopes by altering residues likely to act as epitope determinants. During resurfacing, buried framework residues that may contribute to antigen binding are retained and the number of substitutions to the Parental antibody are minimized. The resurfacing protocol used here follows the concepts of Staelens et al (Staelens et al., 2006).

Further, most therapeutic proteins are, to a varying extent, immunogenic (Stas and Lasters, 2009) and even so called fully-human antibody therapeutics may contain immunogenic regions (Harding et al., 2010) . Immunogenicity is the ability to induce a Th (T-helper) response, which is triggered when a unique T-cell receptor recognizes a peptide bound to a HLA classII molecules displayed an antigen presenting cells. However, it is possible to lower the immunogenicity potential by removing Th epitopes, a process known as de-immunisation (Baker and Jones, 2007) . This is achieved by predicting which peptides in the therapeutic protein can bind to HLA classII molecules, and subsequently introduce substitutions that eliminate or reduce the peptide bonding affinity for HLA classII molecules.

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The focus during a de-immunisation is on the DR allotypes which are known to express at higher level than DQ and DP (Gansbacher and Zier, 1988) (Berdoz et al., 1987) (Stunz et al., 1989). The assessment of severity for individual epitopes is based on the criteria of promiscuity, as well as the importance (frequency) of the allotypes in the population and a qualitative assessment of the HLA :peptide complex binding strength.

As the most significant property of a therapeutic antibody is the activity, it is important that substitutions proposed during the resurfacing and de-immunisation do not affect the affinity or stability of the antibody. A large amount of information has been collected in the last 20 years on humanization and grafting of the CDRs (Jones et al., 1986)(Ewert et al., 2003), the biophysical properties of antibodies (Ewert et al., 2003), the conformation of the CDR-loops (Chothia and Lesk, 1987) (Al-Lazikani et al., 1997) (North et al., 2011) and for the framework (Vargas-Madrazo and Paz-García, 2003) (Honegger et al., 2009), which along with advances in protein modelling (Desmet et al., 2002) (Almagro et al., 2014) makes it possible to accurately humanize and de-immunise antibodies with retained binding affinity and stability.

By way of example, the method known as "resurfacing" consists in replacing the set of surface residues in the frameworks of the variable region of a nonhuman antibody with a human set of surface residues, while the method known as CDR grafting consists of transferring the CDRs from a non-human antibody into the framework regions of a human antibody. CDR grafting is generally completed by framework optimization, consisting in the replacement of some residues of the human framework, in order to optimize the binding affinity.

The step of framework optimization has been recently simplified by the use of combinatorial libraries (Rosok. et al. J. Biol. Chem. 271, 22611-22618, 1996; Baca et al. J. Biol. Chem. 272, 10678-10684, 1997). Another recent strategy available for antibody humanization preserves only the original nonhuman CDR3 sequences of light and heavy chain while the remaining sequence is selected from naïve human V gene libraries (Rader et al, Proc. Natl. Acad. ScL U.S.A. 95, 8910-8915, 1998).

The humanized and/or de-immunized antibodies of the invention can belong to any class of immunoglobulins, like the non-modified antibodies. Preferably, they belong to a subclass of the IgG class such as IgG1, IgG2, IgG3 or IgG4.

In a particular embodiment of the invention, an anti-SIRPa antibody of the invention is also defined by its functional properties.

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In particular, the antibody of the invention presents a binding affinity for SIRPa of at least 1.10^{-9} M, preferably of at least 1.10^{-10} M and more preferably of at least 1.10^{-11} M.

An antibody of the invention specifically antagonizes the interaction between SIRPa and CD47. In a preferred embodiment, the antibody of the invention recognizes an epitope included in the D1 domain of SIRPa.

Compare to antagonist targeting Sirpa, blocking CD47 does not induce IL12 secretion by MDCS or macrophages (data not shown).

In addition, an anti-SIRPa antibody of the invention is able to induce the differentiation of MDSC into non suppressive cells, more particularly of Mo-MDSC into non suppressive cells.

In a particular embodiment, said non suppressive cells are inflammatory effectors which secret pro-inflammatory cytokines such as IL6, IL12p40, TNFa but low level of IL10 and TGFb. Those cells can express iNOS or iNOS-associated marker.

In a particular embodiment, the resulting non suppressive cells are non suppressive cells that do not express the MHC Class II markers and express the markers CD80 and CD86. Said non-suppressive cells may express at least one marker of the natural killer (NK) cells. Examples of such markers of the NK cells are provided in Table 1 below.

Table 1 : Examples of NK cells markers

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Rat	Mice	Human
CD161 (NKRP1)	CD161 (NKRP1)	CD161 (NKRP1)
	CD335 (NKp46)	CD335 (NKp46)
	CD122 (IL2Rbeta)	CD122 (IL2Rbeta)
CD94 (NKG2)	CD94 (NKG2)	CD94 (NKG2)
	CD314 (NKG2D)	CD314 (NKG2D)
Ly49 family	Ly49 family	CD336 (NKp44)
NKG2A family	NKG2A family	CD337 (NKp30)
	CD49b	CD158 (KIR family)
		CD16 (FcgIIIA)
		CD56
		CD57

In another embodiment, an anti-SIRPa antibody of the invention is able to inhibit the M2 phenotypic polarization of macrophages and/or favors the pro-inflammatory M1-type macrophage function.

As used herein, the term "modifying macrophage polarization" relates to a SIRPa antibody able to modify the balance between the different subtypes of macrophages in the treated individual, at least at the functional level. Hence, according to the present invention, the treatment of an individual with an anti-SIRPa compound leads to a modification in the profile of chemokines and cytokines expressed by the individual's macrophages (including a decrease of CCL17 expression and an increase of IL6 and IL12 expression).

According to a particular embodiment of the present invention, the modification of macrophage polarization by the anti-SIRPa antibody specifically binding to the extracellular domain of SIRPa, includes an inhibition of M2 polarization of macrophages and/or an increase of pro-inflammatory M1-type macrophage functions. In particular, it can include an inhibition of M2 phenotypic polarization of macrophages, leading to a decrease of the proportion of macrophages overexpressing cellular markers such as CD206 and CD200R, and/or to a decrease of the expression level of these markers on macrophages which overexpress these.

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In a further embodiment, an anti-SIRPa antibody of the invention is able both to induce the differentiation of monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells and to inhibit M2 phenotypic polarization of macrophages and/or favors pro-inflammatory M1-type macrophage function.

A second object of the invention consists in a composition comprising anti-SIRPa antibodies or functional fragments thereof as defined herein, wherein these antibodies or functional fragments thereof are a homogeneous population of antibodies or functional fragments thereof or are monoclonal antibodies or functional fragments thereof.

Another object of the invention is a pharmaceutical composition comprising an antibody or an antigen-binding fragment thereof or a chimeric molecule, according to the invention, with a pharmaceutical vehicle, wherein said pharmaceutical composition optionally further comprises a different active ingredient.

A composition of the invention may further comprise an additional compound having a therapeutic immunomodulator effect, in particular on cells involved in the immune regulation as a check point inhibitor or activators as described below.

The invention concerns also an antibody or an antigen-binding fragment thereof or a chimeric molecule as defined or illustrated herein, for use as active ingredient in a combination or add-on therapeutic regimen in a patient in need thereof.

A third object of the invention consists in the use of an anti-SIRPa antibody or antigen-binding fragment thereof for use in therapeutic applications.

An antibody or an antigen-binding fragment thereof or a chimeric molecule according to the invention, a pharmaceutical composition or a composition as defined herein are in particular proposed for use in a human patient for treating pathologic conditions influenced by immune responses, especially by memory T cells responses.

In a particular embodiment, the invention concerns the use of an anti-SIRPa antibody or antigen-binding fragment thereof in the treatment of any condition susceptible of being improved or

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prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

As defined herein, "a condition susceptible of being improved or prevented by differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells" corresponds to is a cancer, an infectious disease, a trauma, an auto-immune disease (such as rheumatoid arthritis, type 1 diabetes, lupus, psoriasis), a vaccination, a chronic inflammatory diseases (such as inflammatory bowel diseases including Crohn disease and Ulcerative colitis), a transplant dysfunctions.

In another embodiment, the invention concerns the use of an anti-SIRPa antibody or antigen-binding fragment thereof in the treatment of any condition susceptible of being improved or prevented by modifying macrophage polarization to favor pro-inflammatory macrophages.

As defined herein, "a condition susceptible of being improved or prevented by modifying macrophage polarization to favor pro-inflammatory macrophages" corresponds to a solid cancer, a liquid cancer, an infectious disease, a trauma, an auto-immune disease, a vaccination, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis or a chronic inflammatory disease.

Modifying the polarization of macrophages to favor pro-inflammatory cells can be useful in a number of pathologies or situations. As described above, this modification is particularly useful in the context of cancers, to restore an anti-tumor activity of macrophages and/or prevent the pro-tumoral activity of M2-type macrophages. Inappropriate immune responses due to an excess of M2-type macrophage polarization also occur in infectious diseases, vaccination, trauma and chronic inflammatory diseases.

Thus, according to a particular embodiment, anti-SIRPa antibody ban be used to treat an individual who has a cancer selected from the group consisting of lung cancers, ovary cancers, liver cancers, bladder cancers, brain cancers, breast cancers, colon cancers, thymomas, gliomas, melanomas and hematologic cancers such as lymphomas (Hodgkin's lymphoma and non Hodgkin's lymphoma), leukemias such as T and B Acute or Chronic Lymphoblastic Leukemia (ALL or CLL) or Acute or Chronic myeloid leukemia (AML or CML) and Myelomas.

In another embodiment, the antibody of the invention is combined to a second therapeutic agent.

Modulation macrophage polarization is a very attractive approach to treat cancer, especially in a combined therapy of cancer. Antonia et al. (Antonia et al., 2014) defined immuno-oncology combination that could be interesting for cancer treatment using checkpoint inhibitor approaches.

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Immuno-oncology is an evolving treatment modality that includes immunotherapies designed to harness the patient's own immune system.

In this context, anti-SIRPa antibodies of the present invention can be combined with some other potential strategies for overcoming tumor immune evasion mechanisms with agents in clinical development or already on the market (see table 1 from Antonia et al., 2014):

1- Reversing the inhibition of adaptive immunity (blocking T-cell checkpoint pathways), for example by using an anti-CTLA4, an anti-PD1 or an PD-L1 molecule;

2- Switching on adaptive immunity (promoting T-cell costimulatory receptor signaling using agonist antibodies), for example by using an anti-CD137 molecule;

3- Improving the function of innate immune cells;

4- Activating the immune system (potentiating immune-cell effector function), for example through vaccine-based strategies.

The administration of the second agent can be simultaneous or not with the administration of the anti-SIRPa antibody. Depending on the nature of the second agent, a co-administration can be prepared in the form of a combination drug, also known as a "combo". A combo is a fixed-dose combination that includes two or more active pharmaceutical ingredients combined in a single dosage form, which is manufactured and distributed in fixed doses. But the dose regimen and/or the administration route can also differ.

In a preferred embodiment, this second therapeutic agent is selected from the group consisting of chemotherapeutic agents, radiotherapy, surgery, cell therapy, immunotherapeutic agents, cell therapy (such as CAR-T cells), antibiotics and probiotics. In particular, immunotherapeutic agents useful in the context of the invention are selected from the group consisting in therapeutic vaccines (DNA, RNA or peptide vaccins), immune checkpoint blockers or activators or immunoconjugates such as antibody-drug conjugates (Donaghy, 2016).

As used herein, the term "immunotherapeutic agents" refers to agents that could take cancer vaccines from interesting biological phenomena to effective therapeutic agents include: T-cell growth factors to increase number and repertoire of naive T cells, growth factors to increase the number of dendritic cells (DCs), agonists to activate DCs and other antigen-pre senting cells (APCs), adjuvants to allow and augment cancer vaccines, agonists to activate and stimulate T cells, inhibitors of T-cell checkpoint blockade, T-cell growth factors to increase the growth and survival of immune T cells, agents to inhibit, block, or neutralize cancer cell and immune cell-derived immunosuppressive cytokine (Mac Chevers et al, 2008).

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Numerous immune checkpoint blocker or activator are known in the art. In the context of the invention, examples of immune checkpoint blockers or activators that could be useful are anti-PDL1, anti-PD1, anti-CTLA4, anti-CD137, anti-Her2, anti-EGFR, anti-CD20, anti-CD19, anti-CD52, CD-137 and OX40.

Another aspect of the present invention is a method for *ex vivo* obtaining pro-inflammatory macrophages, comprising a step of incubating macrophages with an anti-SIRPa antibody as described above. This method can be useful, for example, in cell therapy, especially for cancer patients. The present text also describes a method for treating a cancer patient, comprising a step of obtaining macrophages from said patient, followed by a step of modifying their polarization to favor pro-inflammatory functions through incubation with an anti-SIRPa antibody, and a step of re-administering the obtained pro-inflammatory cells to the patient. Of course, additional steps (such as expanding the cells, selecting the cells which exhibit a M1-type phenotype and/or counter-selecting those which exhibit a M2-type phenotype) can be introduced in such a method.

According to another of its aspects, the present invention pertains to a method for *in vivo* determining the efficacy of a treatment by an anti-SIRPa antibody as defined above, comprising measuring the presence of pro-inflammatory macrophages and/or measuring the presence of M2-type macrophages in a sample from an individual treated by said compound. When performing the method, the presence of pro-inflammatory macrophages can be measured, for example, by measuring the levels of IL6 and IL12 secreted by the macrophages present in the sample. The presence of M2-type macrophages can be measured, for example, by measuring the expression of CD206 and/or CD200R on the surface of macrophages.

In order to avoid an unnecessary treatment with an anti-SIRPa antibody, it is of major importance to correctly identify patients who are likely to benefit from such a treatment, *i.e.*, patients for whom this treatment will be efficient. Patients exhibiting high levels of M2-type macrophages, especially cancer patients having a high quantity of tumor-infiltrating M2-type macrophages, are those who are the most likely to respond to a treatment with an anti-SIRPa antibody according to the present invention. The present invention hence also pertains to a method for *in vivo* determining if an individual is likely to be a good responder to a treatment by an anti-SIRPa antibody in the sense of the present invention, comprising measuring the presence of M2-type macrophages in a sample from said individual, for example by measuring the expression of CD206 and/or CD200R on the surface of macrophages present in the sample.

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The invention can, in a particular embodiment, be defined as a method of treatment of cancer, infectious disease, immune disease and other diseases as described below consisting in administrating a therapeutic amount of an anti-SIRPa antibody able to induce the differentiation of Mo-MDSC into non suppressive cells and/or to modulate the polarization of macrophages, to a patient in need thereof. This method can further includes the administration of a second therapeutic agent as defined above.

DESCRIPTION OF THE FIGURES

Figure 1 : Binding study of SIRPa antibodies by ELISA: Represents the binding to SIRPa of different antibodies at different concentrations (ng/ml): m18D3 clone (Cercle) (n=7), m18D5 clone (square) (n=4), SE5A5 commercial clone (Triangle) (n=7), 6G10 clone (empty triangle) (n=3) and 12D7 clone (empty square) (n=4).

Figure 2 : Binding study of SIRPa antibodies on human monocytes by Flow cytometry (FACS): different anti-SIRPa antibodies were tested: m18D3 (black cercle) (n=4), m18D5 (black square) (n=1), SE7C2 (black triangle)(n=2), 12D7 (empty square) (n=2), 6G10 (black diamond) (n=4) : A) Represents the Mean Fluorescence Intensity (MFI) of the different antibodies over a dose response. B) Represents percentage of stained monocytes over antibody dose response. Statistical analysis were performed when it was possible.

Figure 3 : Antagonist activity study of anti-SIRPa antibodies on Sirpa-CD47 interaction by ELISA and FACS: The different anti-SIRPa antibodies were tested over a dose response: m18D3 clone (black cercle) (n=7), m18D5 clone (black square) (n=1), commercial antibodies: SE5A5 or SE7C2 (black triangle) (n=2) and m12D7 (inverted black triangle) (n=2). A) Represents the percentage of CD47 positive Sirpa-CD47 interactions measured by ELISA during a dose response of anti-hSIRPa antibodies, the IC50 was calculated B) Represents the percentage of CD47 positive cells measured by FACS after competition with anti-hSIRPa antibodies. The IC50 was calculated.

Figure 4 : SIRPa variants binding in the population by anti-h SIRPa antibodies: The capacity of different anti-hSIRPa antibodies to bind SIRPa variants in the 32 volunteers was measured by FACS with a PE-anti mouse IgG. All clones were tested at 10 µg/ml: m18D3 (black cercle), m18D5 (black square), 12D7 (inverted black triangle), 6G10 (black diamond) and commercial antibodies SE5A5 (empty square), SE7C2 (empty triangle). A) Represents the homozygote Variant 1 volunteers (n=16)

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B) Represents the homozygote variant 2 volunteers (n=8) and C) Represents the heterozygote V1/V2 volunteers (n=8).

EXPERIMENTAL DATA

Exemple 1: Mice immunization for anti-hSIRPa antibodies generation

Method: Mice were immunized using the cDNA coding for the human transmembrane protein CD172a variant V1 (NM_080792) (Takenaka and al. Nat Immunol. 2007 Dec) to generate murine monoclonal antibodies directed against this antigen. To obtain conformational and functional antibody, a DNA immunization approach is proposed using lipid formulation approach. The full procedure was performed as previously described by Arnaoty et al. (2012). Briefly, nanospheres were assembled using the ICANtibodies™ technology. Intramuscular injections of nanospheres were administered at a single site into shaved muscles in five anesthetized Swiss and BALB/c adult mice. Twenty-one, 42, and 63 days after the primo injection, the animals were given a booster with a formulation containing the same amount of DNA formulated with the synthetic vector. For each mouse, pre-immune serum and antiserum samples were collected on days 0, 21, 42, 63, and in some cases also on day 84, and their antibody titers were determined by ELISA and Western blot. All mouse experiments were performed in accordance with national guidelines

Mice (C57BL6 5 and 5 SWISS-CD1) were immunized according to a protocol of immunization with a minimum of 3 injections. These mice received a boost pre melting before being used for the production of mAbs

Results :

This immunization campaign has resulted in the selection of two murine antibodies:

- m18D3 which is an IgG2a mouse monoclonal anti-human SIRPa antibody.
- m18D5 which is an IgG2a mouse monoclonal anti-human SIRPa antibody.

The experiments presented in this application also cited the antibodies 6G10 and 12D, two antibodies belonging to the Applicant; these antibodies have been obtained by the same method than m18D" and m18D5 and are used as control (Effimune antibodies). These control antibodies are IgG2a mouse monoclonal anti-human SIRPa antibodies.

In addition, two commercial antibodies were used for comparison. The first one is an anti-SIRPa antibody, named SE7C2 (Santa Cruz sc-23863); the second antibody is an antibody able to recognize both SIRP α /β and is named SE5A5 (BioLegend BLE323802).

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The sequence of the heavy chain of both the m18D3 and m18D5 (having the same heavy chain) is the following :

QVQLQQPGAELVRPGSSVKLSCKASGYTFTSYWVHWVKQRPIQGLEWIGNIDPSDSDTHYNQKFKDKASLTVDKS
SSTAYMQLSSLTFEDSAVYYCVRGGTGTMAWFAYWGQGTLVTVSA (SEQ ID NO.127)

The sequence of the light chain of the m18D5 is the following:

DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSYGNTYLYWYLQKPGQSPKLLIYRVSNRFSGVPDRFSGSGSGTDFTL
KISRVEAEDLGVYFCFQGTHVPYTFGSGTKLEIKR (SEQ ID NO.128)

The sequence of the light chain of the m18D3 is the following:

DIVMTQSQNFMSTSVGDRVSVTCKASQYVGTYVAWYQQKPGQSPKALIYSASYRHTGVPDRFTGSGSGTDFTLTIS
NVQSEDLADYFCQQYSSSPYTFGGGTKLEIKR (SEQ ID NO.129)

Example 2: Humanization by CDR grafting approach

Method: The murine m18D3 and m18D5 antibodies were humanized using in silico CDR grafting method as described above, a method of humanization well known in the art.

Results: The resulting humanized sequences from the CDR and FR regions, as well as from the variable heavy chain and light chain are described in the following Tables:

The humanization has allowed to identify some particular amino-acids in the wild type sequence.

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Kabbat nomenclature (IMGT)		FR1	FR2	FR3	FR4	CDR1	CDR2	CDR3
m18D3_VH	Canonic aa	H24, H26, H27, H29		H71, H94		H34	H52A, H55	
	Conserved aa	H2, H14, H22, H28, H30	H36, H49	H73, H92, H93,				
	VL/VH interface aa		H37, H45, H47	H89, H90, H91	H103	H35		H95, H100C
m18D5_VL (	Canonic aa	L2	L48(L53)	L64(L69), L71 (L76)		L25, L27B (L29), L27C (L30), L33 (L38)	L51(L56), L52(L57)	L90(L95), L95(L100)
	Conserved aa	L8, L23	L35 (L40)	L73(L78), L88(L93)				
	VL/VH interface aa		L48(L43), L44(L49),	L87(L92),	L98			L89(L94), L91(L96), L96(L101)
m18D3_VL	Canonic aa	L2	L48(53)	L64(69), L71(76)		L25, L33 (38)	L51(56), L52(57)	L90(95), L95(100)
	Conserved aa	L23	L35(40)	L88(93)				
	VL/VH interface aa		L38(43), L44(49)	L73(78)	L98			L89(94), L91(96)

Table 2: humanized amino acids into the CDRs

Kabbat (IMGT)	CDR1	CDR2	CDR3
h18D3_VH	W33Y or F;	N60A or S; Q61P; K62S; K64Q; D65G	M100L or F or I; W100BY or F or L
h18D5_VL	T31S(T36S); Y34N (Y39N)	R50K or A or D (R55K or A or D); N53S (N58S); R54L (R59L); F55D or Q or A(F60D); S56T (S61T)	F89Q (F94Q); G91S or R (G96S or R); T92Y or S (T97Y or S); H93S or N (H98S or N); V94T (V99T)
h18D3_VL	K24R; Y28G or S (Y33G or S); V29I(V34I); G30S(G35S); T31N or S (T36N or S); Y32N(Y37N); V33L(V38L)	S50A or D or G (S55A or D or G); Y53S or N(Y58S or N); R54L(R59L); H55Q or A (H60Q or A); T56S(T61S)	Y91R(Y96R); S94Y(S99Y)

Table 3 : humanized amino acids into the frameworks

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Kabbat (IMGT)	FR1	FR2	FR3	FR4
h18D3_VH	Q1E; Q5V; P7S; L11V; V12K; R13K; S16A or E; V18L; K19R; L20V or I; T28S	K38R; R40A or M; I42G; R40M; Q43K; I48M	K66R; S68T; A67V; L69M; T70S; S75I or T; A78V; M80L; Q81E; T83R or K; F84S or A; E85S; S87T; V89M;	A113S
h18D5_VL	D1E; V2I; V3Q; M4L; T7S; L9S; P12S; V13A; S14T; L15V; D17Q; Q18P or R; A19V; S20T; V2I; S22T	Y36F (Y41F); L37Q(L42Q); K39R(K44R); Q42K(Q47K); S43A(S48A); K45R(K50R);	V58I (V63I); D60S(D65S); K74T(K79T); R77S(R82S);V78L(V83L); E79Q(E84Q); A80P(A85P); L83V or F(L88V or F); G84A(G89A); V85T(V90T); F87Y(F92Y)	S100G; L104V;
h18D3_VL	D1E; V3Q; M4L; Q8P;N9S or A; F10S or T; M11L; T13A or L or V; V15P; D17E; V19A; S20T; V21I or L; T22S	Y36F(Y41F); Q42K(Q47K); S43A(S48A); K45R(K50R); A46S or L (A51S or L)	V58I(V63I); D60S or A(D65S or A); T63S(T68S); D70E(D75E); N77S(N82S); V78L(V83L); Q79E(Q84E); S80P(S85P); L83F(L88F); D85T or V(D90T or V); F87Y(F92Y)	G100Q

Table 4 : Sequences of the CDRs of the anti-SIRPa antibodies

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	Sequence	SEQ ID NO.
CDR1 wt	SYWVH	1
CDR1 variant 1	SYYVH	10
CDR1 variant 2	SYFVH	13
CDR2 wt	NIDPSDSDTHYNQKFKD	2
CDR2 variant 1	NIDPSDSDTHYAQKFQG	11
CDR2 variant 2	NIDPSDSDTHYSPSFQG	14
CDR3 wt	GGTGTMAWFAY	3
CDR3 variant 1	GGTGTLAYFAY	12
CDR3 variant 2	GGTGTFAFFAY	15
CDR3 variant 3	GGTGTIALFAY	16
CDR4 wt	RSSQSLVHSYGNTYLY	4
CDR4 variant 1	RSSQSLVHSYGNTYLN	17
CDR4 variant 2	RSSQSLVHSYGNSYLY	20
CDR5 wt	RVSNRFS	5
CDR5 variant 1	KVSNRDS	18
CDR5 variant 2	AVSSLQS	21
CDR5 variant 3	DVSNRAT	23
CDR6 wt	FQGTHVPYT	6
CDR6 variant 1	QQSYSTPYT	19
CDR6 variant 2	QQRSNVPLT	22
CDR7 wt	KASQYVGTYVA	7
CDR7 variant 1	RASQGISNYLA	24
CDR7 variant 2	RASQSVSSYLA	27

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CDR7 variant 3	RASQSVSSNLA	30
CDR8 wt	SASYRHT	8
CDR8 variant 1	AASSLQS	25
CDR8 variant 2	DASNRAT	28
CDR8 variant 3	GASTRAT	31
CDR9 wt	QQYSSSPYT	9
CDR9 variant 1	QQYSSYPYT	26
CDR9 variant 2	QQRSSSPYT	29

Table 5: Sequences of the FR regions of the anti-SIRPa antibodies

	Sequence	**SEQ ID NO.**
FR1 wt	QVQLQQPGAELVRPGSSVKLSCKASGYTFT	32
FR1 variant 1	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	35
FR1 variant 2	EVQLVQSGAEVKKPGESLRISCKASGYTFT	38
FR1 variant 3	EVQLVQSGAEVKKPGESLRISCKASGYSFT	41
FR2 wt	WVKQRPIQGLEWIG	33
FR2 variant 1	WVRQAPGQGLEWIG	36
FR2 variant 2	WVRQMPGKGLEWIG	39
FR2 variant 3	WVRQMPGKGLEWMG	42
FR3 wt	KASLTVDKSSSTAYMQLSSLTFEDSAVYYCVR	34
FR3 variant 1	RATLTVDKSTSTAYMELSSLRSEDTAVYYCVR	37
FR3 variant 2	RVTMTVDKSTSTVYMELSSLRSEDTAVYYCVR	40

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FR3 variant 3	HVTLSVDKSISTAYLQLSSLKASDTAMYYCVR	43
FR4 wt	WGQGTLVTVSA	80
FR4	WGQGTLVTVSS	37
FR5 wt	DVVMTQTPLSLPVSLGDQASISC	44
FR5 variant 1	DVVMTQSPLSLPVTLGQPASISC	48
FR5 variant 2	DVQMTQSPSSLSASVGDRVTITC	55
FR5 variant 3	DIQMTQSPSSLSASVGDRVTITC	53
FR5 variant 4	EVVMTQSPATLSLSPGERATLSC	54
FR5 variant 5	EIVLTQSPATLSLSPGERATLSC	57
FR6 wt	WYLQKPGQSPKLLIY	45
FR6 variant 1	WYQQRPGQSPRLLIY	49
FR6 variant 2	WFQQRPGQSPRLLIY	51
FR6 variant 3	WYQQKPGKAPKLLIY	54
FR6 variant 4	WYQQKPGQAPRLLIY	55
FR7 wt	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC	46
FR7 variant 1	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYFC	50
FR7 variant 2	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	52
FR7 variant 3	GVPSRFSGSGSGTDFTLTISSLQPEDFATYFC	53
FR7 variant 4	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	56
FR7 variant 5	GIPARFSGSGSGTDFTLTISSLEPEDFAVYFC	58
FR7 variant 6	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	59
FR8 wt	FGSGTKLEIK	47
FR8 variant 1	FGGGTKVEIK	54
FR9 wt	DIVMTQSQNFMSTSVGDRVSVTC	61

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FR9 variant 1	DIQMTQSPSSLSASVGDRVTITC	65
FR9 variant 2	EIVMTQSPATLSLSPGERATLSC	69
FR9 variant 3	EIVLTQSPATLSLSPGERATLSC	72
FR9 variant 4	EIVMTQSPATLSVSPGERATLSC	75
FR10 wt	WYQQKPGQSPKALIY	62
FR10 variant 1	WYQQKPGKAPKALIY	66
FR10 variant 2	WFQQKPGKAPKSLIY	70
FR10 variant 3	WYQQKPGQAPRALIY	73
FR10 variant 4	WYQQKPGQAPRLLIY	76
FR11 wt	GVPDRFTGSGSGTDFTLTISNVQSEDLADYFC	63
FR11 variant 1	GVPSRFSGSGSGTDFTLTISSLQPEDFATYFC	67
FR11 variant 2	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	71
FR11 variant 3	GIPARFSGSGSGTDFTLTISSLEPEDFAVYFC	74
FR11 variant 4	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	77
FR11 variant 5	GIPARFSGSGSGTEFTLTISSLQSEDFAVYFC	78
FR11 variant 6	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC	79
FR12 wt	FGGGTKLEIK	64
FR12 variant 1	FGQGTKLEIK	68

Table 6: Sequences of the heavy chain of the anti-SIRPa antibodies

	Sequence	**SEQ ID NO.**
h18D3_VH_V7	EVQLVQSGAEVKKPGESLRISCKASGYTFTSYWVHWVRQMPGKGLEWIG NIDPSDSDTHYNQKFKDHVTLSVDKSISTAYLQLSSLKASDTAMYYCVRGG TGTMAWFAYWGQGTLVTVSS	94
h18D3_VH_V8	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQMPGKGLEWM	95

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	GNIDPSDSDTHYNQKFKDHVTLSVDKSISTAYLQLSSLKASDTAMYYCVRG GTGTMAWFAYWGQGTLVTVSS	
h18D3_VH_V9	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQMPGKGLEWM GNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYLQLSSLKASDTAMYYCVRG GTGTMAWFAYWGQGTLVTVSS	96
h18D3_VH_V10	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYYVHWVRQMPGKGLEWMG NIDPSDSDTHYSPSFQGHVTLSVDKSISTAYLQLSSLKASDTAMYYCVRGGT GTMAWFAYWGQGTLVTVSS	97
h18D3_VH_V11	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQMPGKGLEWM GNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYLQLSSLKASDTAMYYCVRG GTGTLAWFAYWGQGTLVTVSS	98
h18D3_VH_V12	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYWVHWVRQMPGKGLEWM GNIDPSDSDTHYSPSFQGHVTLSVDKSISTAYLQLSSLKASDTAMYYCVRG GTGTMAYFAYWGQGTLVTVSS	99
h18D3_VH_V13	EVQLVQSGAEVKKPGESLRISCKASGYSFTSYYVHWVRQMPGKGLEWMG NIDPSDSDTHYSPSFQGHVTLSVDKSISTAYLQLSSLKASDTAMYYCVRGGT GTLAYFAYWGQGTLVTVSS	100
h18D3_VH_V1	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWVHWVRQAPGQGLEWI GNIDPSDSDTHYNQKFKDRVTMTVDKSTSTVYMELSSLRSEDTAVYYCVR GGTGTMAWFAYWGQGTLVTVSS	101
h18D3_VH_V2	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWVHWVRQAPGQGLEW MGNIDPSDSDTHYAQKFQGRVTMTVDKSTSTVYMELSSLRSEDTAVYYCV RGGTGTMAWFAYWGQGTLVTVSS	102
h18D3_VH_V3	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYVHWVRQAPGQGLEWM GNIDPSDSDTHYAQKFQGRVTMTVDKSTSTVYMELSSLRSEDTAVYYCVR GGTGTMAWFAYWGQGTLVTVSS	103
h18D3_VH_V4	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWVHWVRQAPGQGLEW MGNIDPSDSDTHYAQKFQGRVTMTVDKSTSTVYMELSSLRSEDTAVYYCV RGGTGTLAWFAYWGQGTLVTVSS	104
h18D3_VH_V5	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWVHWVRQAPGQGLEW MGNIDPSDSDTHYAQKFQGRVTMTVDKSTSTVYMELSSLRSEDTAVYYCV RGGTGTMAYFAYWGQGTLVTVSS	105
h18D3_VH_V6	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYVHWVRQAPGQGLEWM	106

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	GNIDPSDSDTHYAQKFQGRVTMTVDKSTSTVYMELSSLRSEDTAVYYCVR GGTGTLAYFAYWGQGTLVTVSS	
h18D3_VH_V7	EVQLVQSGAEVKKPGESLRISCKASGYTFTSYWVHWVRQMPGKGLEWIG NIDPSDSDTHYNQKFKDHVTLSVDKSISTAYLQLSSLKASDTAMYYCVRGG TGTMAWFAYWGQGTLVTVSS	107

Table 7: Sequences of the light chain of the anti-SIRPa antibodies

	Sequence	**SEQ ID NO.**
h18D5_VL_V1	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSYGNTYLYWYQQRPGQSPRLL IYRVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYFCFQGTHVPYTFGG GTKVEIK	108
h18D5_VL_V2	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSYGNTYLYWFQQRPGQSPRLL IYRVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGTHVPYTFGG GTKVEIK	109
h18D5_VL_V3	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSYGNTYLNWFQQRPGQSPRL LIYKVSNRDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGTHVPYTFG GGTKVEIK	110
h18D5_VL_V4	DVQMTQSPSSLSASVGDRVTITCRSSQSLVHSYGNTYLYWYQQKPGKAPKL LIYRVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCFQGTHVPYTFGG GTKVEIK	111
h18D5_VL_V5	DIQMTQSPSSLSASVGDRVTITCRSSQSLVHSYGNSYLYWYQQKPGKAPKLLI YRVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCFQGTHVPYTFGGG TKVEIK	112
h18D5_VL_V6	DIQMTQSPSSLSASVGDRVTITCRSSQSLVHSYGNSYLYWYQQKPGKAPKLLI YAVSNRQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTHVPYTFGG GTKVEIK	113
h18D5_VL_V7	DIQMTQSPSSLSASVGDRVTITCRSSQSLVHSYGNSYLYWYQQKPGKAPKLLI YAVSNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTHTPYTFGGG TKVEIK	114
h18D5_VL_V8	DIQMTQSPSSLSASVGDRVTITCRSSQSLVHSYGNSYLYWYQQKPGKAPKLLI YAVSSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPYTFGGGT	115

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	KVEIK	
h18D5_VL_V9	EVVMTQSPATLSLSPGERATLSCRSSQSLVHSYGNTYLYWYQQKPGQAPRLLIYRVSNRFSGIPARFSGSGSGTDFTLTISSLEPEDFAVYFCFQGTHVPYTFGGGTKVEIK	116
h18D5_VL_V10	EIVLTQSPATLSLSPGERATLSCRSSQSLVHSYGNTYLYWYQQKPGQAPRLLIYRVSNRFSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCFQGTHVPYTFGGGTKVEIK	117
h18D5_VL_V11	EIVLTQSPATLSLSPGERATLSCRSSQSLVHSYGNTYLYWYQQKPGQAPRLLIYDVSNRFTGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCFQGTHVPYTFGGGTKVEIK	118
h18D5_VL_V12	EIVLTQSPATLSLSPGERATLSCRSSQSLVHSYGNTYLYWYQQKPGQAPRLLIYDVSNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRTHVPYTFGGGTKVEIK	119
h18D5_VL_V13	EIVLTQSPATLSLSPGERATLSCRSSQSLVHSYGNTYLYWYQQKPGQAPRLLIYDVSNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNVPYTFGGGTKVEIK	120
h18D3_VL_V1	DIQMTQSPSSLSASVGDRVTITCKASQYVGTYVAWYQQKPGKAPKALIYSASYRHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQYSSSPYTFGQGTKLEIK	121
h18D3_VL_V2	DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSSYPYTFGQGTKLEIK	122
h18D3_VL_V3	EIVMTQSPATLSLSPGERATLSCKASQYVGTYVAWYQQKPGQAPRALIYSASYRHTGIPARFSGSGSGTDFTLTISSLEPEDFAVYFCQQYSSSPYTFGQGTKLEIK	123
h18D3_VL_V4	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSSSPYTFGQGTKLEIK	124
h18D3_VL_V5	EIVMTQSPATLSVSPGERATLSCKASQYVGTYVAWYQQKPGQAPRALIYSASYRHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYFCQQYSSSPYTFGQGTKLEIK	125
h18D3_VL_V6	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQRSSSPYTFGQGTKLEIK	126

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Example 3: SIRPa bindings assay with monoclonal anti-hSIRPa antibodies by ELISA

Method: The binding activity of the anti-hSIRPa antibodies was assessed by ELISA (Enzyme-linked immunosorbent assay). For the ELISA assay, recombinant hSIRPa (Sino Biologicals, Beijing, China; reference 10975-H08H) was immobilized on plastic at 0,5 µg/ml in carbonate buffer (pH9.2) and purified antibodies were added at different concentrations to measure binding. After incubation and washing, peroxidase-labeled goat anti-mouse Fc chain (Jackson Immunoresearch; reference 115-036-071) was added and revealed by conventional methods.

Results : As shown in Figure 1, the binding activity of different anti-SIRPa antibodies on SIRPa as measured by ELISA showed an effective dose (ED50) of 0.16nM (24ng/ml) for SE5A5, of 0.11nM (17ng/ml) for the clone m18D3 and 0.06nM (9ng/ml) for the clone m18D5. The clones 6G10 and 12D7 didn't seem to bind SIRPa by ELISA assay. Those results indicate that the clones m18D3 and m18D are good SIRPa binders by ELISA compare to a commercial antibody and indicate that the epitope recognized by these clones is accessible when SIRPa is coated on a plastic well compared to clones 6G10 and 12D7.

Example 4: SIRPa binding assay on human monocytes to compare different anti-hSIRPa antibodies by flow cytometry.

Method: To measure binding of anti-SIRPa on human monocytes, antibodies at different concentrations were incubated for 30min at 4°C, and washed before stained 30min at 4°C with PE-labelled anti-mouseIgG (Jackson Immunoresearch; reference 715-116-151). Samples were analyzed on BD LSRII or Canto II cytofluorometer. The MFI of anti-Sirpa was compared between all anti-SIRPa antibodies (Figure 2.A.) and percentage of stained monocytes was analysed in all conditions (Figure 2.B.).

Results: The results indicate a strong binding (high MFI) of clones m18D3 and m18D5 with 100% of stained monocytes at 0.5 ng/ml compared to 6G10, 12D7 and SE7C2.

Example 5: Ccompetitive study between CD47 and mouse anti-hSIRPa antibodies using antagonist assays.

Methods:

a) In vitro by ELISA assay

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For competitive ELISA assay, recombinant hSIRPa (Sino Biologicals, Beijing, China; reference 11612-H08H) was immobilized on plastic at 0.5µg/ml in carbonate buffer (pH9.2). Each purified anti-SIRPa antibodies were mixed with 0.04µg/ml of CD47Fc (Sino Biologicals, Beijing, China; reference 12283-H02H) to measure competitive binding for 2h at 37°C. After incubation and washing, peroxidase-labeled donkey anti-human Fc chain (Jackson Immunoresearch; reference 709-035-149) was added to detect CD47Fc binding and revealed by conventional methods.

b) In vitro on human monocytes by FACS

To measure competition between CD47 and mouse anti-hSIRPa on human monocytes, purified antibody were added on monocytes for 15min at 4°C then cells were mixed with 5µg/ml final of CD47Fc (Sino Biologicals, Beijing, China; reference 12283-H02H) and incubated for 15min at 4°C to measure the competitive binding antibody. After incubation and washing, FITC-labelled anti-human Fc (Beckman Coulter; reference IM1627) was added for 30min at 4°C to detect CD47Fc binding and analyzed on BD LSRII or Canto II cytofluorometer.

Results: Figure 3 showed the antagonistic activity of the anti-hSIRPa antibodies. As shown figure 3.A by ELISA, the CD47 signal is inhibited by clones 18D3 and 18D5 with an IC50 of 4nM and 3nM respectively compared to an IC50 > 2000nM for the SE5A5. Then, the antagonistic activity was measured by FACS to compare clones 18D3 and 18D5 with the SE7C2 commercial antibody. As shown Figure 3.B. clones 18D3 and 18D5 present a better antagonistic activity regarding the Sirpa-CD47 interaction with an IC50 of 25nM and 74 nM respectively compared to the SE7C2 antibody presenting an IC50 > 1000nM.

All together these results showed that clones m18D3 and m18D5 presented the strongest binding to SIRPa with the best antagonistic activity on Sirpa-CD47 interaction.

Example 6: Competitive analysis of mouse anti-hSIRPa antibodies on SIRPa-SpD interaction

Method: This method was performed with a Blitz (Forté Bio, C22-2 No 61010-1). SIRP-a recombinant protein (Sino Biological Cat: 11612-H08H) was immobilized by histidine tail into a Ni-NTA biosensor (Forté Bio, 18-0029) for 30 seconds.

Condition A: SIRPa + antiSirpa Abs + SP-D. Mouse anti-SIRPa antibodies were added at 20µg/mL (saturating concentration) for 120 seconds. Then, human SP-D (R et D Systems cat: 1920-SP-050) was associated at 100 µg/mL in competition with mouse anti-SIRPa antibodies for 120 seconds. The dissociation of SP-D was made in kinetics buffer for 120 seconds.

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Condition B: sirpa + SP-D+antiSirpa Abs. A human SP-D (R et D Systems cat: 1920-SP-050) was added at 100µg/mL for 120 seconds. Then, mouse anti-SIRPa antibodies were associated at 20µg/mL (saturating concentration) for 120 seconds. The dissociation of mouse anti-SIRPa antibody was made in kinetics buffer for 120 seconds.

Analysis was made with Blitz pro 1.2 software, which calculates association constant (ka) and dissociation constant (kd) and determined the affinity constant KD (ka/kd).

Results: are shown in Table 8 for condition A and in Table 9 for condition B.

Table 8 : Association/Dissociation constants measured in condition A.

	KD(M)	ka(1/Ms)	kd (1/s)
SP-D	4,37E-06	2,46E+03	1,07E-02
18D3+SP-D	3,55E-06	1,96E+03	6,00E-03
18D5+SP-D	1,35E-07	3,27E+03	2,41E-03

This result shows that the binding of clones m18D3 and m18D5 to Sirpa does not block the binding of SP-D to the target. m18D3 and m18D5 antibodies do not inhibit the interaction of SIRPa-SP-D.

Table 9: Association/Dissociation constants measured in condition B.

	KD(M)	ka(1/Ms)	kd (1/s)
SP-D +18D3	2,10E-08	4,83E+05	1,01E-02
SP-D + 18D5	2,87E-08	3,80E+05	1,09E-02
18D3	1,13E-08	1,31E+05	1,48E-03
18D5	1,11E-07	1,58E+04	1,76E-03

This results confirmed that clones m18D3 and m18D5 were still able to bind SIRPa even in presence of SP-D.

All together, the anti-Sirpa antibodies 18D3 and 18D5 do not block the interaction of SP-D with Sirpa, indicating that the anti-Sirpa antibodies do not bind to SP-D binding domain into the domain 3 of the Sirpa protein. Indeed, it has been shown that Sp-D binds to Membrane-proximal Domain (D3) of

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SIRPa (Fournier et al., 2012), a site distant from binding domain of CD47 which is exclusively located on D1 domain (Lee et al., 2007). The anti-Sirpa antibodies of the invention antagonize the binding of CD47 to Sirpa without modifying the Sirpa-Sp-D interaction and suggest that those antibodies are susceptible to recognize an epitope located at least on the D1 domain of Sirpa. Their functional activities regarding the modulation of Mo-MDSC suppressive function and macrophage polarization could involve the inhibition of the binding of Sirpa to other ligands such as CD47.

Example 7 : Study of SIRPa variants recognition

Method: SIRPa polymorphisms were studied on 32 volunteers with anti-SIRPa antibodies by flow cytometry. Binding of four monoclonal anti-human SIRPa antibodies (m18D3; m18D5; m12D7; m6G10) were analyzed and compared to the two commercial anti-human Sirpa antibodies. Flow cytometry was performed on frozen human monocytes from 32 healthy volunteers. All the anti-human SIRPa antibodies were used at 10µg/ml and binding was detected by PE labelled anti-mouse IgG (Jackson #715-116-151). The analysis was made using a FACS Canto II (BD).

SIRPa variant 1 (v1) and variant 2 (v2) are the most common variants (accession numbers NP_542970 (P78324) and CAA71403), as described by Hatherley *et al.* (2014).

For analysis of exon 3 of human SIRPa PCR and sequencing, genomic DNA were purified by phenol-chloroform extraction of frozen human monocytes from 32 healthy volunteers obtained by centrifugal elutriation. In order identify Sirpa variants into the volunteers the Exon 3 of human sirpa gene was amplified by PCR with Herculase polymerase (Agilent Technologies #600679) using the following primers: Forward: TAGAATACAGGCTCATGTTGCAGGT (SEQ ID NO.130) Reverse: GCCTTCAGCAAATAGCATGACGT (SEQ ID NO.131) (Takenaka and al. Nat Immunol. 2007 Dec). Amplified PCR products were sequenced by the "Plateforme Génomique de Nantes". Sequences were analyzed with Chromas software.

Results : The results on Sirpa PCR and sequencing shown Figure 4 indicated that between 32 volunteers, 16 were Sirpa v1 homozygotes, 8 were Sirpa v2 homozygotes and 8 were v1/v2 heterozygotes.

In order to test if the m18D3 and m18D5 clones were able to recognize Sirpa variants in the population, we tested antibodies binding on monocytes of 32 healthy volunteers. Figure 4 showed FACS results and indicated that SE7C2 antibody was not able to recognize Sirpa V1 volunteers whereas m18D3; m18D5; m12D7; m6G10 and SE5A5 bind both variants v1 and v2, the only two Sirpa variants that were identified between the 32 volunteers. Figure 5A, B and C showed that m18D3 and

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m18D5 were able to bind both variants with very high MFI (> 10^4 to 10^5) compared to the other antibodies and presented a difference in the binding intensity between V1, V2 and V1/V2 that could be used to differentiate V1 homozygote versus V2 homozygote without a sequencing step.

Example 8 : Anti-hSirpa antibodies affinity study

The affinity of the anti-hSirpa antibodies, clones m18D3 and m18D5, IgG4 chimeric 18D3 and 18D5, SE7C2 and SE5A5, was measured by surface plasmon resonance on a Biacore 3000 (GE Healthcare). A CM5 chip (GE healthcare) was activated by injection of NHS / EDC mix for 7 min. The Sirpa protein (5µg/mL) was diluted in 5 mM maleate buffer pH6.2 was injected and the succinimide ester residues until a hooking 300RU signal. The free reactive residues were inactivated by the injection of 1M ethanolamine pH8.5. Antibodies were diluted in a HBSEP buffer and injected over the immobilized recombinant human Sirpa in the concentration range from 3.125nM to 500nM. The injection rate was set at 40µL/min, the association was measured for 3 min and dissociation for 10 min. Between each cycle of the analysis, the chip was regenerated by injection of a solution of 5M MgCl2 for 60s. The obtained sensorgrams were analyzed with model "Bivalent analyte" on BIAeval 4 software.

Table 10: Affinity constants of anti-Sirpa antibodies

	ka (1/Ms)	kd (1/s)	Rmax (RU)	KD (M)
18D5-G4m chim	4.00E+04	6.39E-06	5.51E+02	**1.60E-10**
18D3 ms	7.07E+04	1.89E-05	7.65E+02	**2.67E-10**
18D5 ms	6.23E+04	1.20E-05	7.36E+02	**1.93E-10**
SE7C2 ms	5.98E+03	0.01	2.33E+02	**1.67E-06**
Se5A5 ms	2.45E+04	9.49E-04	2.97E+02	**3.87E-08**

Results showed a strong affinity of clones m18D3, m18D5 as well as the chimeric 18D5-IgG4 to recombinant human Sirpa compare to the commercial antibodies SE7C2 and SE5A5 with an affinity between 1.60E-10 and 2.7 E-10 M. The determination of the chimeric 18D3 clone affinity was not possible because of technical problem.

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CLAIMS

1. An anti-SIRPa antibody or antigen-binding fragment thereof comprising:
    a) A heavy chain comprising CDR1, CDR2 and CDR3, and
    b) A light chain comprising 3 CDR chosen among CDR4, CDR5, CDR6, CDR7, CDR8 and CDR9

wherein said CDR are defined as follows:

- CDR1 comprises the amino acid sequence SYX$_1$VH wherein X$_1$ is W, Y or F (SEQ ID NO. 81);
- CDR2 comprises the amino acid sequence NIDPSDSDTHYX$_2$X$_3$X$_4$FX$_5$X$_6$ where X$_2$ is N, A or S; X$_3$ is Q or P; X$_4$ is K or S; X$_5$ is K or Q and X$_6$ is D or G (SEQ ID NO. 82);
- CDR3 comprises the amino acid sequence GGTGTX$_7$A X$_8$FAY where X$_7$ is M, L, F or I and X$_8$ is W or Y or F or L (SEQ ID NO. 83);
- CD4 comprises the amino acid sequence RSSQSLVHSYGNX$_9$YLX$_{10}$ wherein X$_9$ is T or S and X$_{10}$ is Y or N (SEQ ID NO. 84);
- CD5 comprises the amino acid sequence X$_{11}$VS X$_{12}$ X$_{13}$ X$_{14}$ X$_{15}$, wherein X$_{11}$ is R, K, A or D; X$_{12}$ is N or S; X$_{13}$ is R or L; X$_{14}$ is F, D, Q or A and X$_{15}$ is S or T (SEQ ID NO. 85);
- CD6 comprises the amino acid sequence X$_{16}$Q X$_{17}$ X$_{18}$ X$_{19}$ X$_{20}$P X$_{21}$T, wherein X$_{16}$ is F or Q; X$_{17}$ is G, S or R; X$_{18}$ is T, Y or S; X$_{19}$ is H, S or N, X$_{20}$ is V or T and X$_{21}$ is Y or L (SEQ ID NO. 86);
- CD7 comprises the amino acid sequence X$_{22}$ASQX$_{23}$ X$_{24}$ X$_{25}$ X$_{26}$ X$_{27}$ X$_{28}$A wherein X$_{22}$ is K or R; X$_{23}$ is Y, G or S; X$_{24}$ is V or I; X$_{25}$ G or S; X$_{26}$ is T, N or S, X$_{27}$ is Y or N and X$_{28}$ is V or L (SEQ ID NO. 87);
- CD8 comprises the amino acid sequence X$_{29}$ASX$_{30}$ X$_{31}$ X$_{32}$ X$_{33}$, wherein X$_{29}$ is S, A, D or G, X$_{30}$ is Y, S, N or T; X$_{31}$ is R or L; X$_{32}$ is H, Q or A and X$_{33}$ is T or S (SEQ ID NO. 88);
- CD9 comprises the amino acid sequence QQX$_{34}$SSX$_{35}$PYT wherein X$_{34}$ is Y or R and X$_{35}$ is S or Y (SEQ ID NO. 89).

2. The antibody of claim 1 wherein the CDRs of the light chain correspond to CDR4, CDR5 and CDR6 or to CDR7, CDR8 and CDR9.

3. The antibody or antigen-binding fragment thereof of claim 1 or 2, wherein the CDRs of the heavy chain are defined as follows :
    d) A CDR1 sequence corresponding to SYWVH (SEQ ID NO.1); a CDR2 sequence corresponding to NIDPSDSDTHYNQKFKD (SEQ ID NO.2) and a CDR3 sequence corresponding to GGTGTMAWFAY (SEQ ID NO.3), or

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e) A CDR1 sequence corresponding to SYYVH (SEQ ID NO.10); a CDR2 sequence corresponding to NIDPSDSDTHYAQKFQG (SEQ ID NO.11) and a CDR3 sequence corresponding to GGTGTLAYFAY (SEQ ID NO.12), or

f) A CDR1 sequence corresponding to SYFVH (SEQ ID NO.13); a CDR2 sequence corresponding to NIDPSDSDTHYSPSFQG (SEQ ID NO.14) and a CDR3 sequence corresponding to GGTGTFAFFAY (SEQ ID NO.15).

4. The antibody or antigen-binding fragment thereof of claim 1 or 2, wherein the CDRs of the light chain are defined as follows:

g) A CDR4 sequence corresponding to RSSQSLVHSYGNTYLY (SEQ ID NO.4); a CDR5 sequence corresponding to RVSNRFS (SEQ ID NO.5) and a CDR6 sequence corresponding to FQGTHVPYT (SEQ ID NO.6), or

h) A CDR4 sequence corresponding to RSSQSLVHSYGNTYLN (SEQ ID NO.17); a CDR5 sequence corresponding to KVSNRDS (SEQ ID NO.18) and a CDR6 sequence corresponding to QQSYSTPYT (SEQ ID NO.19), or

i) A CDR4 sequence corresponding to RSSQSLVHSYGNSYLY (SEQ ID NO.20); a CDR5 sequence corresponding to AVSSLQS (SEQ ID NO.21) and a CDR6 sequence corresponding to QQRSNVPLT (SEQ ID NO.22), or

j) A CDR7 sequence corresponding to KASQYVGTYVA (SEQ ID NO.7); a CDR8 sequence corresponding to SASYRHT (SEQ ID NO.8) and a CDR9 sequence corresponding to QQYSSSPYT (SEQ ID NO.9), or

k) A CDR7 sequence corresponding to RASQGISNYLA (SEQ ID NO.24); a CDR8 sequence corresponding to AASSLQS (SEQ ID NO.25) and a CDR9 sequence corresponding to QQYSSYPYT (SEQ ID NO.26), or

l) A CDR7 sequence corresponding to RASQSVSSYLA (SEQ ID NO.27); a CDR8 sequence corresponding to DASNRAT (SEQ ID NO.28) and a CDR9 sequence corresponding to QQRSSSPYT (SEQ ID NO.29).

5. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein said antibody presents a binding affinity for SIRPa of at least 1.10^{-10} M, preferably 1.10^{-11} M.

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6. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein said antibody specifically antagonizes the interaction between SIRPa and CD47.

7. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein said antibody recognizes an epitope included in the D1 domain of SIRPa.

8. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein said antibody or antigen-binding fragment thereof is able to induce the differentiation of monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

9. The antibody or antigen-binding fragment thereof of claim 8, wherein said non suppressive cells secret pro-inflammatory such as cytokines IL6, IL12 and/or TNF, and no or low level of anti-inflammatory cytokines such as IL10 and TGFb.

10. The antibody or antigen-binding fragment thereof of claim 9, wherein said non suppressive cells express iNOS.

11. The antibody or antigen-binding fragment thereof of any one of claims 8, 9 or 10, wherein said non suppressive cells do not express the MHC Class II markers and express the markers CD80 and CD86.

12. The antibody or antigen-binding fragment thereof of claim 8 to 11, wherein said non suppressive cells express at least one marker of the natural killer (NK) cells.

13. The antibody or antigen-binding fragment thereof of any one of claims 1 to 7, wherein said antibody or antigen-binding fragment thereof is able to inhibit M2 phenotypic polarization of macrophages and/or favors pro-inflammatory M1-type macrophage function.

14. The anti-SIRPa antibody or antigen-binding fragment thereof of any one of claims 1 to 12, for use in the treatment of any condition susceptible of being improved or prevented by

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differentiating monocytic myeloid-derived suppressor cells (Mo-MDSC) into non suppressive cells.

15. The anti-SIRPa antibody or antigen-binding fragment thereof of any one of claims 1 to 7 or 13, for use in the treatment of any condition susceptible of being improved or prevented by modifying macrophage polarization to favor pro-inflammatory macrophages.

16. The antibody or antigen-binding fragment thereof of claim 14 or 15, for use according to claim 14 or 15, wherein said condition is a cancer, an infectious disease, a chronic inflammatory disease, auto-immune disease, a trauma or a vaccination.

17. The antibody or antigen-binding fragment thereof of claim 14, for use according to claim 14, wherein said condition is a cancer, an infectious disease, a trauma, an auto-immune disease, a vaccination, a chronic inflammatory disease or a transplant dysfunction.

18. The antibody or antigen-binding fragment thereof of claim 15 for use according to claim 15, wherein said condition is a solid cancer, a liquid cancer, an infectious disease, a trauma, an auto-immune disease, a vaccination, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis or a chronic inflammatory disease.

19. The antibody or antigen-binding fragment thereof of any one of claims 14 to 18, for use according to any one of claims 14 to 18, wherein said antibody or antigen-binding fragment thereof is combined to a second therapeutic agent.

20. The antibody or antigen-binding fragment thereof of claim 19, for use according to claim 19, wherein said second therapeutic is selected from the group consisting of chemotherapeutic agents, radiotherapy, surgery, cell therapy, immunotherapeutic agents, antibiotics and probiotics.

21. The antibody or antigen-binding fragment thereof of claim 20, for use according to claim 20, wherein said immunotherapeutic agent is selected from the group consisting in therapeutic vaccines, immune checkpoint blockers or activators or antibody-drug conjugates.

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22. The antibody or antigen-binding fragment thereof of claim 21, for use according to claim 21, wherein said immune checkpoint blocker or activator is selected from the group consisting in anti-PDL1, anti-PD1, anti-CTLA4, anti-CD137, anti-Her2, anti EGFR, anti-CD20, anti-CD19, anti-CD52 and OX40.

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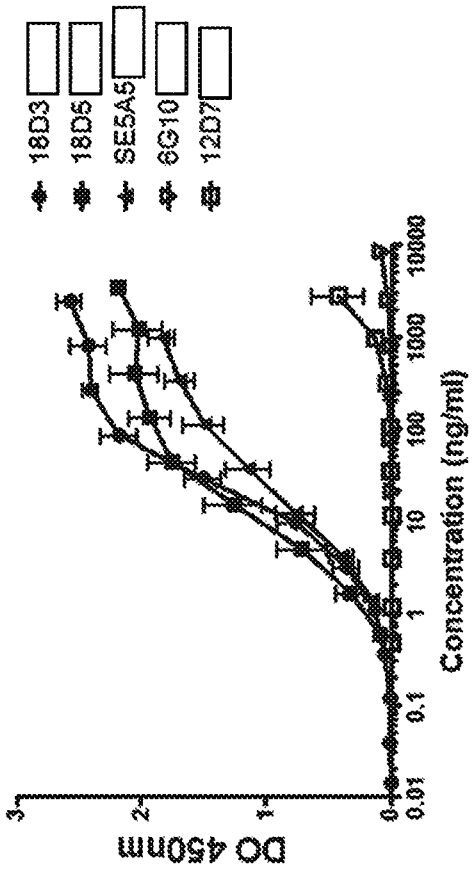


Figure 1

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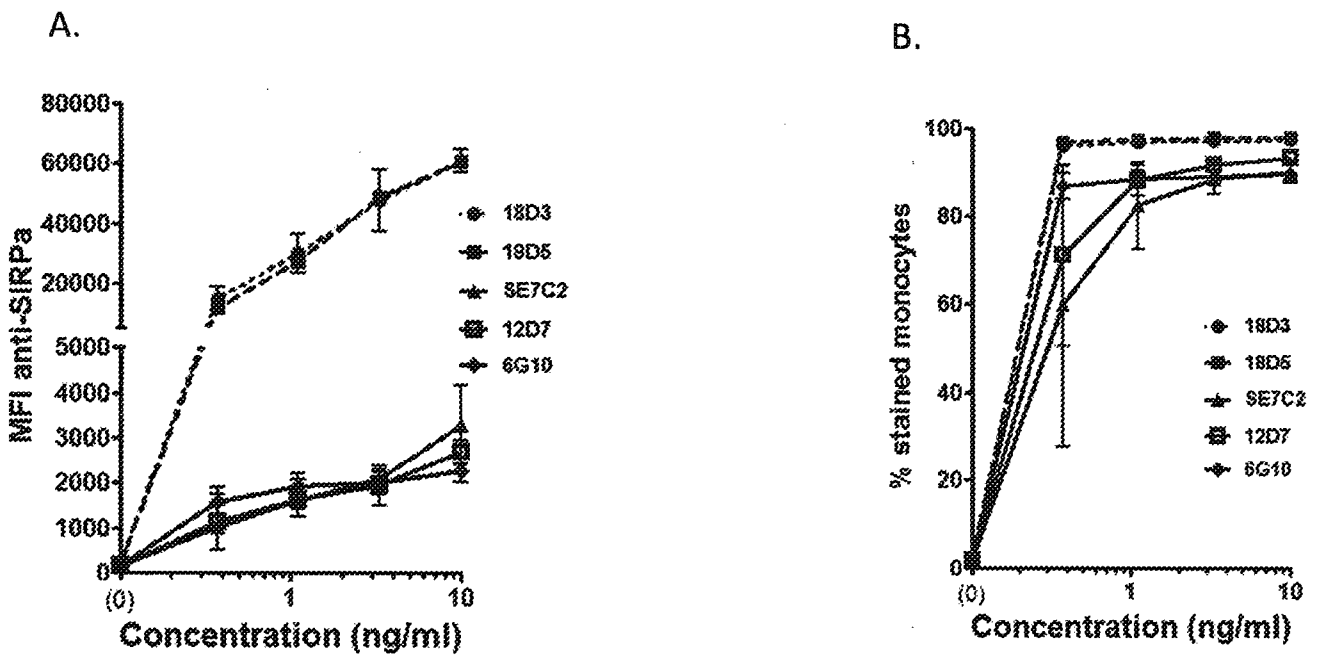
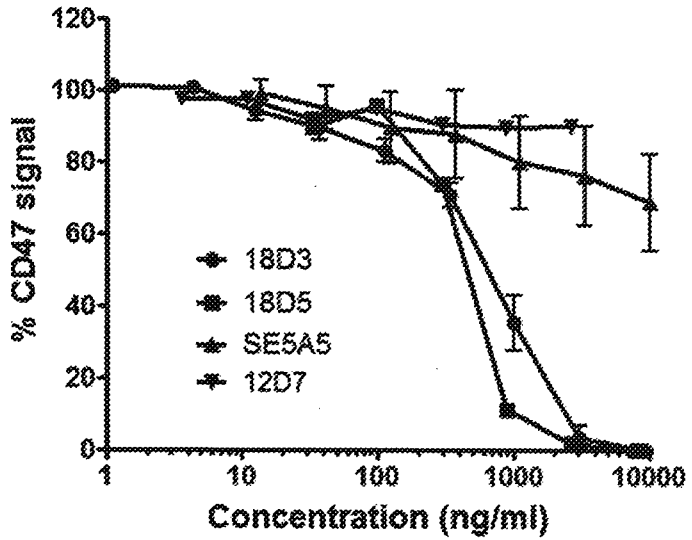


Figure 2

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A.



B.

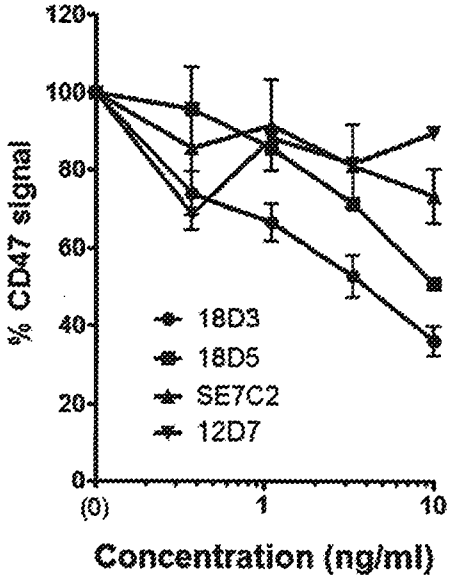


Figure 3

Figure 4

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	045636-5291
	Application Number	
Title of Invention	Human SIRPA Antagonist Antibodies and their Therapeutic Applications	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

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☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	045636-5291
	Application Number	
Title of Invention	Human SIRPA Antagonist Antibodies and their Therapeutic Applications	

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Title of Invention	Human SIRPA Antagonist Antibodies and their Therapeutic Applications	

Application Information:

Title of the Invention	Human SIRPA Antagonist Antibodies and their Therapeutic Applications		
Attorney Docket Number	045636-5291	**Small Entity Status Claimed** ☐	
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	4	**Suggested Figure for Publication (if any)**	

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Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

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☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

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Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status		Remove	
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This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

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Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

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Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	045636-5291
	Application Number	
Title of Invention	Human SIRPA Antagonist Antibodies and their Therapeutic Applications	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	045636-5291
	Application Number	
Title of Invention	Human SIRPA Antagonist Antibodies and their Therapeutic Applications	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:	
If the Applicant is an Organization check here.	☒
Organization Name	Effimune

Mailing Address Information For Applicant:

Address 1	1 rue Gaston Veil		
Address 2			
City	Nantes	**State/Province**	
Country	FR	Postal Code	44035
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	045636-5291
	Application Number	
Title of Invention	Human SIRPA Antagonist Antibodies and their Therapeutic Applications	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/robert smyth/			Date (YYYY-MM-DD)	2016-04-14
First Name	Robert	Last Name	Smyth	Registration Number	50801

Additional Signature may be generated within this form by selecting the Add button.

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From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION, OBTENTION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

GEVERS & ORES
Association N° 92
41, avenue de Friedland
75008 PARIS
FRANCE

Date of mailing *(day/month/year)* 15 May 2017 (15.05.2017)	
Applicant's or agent's file reference ERBIsafl-F2173/8/WO	**IMPORTANT NOTIFICATION**
International application No. PCT/EP2017/059071	International filing date *(day/month/year)* 14 April 2017 (14.04.2017)
International publication date *(day/month/year)* **Not yet published**	Priority date *(day/month/year)* 14 April 2016 (14.04.2016)
Applicant OSE IMMUNOTHERAPEUTICS	

The applicant is hereby notified of the date of receipt (or of obtaining by the International Bureau) of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to the date of receipt, **the priority document concerned was submitted or transmitted to or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-*bis*)**. This Form replaces any previously issued notification concerning submission, transmittal or obtaining of priority documents.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
14 April 2016 (14.04.2016)	62/322,707	US	09 May 2017 (09.05.2017)
17 February 2017 (17.02.2017)	17305182.2	EP	05 May 2017 (05.05.2017)

The letters "NR" denote a priority document which, on the date of mailing of this Form, had not yet been received or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-*bis*). Where the applicant has failed to either submit, request to prepare and transmit, or to request the International Bureau to obtain the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

An asterisk "*" next to a date of receipt, denotes a priority document submitted or transmitted to or obtained by the International Bureau but not in compliance with Rule 17.1(a), (b) or (b-*bis*) (the priority document was received after the time limit prescribed in Rule 17.1(a); the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b) or the request to the International Bureau to obtain the priority document was made after the applicable time limit under Rule 17.1(b-*bis*)). Even though the priority document was not furnished in compliance with Rule 17.1(a), (b) or (b-*bis*), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

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