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(54) **Title**: HUMANIZED, MOUSE OR CHIMERIC ANTI-CD47 MONOCLONAL ANTIBODIES

(57) **Abstract**: Humanized, mouse or chimeric anti-CD47 monoclonal antibodies are provided. The antibodies bind to human glycosylated and deglycosylated CD47 with an optimized Koff value, they disrupt the human CD47-SIRPα interaction, and find use in various therapeutic, preventive or diagnostic methods. The invention includes isolated antibodies and derivatives and fragments thereof, pharmaceutical formulations comprising one or more of the humanized or chimeric anti-CD47 monoclonal antibodies; and cell lines that produce these monoclonal antibodies. Also provided are amino acid and nucleotide sequences of the antibodies.

HUMANIZED, MOUSE OR CHIMERIC ANTI-CD47 MONOCLONAL ANTIBODIES

FIELD OF THE INVENTION

Humanized, mouse or chimeric anti-CD47 monoclonal antibodies are provided. The antibodies bind to human glycosylated and deglycosylated CD47 with an optimized Koff value, they disrupt the human CD47-SIRPα interaction, and find use in various therapeutic, preventive or diagnostic methods. The invention includes isolated antibodies and derivatives and fragments thereof, pharmaceutical formulations comprising one or more of the humanized or chimeric anti-CD47 monoclonal antibodies; and cell lines that produce these monoclonal antibodies. Also provided are amino acid and nucleotide sequences of the antibodies.

BACKGROUND OF THE INVENTION

Macrophages clear pathogens and damaged or aged cells from the blood stream via phagocytosis. Cell-surface CD47 interacts with its receptor on macrophages, SIRPα, to inhibit phagocytosis of normal, healthy cells. CD47 is a broadly expressed transmembrane glycoprotein with a single Ig-like domain and five membrane spanning regions, which functions as a cellular ligand for SIRPα with binding mediated through the NH2-terminal V-like domain of SIRPα. SIRPα is expressed primarily on myeloid cells, including macrophages, granulocytes, myeloid dendritic cells (DCs), mast cells, and their precursors, including hematopoietic stem cells (HSCs).

SIRPα inhibits the phagocytosis of host cells by macrophages, where the ligation of SIRPα on macrophages by CD47 expressed on the host target cell generates an inhibitory signal mediated by SHP-1 that negatively regulates phagocytosis. SIRPα acts to detect signals provided by "self", to negatively control innate immune effector function against these cells.

In keeping with the role of CD47 to inhibit phagocytosis of normal cells, there is evidence that it is upregulated on a number of cancers. Overexpression of CD47 on a cancer cell line, such as e.g. myeloid leukemia, increases its pathogenicity by allowing it to evade phagocytosis. Thus, CD47 up-regulation is an important mechanism that provides protection to normal HSCs during inflammation-mediated mobilization, and that cancer cells co-opt in order to evade macrophage killing.

The present invention provides anti-CD47 antibodies showing various desirable characteristics for the treatment of a disease such as cancer in a subject, e.g. a human.

SUMMARY

In one aspect, the present invention relates to an antibody that binds to glycosylated and non-glycosylated CD47, wherein binding of the antibody to CD47 is not dependent on glycosylation of CD47. In one embodiment, the antibody binds glycosylated and deglycosylated forms of human CD47. In another embodiment, the glycosylated form of human CD47 comprises one or more N-glycosylated residues at positions N23, N34, N50, N73, N111 and/or N206 in the amino acid sequence of human CD47. The deglycosylated form of human CD47 may comprise glycosylated human CD47 that has been treated with a peptide N-glycosidase (PNGase) for removal of N-glycosylations.

In one embodiment, a ratio of EC50s of binding of the antibody to non-glycosylated versus glycosylated forms of CD47 is less than 4:1, 3:1 or 2:1, preferably in a range from 4:1 to 1:4, more preferably 3:1 to 1:3, most preferably 2:1 to 1:2; or a ratio of EC95s of binding of the antibody to non-glycosylated versus glycosylated forms of CD47 is less than 25:1, 20:1 or 10:1, preferably in a range from 10:1 to 1:10, more preferably 9:1 to 1:9, most preferably 10:1 to 1:10.

In another embodiment, the antibody binds to each of glycosylated and non-glycosylated CD47 with an equilibrium binding constant of 80 pM or lower, preferably 70 pM or lower, more preferably 60 pM or lower.

In another embodiment, a maximum binding capacity ($Bmax_2$) of the antibody to non-glycosylated CD47 is at least 60% of a maximum binding capacity ($Bmax_1$) of the antibody to glycosylated CD47.

Preferably the antibody has a Koff value for binding to glycosylated and/or non-glycosylated CD47 of about 1.0×10^{-4} s^{-1} (1/s) or more. More preferably the antibody has a Koff value for binding to glycosylated and/or non-glycosylated CD47 of from 1.0×10^{-4} s^{-1} to 1.0×10^{-3} s^{-1}. Most preferably the antibody has a Koff value for binding to glycosylated and/or non-glycosylated CD47 of from 2.5×10^{-4} s^{-1} to 5.0×10^{-4} s^{-1}.

In another aspect, the invention provides an antibody that binds to CD47, wherein the antibody has a Koff value for binding to CD47 of from 1.0×10^{-4} s-1 to 1.0×10^{-3} s^{-1}.

Compositions and methods are provided relating to humanized or chimeric anti-CD47 monoclonal antibodies. The antibodies of the invention bind to CD47 and have a unique functional profile. In particular said antibodies have at least one of the following characteristics: disrupt the human CD47-SIRPα interaction, inhibit the CD47-SIRPα signal transduction, increase phagocytosis of certain CD47 expressing cells, do not cause a significant level of agglutination of cells, and find use in various therapeutic methods. Preferred are antibodies that bind to human CD47 and that are in the IgG4 format. Preferred are antibodies in the bispecific format that bind to human CD47 may also be in the IgG1 format. Preferred are non-activating antibodies with high Koff values (suggesting rapid dissociation kinetics) and weak or absent apoptosis profiles and red blood cell agglutination activity (suggesting low toxicity). Furthermore, preferred are antibodies that bind to human CD47 of different glycosylation pattern and/or to de-glycosylated forms of human CD47. Embodiments of the invention include isolated antibodies and derivatives and fragments thereof, pharmaceutical formulations comprising one or more of the humanized or chimeric anti-CD47 monoclonal antibodies; and cell lines that produce these monoclonal antibodies. Also provided are amino acid and nucleotide sequences of the antibodies.

Antibodies of interest include the provided humanized, engineered or chimeric antibodies, and variants thereof. The monoclonal antibodies of the invention find particular utility as reagents for the diagnosis and immunotherapy of disease associated with CD47 in humans, particularly in cancer therapy such as hematological and solid tumors. An advantage of the monoclonal antibodies of the invention derives from the humanization process. Thus, *in vivo* use of the monoclonal antibodies of the invention for immunotherapy greatly reduces the problem of significant host immune responses to the antibodies.

Various forms of the antibodies are contemplated herein. For example, the anti-CD47 antibody may be a full length chimeric or humanized antibody, e.g. having a human immunoglobulin constant region of any isotype, e.g. lgG1, lgG2, lgG3, lgG4, IgA, etc., or an antibody fragment, e.g. a single chain antibody, an F(ab')2 fragment, an F(ab) fragment, etc. Fragments comprising CDR regions are also of interest, e.g. for imaging purposes, although the IgG4 format is a clearly preferred form. For a bi-specific approach, the IgG1 format may also be suitable. Furthermore, the antibody may be labeled with a detectable label, immobilized on a solid phase and/or conjugated with a heterologous compound. The antibody may also be provided as a bi-specific or multi-specific antibody reactive with a second antigen, particularly including cancer antigens. A preferred bi-specific antibody is an antibody that is functionally reactive with the Her-2 antigen. Bi-specific antibodies of preferred use are those with a medium binding to human CD47 such as e.g. candidate 20 and 22 and variants thereof (e.g. humanized version thereof).

Diagnostic and therapeutic uses for the antibody are contemplated, particularly relating to the detection and elimination of undesirable cells expressing CD47. In one diagnostic application, the invention provides a method for determining the presence of CD47-expressing cancer cells, comprising exposing a patient sample suspected of containing CD47-expressing cancer cells to the anti-CD47 antibody and determining binding of the antibody to the sample. For this use, the invention provides a kit comprising the antibody and instructions for using the antibody.

The antibodies of the invention are particularly efficacious in the treatment of disease, e.g. increasing the phagocytosis of CD47-expressing cells. Treatment may be systemic or localized, e.g. delivery by intra-tumoral injection, etc.

Embodiments of the invention include isolated antibodies and derivatives and fragments thereof that comprise at least 3 CDR sequences as provided herein, usually in combination with framework sequences from a human variable region. In some embodiments an antibody comprises at least one light chain comprising the 3 light chain CDR sequences provided herein situated in a variable region framework, which may be, without limitation, a human or mouse variable region framework, and at least one heavy chain comprising the 3 heavy chain CDR sequences provided herein situated in a variable region framework, which may be, without limitation, a human or mouse variable region framework.

In other embodiments, the antibody comprises an amino acid sequence variant of the CDRs of the provided 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). Such variants will normally having a binding affinity for human CD47 of at least about 10^{-8} M, such as e.g. a binding affinity to human CD47 between 2 nM to 15 nM, disrupting the human CD47-SIRPα interaction and will bind to the same epitope as an antibody having the amino acid sequence of those set forth herein. Various forms of the antibodies are contemplated herein. For example, the antibody may be a full length antibody, e.g. having a human immunoglobulin constant region of any isotype, e.g. lgG1, lgG2, lgG3, lgG4, IgA, more preferably IgG4 optionally with mutation(s) or an antibody fragment, e.g. an F(ab')2 fragment, an F(ab) fragment, etc. Furthermore, the antibody may be labeled with a detectable label, immobilized on a solid phase and/or conjugated with a heterologous compound.

Embodiments of the invention include anti-CD47 antibodies (i) with potent disruption of the human CD47-SIRPα interaction regardless of the antibody isotype (and effector function), (ii) enabling efficient phagocytosis of CD47-expressing tumor cells by human monocyte-derived macrophages, (iii) with an optimized Koff value of between 1.0 x 10^{-4} s-1 to 1.0 x 10^{-3} s-1 (enabling low sink effect and greater availability of antibodies to higher density CD47-carrying cancer cells), and/or (iv) showing inhibition of tumor growth in xenograft mouse models overexpressing the target CD47. A preferred embodiment of the invention is a group of anti-CD47 antibodies that additionally show weak red blood cell agglutination activity suggesting additional low toxicity (candidate NOs 19, 33, 20) and/or do not induce (or only slightly induce) apoptosis of Jurkat cells (candidate NOs 7, 19, 20, 22, 33). Furthermore, a more preferred embodiment of the invention is a group of anti-CD47 antibodies that additionally bind to glycosylated and non-glycosylated CD47 extracellularly or bind to an immunologically active and glycosylated and non-glycosylated fragment thereof. Furthermore, a more preferred embodiment of the invention is a group of anti-CD47 antibodies that bind the monomer and dimer of CD47. Furthermore, a more preferred embodiment of the invention is a group of anti-CD47 antibodies that bind to a particular epitope on CD47, i.e. in particular a discontinuous epitope that comprises K59, R63, Y66, T67, H108, T109, T117 and T120 of human CD47 when numbered in accordance with SEQ ID NO: 151 (e.g. candidate 20 and humanized and engineered version thereof). Another example of an embodiment of the invention is a group of anti-CD47 antibodies that bind to a particular epitope on CD47, i.e. in particular a discontinuous epitope that comprises K59, K61, S107, H108, T117, T120 and R121 of human CD47 when numbered in accordance with SEQ ID NO: 151 (e.g. candidate 22 and humanized and engineered version thereof).

Embodiments of the invention include anti-CD47 antibodies (i) with potent disruption of the human CD47-SIRPα interaction regardless of the antibody isotype (and effector function), (ii) enabling efficient phagocytosis of CD47-expressing tumor cells by human monocyte-derived macrophages, (iii) with an optimized Koff value of between 1.0 x 10^{-4} s-1 to 1.0 x 10^{-3} s-1 (enabling low sink effect and greater availability of antibodies to higher density CD47-carrying cancer cells), (iv) showing inhibition of tumor growth in xenograft mouse models overexpressing the target CD47, and/or (v) and CDR combinations as set out in tables 2 and 3 below, wherein up to 5, more preferably up to 4, 3, 2 or 1 of the amino acids in one or more of the CDRs are replaced by other amino acids such as e.g. conservative substitutions, wherein however the overall profile of the antibody such as the elements (i) to (iv) above is unchanged or similar, i.e. within a 10% range of standard biological variance.

The invention further provides: an isolated nucleic acid encoding the antibodies and variants thereof; a vector comprising that nucleic acid, optionally operably linked to control sequences recognized by a host cell transformed with the vector; a host cell comprising that vector; a process for producing the antibody comprising culturing the host cell so that the nucleic acid is expressed and, optionally, recovering the antibody from the host cell culture (e.g. from the host cell culture medium). The invention also provides a composition comprising one or more of the anti- human CD47 antibodies and a pharmaceutically acceptable carrier or diluent. This composition for therapeutic use is sterile and may be lyophilized, e.g. being provided as a pre-pack in a unit dose with diluent and delivery device, e.g. inhaler, syringe, etc.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are not intended to be drawn to scale. The Figures are illustrative only and are not required for enablement of the disclosure. For purposes of clarity, not every component may be labeled in every drawing. In the drawings:

Figure 1 Inhibition of hSIRPα binding to hCD47 expressed on CHO cells by anti-CD47 antibodies.

Figure 2 Binding profiles of anti-CD47 antibodies to hCD47 expressed on Raji cells.

Figure 3 Inhibition of hSIRPα binding to hCD47 expressed on Raji cells by anti-CD47 antibodies.

Figure 4 Enhancement of Raji cell phagocytosis by human macrophages with anti-CD47 antibodies.

Figure 5 Agglutination of purified human RBCs by anti-CD47 antibodies.

Figure 6 Profiles of association and dissociation of anti-CD47 antibodies with hCD47 as measured by SPR.

Figure 7 The amino-acid sequences of the 5 humanized VH variants (VH1 to VH5) aligned with the sequence of the mouse VH sequence of candidate 20 (VH0). The CDRs are underlined (using the combined definition of KABAT and IMGT).

Figure 8 The amino-acid sequences of the 5 humanized VL variants (VL1 to VL5) aligned with the sequence of the mouse VL sequence of candidate 20 (VL0). The CDRs are underlined (using the combined definition of KABAT and IMGT).

Figure 9 Effect of anti-CD47 antibodies on A) the growth of human Raji lymphoma cells in NOG mice and B) survival of NOG mice after Raji tumor cell engraftment (*p<0.05; **p<0.005; Mantel-Cox test).

Figure 10 Effect of anti-CD47 candidate 20 alone or in combination with Herceptin® or Erbitux® on growth of A2780/Luc human ovarian tumors in NOG mice. A2780/Luc cells were engrafted intraperitoneally (IP) in NOG mice (n=4/group) and the antibody treatment was started 1 day after graft for up to 5 weeks (3 injections/week, IP) at 10 mg/kg. (A) Ventral and dorsal luminescence observed in each mouse at Day 28. (B) Quantification of the bioluminescence intensity (dorsal + ventral) plotted against the time after tumor engraftment. Treatment groups were compared to the vehicle group at day 28 by using the unpaired t-test of the GraphPad Prism software (**p<0.005; ***p<0.001).

Figure 11 Effect of anti-CD47 candidate 20 alone or in combination with Herceptin® or Erbitux® on the growth of A549 human lung tumor cells in NOG mice. A549 cells were engrafted subcutaneously in NOG mice (n=4/group) and the antibody treatment was started when the tumor was palpable (day 10) for up to 10 weeks (3 injections/week, IP) at 10 mg/kg. (A) Tumor cell growth as measured by the mean tumor volume +/- SD (cm^3) for each group plotted against the time after tumor engraftment. Treatment groups were compared to the vehicle group by using the rate-based T/C method described by Hather et al. (Hather G., Liu R., Bandi S., Mettetal J., et al. "Growth Rate Analysis and Efficient Experimental Design for Tumor Xenograft Studies." Cancer Informatics 13(S4):65-72 (2014)), (*p<0.05; **p<0.005). (B) Survival curves of the mice. Treatment groups were compared to the vehicle group by using the Log-rank (Mantel-Cox) Test of the GraphPad Prism software (*p<0.05; **p<0.01).

Figure 12 Binding of humanized variants of anti-CD47 Candidate 20 on Raji cells by flow cytometry. Humanized variants with the VL-CDR2-F56Y mutation (CDR2 with SEQ ID Nos: 152 (Kabat) and 153 (IMGT), i.e. candidates 20.26 (h20-H2-L5Y), 20.27 (h20-H3-L2Y), 20.28 (h20-H3-L3Y), 20.29 (h20-H4-L4Y), 20.30 (h20-H4-L5Y)) were compared with the corresponding variants without the F56Y mutation candidates 20.10 (h20-H2-L5), 20.12 (h20-H3-L2), 20.13 (h20-H3-L3), 20.19 (h20-H4-L4), 20.20 (h20-H4-L5)) and with the chimeric candidate 20 (all antibodies in human IgG4 format).

Figure 13 Inhibition of hSIRPα binding to hCD47 on Raji cells by humanized variants of anti-CD47 Candidate 20. Humanized variants with the VL-CDR2-F56Y mutation (CDR2 with SEQ ID Nos: 152 (Kabat) and 153 (IMGT), i.e. candidates 20.26 (h20-H2-L5Y), 20.27 (h20-H3-L2Y), 20.28 (h20-H3-L3Y), 20.29 (h20-H4-L4Y), 20.30 (h20-H4-L5Y)) were compared with the corresponding variants without the F56Y mutation candidates 20.10 (h20-H2-L5), 20.12 (h20-H3-L2), 20.13 (h20-H3-L3), 20.19 (h20-H4-L4), 20.20 (h20-H4-L5)).

Figure 14 Comparison of VL-CDR2-F56Y humanized variants of candidate 20 (i.e. candidates 20.26 (h20-H2-L5Y), 20.27 (h20-H3-L2Y), 20.28 (h20-H3-L3Y), 20.29 (h20-H4-L4Y), 20.30 (h20-H4-L5Y)) with antibodies AB06.12 and Hu5F9 in the CD47 binding assay and in the CD47/SIRPα interaction assay on Raji cells.

Figure 15 Epitope mapping of the interaction between candidate 20 and hCD47.

Figure 16 Epitope mapping of the interaction between candidate 22 and hCD47.

Figure 17 Analysis of the soluble hCD47 by SEC-HPLC showed heterogeneity in the protein preparation, with the presence of monomer and dimer of CD47.

Figure 18 SDS-PAGE analysis of purified hCD47 monomer and dimer fractions, at ~30kDa and >50 kDa respectively.

Figure 19 Recognition of hCD47 monomer and dimer by candidates 20 and 22 by binding ELISA.

Figure 20 Release of anti-CD47 antibodies after binding to RBCs. Human RBCs were first stained with 1 μg/mL of mouse anti-CD47 candidates 14, 19, 20, 22 or with mouse 2A1 or B6H12 antibody at +4°C. After washing, RBCs were incubated for 6 hours (T+6h) or for 24 hours (T+24h) at +37°C in antibody-free medium, and the residual levels mouse anti-CD47 antibodies fixed on RBCs was revealed with a PE-conjugated anti-mouse IgG antibody and flow cytometry. The results were expressed as the % of decrease of the mean fluorescence intensity (MFI) measured at T+6h or T+24h on the RBCs stained with an anti-CD47 antibody compared to the MFI obtained for the staining of RBCs with the same antibody before the 6 and 24 hour incubations (T0).

Figure 21 Release of anti-CD47 antibodies after binding to Raji cells and decrease of phagocytosis of Raji tumor cells after their staining by anti-CD47 antibodies. (A) Raji lymphoma cells were first stained with 1 µg/mL of mouse anti-CD47 candidates 14, 19, 20, 22 or with mouse 2A1 or B6H12 antibody. After washing, Raji cells were incubated for 24 hours (T+24h) at +37°C in antibody-free medium, then washed and fixed with PFA 4%. The residual levels of mouse anti-CD47 antibodies fixed on Raji cells was then revealed with a PE-conjugated anti-mouse IgG antibody and flow cytometry. The results were expressed as the % of decrease of the mean fluorescence intensity (MFI) measured at T+24h on the cells stained with an anti-CD47 antibody compared to the MFI obtained for the staining of PFA-fixed Raji cells with the same antibody before the 24 hour incubations (T0). (B) CFSE-labelled Raji lymphoma cells were first stained with 0.1 or 1 µg/mL of mouse anti-CD47 candidates 14, 19, 20, 22, or with mouse 2A1 or B6H12 antibody. After washing, Raji cells were incubated for 24 hours (T+24h) at +37°C in antibody-free medium, then washed and tested in a phagocytosis assay by flow cytometry with Far-Red-labelled human macrophages (hMDM). The results were expressed as the % of decrease of the phagocytosis measured at T+24h for Raji cells stained with an anti-CD47 antibody compared to the phagocytosis of the Raji cells stained with the same antibody before the 24 hour incubation period (T0).

Figure 22 Recognition of human glycosylated (A) and N-Deglycosylated (B) CD47 by chimeric candidates 20 and 22, humanized h20-H2L5Y antibody, and humanized Hu5F9 and AB06.12 antibodies, as measured by ELISA on plates coated with glycosylated or N-deglycosylated hCD47. All antibodies were tested in a hu-IgG4 format.

Figure 23 Efficacy of the humanized h20-H2L5Y antibody alone or in combination with Herceptin® in the A549 NSCLC xenograft model in NOG mice. A549 cells were engrafted subcutaneously in NOG mice (n=8 per group) and the antibody treatment was started when the tumor was about 100 mm^3 (day 14) for up to 10 weeks. Antibodies were injected IP (3 injections/week), with h20-H2L5Y injected in a hu-IgG4 format (h20-H2L5Y-G4) at 10 mg/kg/dose and Herceptin® (hu-IgG1) injected at 2.5 mg/kg/dose. Survival of the mice was monitored and survival curves are presented. Treatment groups were compared to the vehicle group by using the Log-rank (Mantel-Cox) Test of the GraphPad Prism software (*p<0.05; ***p<0.005). The survival of mice treated with the combination of h20-H2L5Y-G4 and

Herceptin® was significantly enhanced when compared to the group treated with Herceptin® alone ($p<0.01$).

Figure 24 Efficacy of the humanized antibody h20-H2L5Y alone or in combination with Herceptin® in the NCI-N87 gastric xenograft model in NOG mice. NCI-N87 cells (10 x 10^6) were engrafted subcutaneously in NOG mice (n=8 per group) and the antibody treatment was started when the tumor was about 100 mm^3 (day 7) for up to 5 weeks. Antibodies were injected IP (3 injections/week), with h20-H2L5Y injected in a hu-IgG4-S228P-L235E format (h20-H2L5Y-G4PE) at 10 mg/kg/dose and Herceptin® (hu-IgG1) injected at 2.5 mg/kg/dose. Tumor cell growth was monitored by measuring the tumor volume. The mean tumor volume (cm^3) +/- SD of each group was presented for different times until the first mouse died or was sacrificed into the respective groups. Treatment groups were compared to the vehicle group by using the rate-based T/C method described by Hather et al. (Hather G., Liu R., Bandi S., Mettetal J., et al. “Growth Rate Analysis and Efficient Experimental Design for Tumor Xenograft Studies.” Cancer Informatics 13(S4):65-72 (2014)), (*$p<0.05$; ***$p<0.0005$). The growth of the NCI-N87 tumor cells was also significantly reduced in the group of animals treated with the combination of h20-H2L5Y-G4PE plus Herceptin® when compared to the animals treated with Herceptin® alone ($p<0.05$).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to monoclonal antibodies which are specific for CD47. Also disclosed are nucleic acid and amino acid sequences of such antibodies. The antibodies find use in therapeutic and diagnostic methods associated with CD47.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is a human.

The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multi-specific antibodies (e.g., bi-specific antibodies), and antibody fragments so long as they exhibit the desired biological activity. "Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

As used in this invention, the term "epitope" means any antigenic determinant on an antigen to which the paratope of an antibody binds. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

"Native antibodies and immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains (Clothia C, Novotný J, Bruccoleri R, Karplus M. "Domain association in immunoglobulin molecules. The packing of variable domains." J. Mol. Biol. 186:651-63 (1985); Novotny J. and Haber E. "Structural invariants of antigen binding: comparison of immunoglobulin VL-VH and VL-VL domain dimers." Proc. Natl. Acad. Sci. U.S.A. 82:4592-96 (1985)).

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (for KABAT annotation see Kabat E.A.Sequences of Proteins of Immunological Interest, Fifth Edition, National Institutes of Health, Bethesda, MD (1991) or for IMGT annotation, see http://www.imgt.org). The constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity (ADCC).

The CDR sequences of exemplary anti-CD47 heavy and light chain combinations are set forth in the sequence listing, including SEQ ID NOs: 1-120, 152, 153 (see Table 2 and 3, *supra*).

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')2 fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen recognition and binding site. In a two-chain Fv species, this region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv species (scFv), one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that of a two-chain Fv species. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site. For a review of scFv, see Plückthun A. Antibodies from Escherichia coli, in "The Pharmacology of Monoclonal Antibodies", by Rosenburg and Moore eds., Springer-Verlag, New York, vol. 113, pp. 269-315 (1994).

The Fab fragment also contains the constant domain of the light chain and the first constant domain of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')2 antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these can be further divided into subclasses (isotypes), e.g., IgG1, lgG2, lgG3, lgG4, IgA1, and lgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

"Antibody fragment", and all grammatical variants thereof, as used herein are defined as a portion of an intact antibody comprising the antigen binding site or variable region of the intact antibody, wherein the portion is free of the constant heavy chain domains (i.e. CH2, CH3, and CH4, depending on antibody isotype) of the Fc region of the intact antibody. Examples of antibody fragments include Fab, Fab', Fab'-SH, F(ab')2, and Fv fragments; diabodies; any antibody fragment that is a polypeptide having a primary structure consisting of one uninterrupted sequence of contiguous amino acid residues (referred to herein as a "single-chain antibody fragment" or "single chain polypeptide"), including without limitation (1) single-chain Fv (scFv) molecules (2) single chain polypeptides containing only one light chain variable domain, or a fragment thereof that contains the three CDRs of the light chain variable domain, without an associated heavy chain moiety and (3) single chain polypeptides containing only one heavy chain variable region, or a fragment thereof containing the three CDRs of the heavy chain variable region, without an associated light chain moiety; and multispecific or multivalent structures formed from antibody fragments. In an antibody fragment comprising one or more heavy chains, the heavy chain(s) can contain any constant domain sequence (e.g. CH1 in the IgG isotype) found in a non-Fc region of an intact antibody, and/or can contain any hinge region sequence found in an intact antibody, and/or can contain a leucine zipper sequence fused to or situated in the hinge region sequence or the constant domain sequence of the heavy chain(s).

Unless specifically indicated to the contrary, the term "conjugate" as described and claimed herein is defined as a heterogeneous molecule formed by the covalent attachment of one or more antibody fragment(s) to one or more polymer molecule(s), wherein the heterogeneous molecule is water soluble, i.e. soluble in physiological fluids such as blood, and wherein the heterogeneous molecule is free of any structured aggregate. A conjugate of interest is PEG. In the context of the foregoing definition, the term "structured aggregate" refers to (1) any aggregate of molecules in aqueous solution having a spheroid or spheroid shell structure, such that the heterogeneous molecule is not in a micelle or other emulsion structure, and is not anchored to a lipid bilayer, vesicle or liposome; and (2) any aggregate of molecules in solid or insolubilized form, such as a chromatography bead matrix, that does not release the heterogeneous molecule into solution upon contact with an aqueous phase. Accordingly, the term "conjugate" as defined herein encompasses the aforementioned heterogeneous molecule in a precipitate, sediment, bioerodible matrix or other solid capable of releasing the heterogeneous molecule into aqueous solution upon hydration of the solid.

The term "monoclonal antibody" (mAb) as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Each mAb is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they can be synthesized by hybridoma culture or mammalian cell lines, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made in an immortalized B cell or hybridoma thereof, or may be made by recombinant DNA methods.

The monoclonal antibodies herein include hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of an anti-CD47 antibody with a constant domain (e.g. "humanized" antibodies), or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (e.g., Fab, F(ab')2, and Fv), so long as they exhibit the desired biological activity.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity.

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, the antibody will be purified (1) to greater than 75% by weight of antibody as determined by the Lowry method, and most preferably more than 80%, 90% or 99% by weight, or (2) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The term "epitope tagged" when used herein refers to an anti-CD47 antibody fused to an "epitope tag". The epitope tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the CD47 antibody. The epitope tag preferably is sufficiently unique so that the antibody specific for the epitope does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least 6 amino acid residues and usually between about 8-50 amino acid residues (preferably between about 9-30 residues). Examples include the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan G.I., Lewis G.K., Ramsay G., et al. "Isolation of monoclonal antibodies specific for human c-myc proto-oncogene product", Mol. Cell. Biol. 5(12):3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky L.R., Fendly B.M., Fisher K.L., et al. “Mammalian cell transient expression of tissue factor for the production of antigen", Protein Engineering 3(6):547-553 (1990)).

The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody. The label may itself be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g. controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g. an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Pat. No. 4,275,149.

Glycosylation of CD47

CD47 is subject to post-translational modifications, most notably glycosylation. CD47 has a number of N-terminal glycosylation sites that directly affect cell surface display and regulate interaction with extracellular ligands. For instance, deglycosylated CD47 has a higher avidity for SIRPα than glycosylated CD47 and, vice versa, deglycosylated SIRPα has a higher avidity for CD47 (Subramanian S., Boder E.T., and Discher D. E. "Phylogenetic divergence of CD47 interactions with human signal regulatory protein α reveals locus of species specificity." J. Biolog. Chem. 282(3):1805-18 (2007); Subramanian S., Parthasarathy R., Sen S., Boder E.T., and Discher D.E. "Species and cell type-specific interactions between CD47 and human SIRPα." Blood 107(6):2548-56 (2006)). Reversely, hyperglycosylated SIRPα can disrupt CD47/SIRPα interactions (Ogura T., Noguchi T., Murai-takebe R., Hosooka T., Honma N., and Kasuga M. "Resistance of B16 melanoma cells to CD47-induced negative regulation of motility as a result of aberrant N-glycosylation of SHPS-1." J Biol Chem 279(14): 13711-20 (2004)). Of note, site-directed mutagenesis of N-linked glycosylation sites inhibited cell surface localization of CD47 in yeast models (Parthasarathy R., Subramanian S., Boder E.T., and Discher D.E. "Post-Translational Regulation of Expression and Conformation of an Immunoglobulin Domain in

Yeast Surface Display." Biothech Bioin 93(1):159-68 (2006)), although similar mutagenesis did not affect membrane localization of human CD47 in CHO cells (Subramanian et al., 2006, *supra*). Aberrant glycosylation of either CD47 or SIRPα can also alter downstream responses, with differentially glycosylated SIRPα rendering B16 melanoma cells resistant to CD47-induced inhibition of motility (Ogura et al., 2004, *supra*). In addition, a heavily glycosylated (>250 kD) form of CD47 has been detected in primary and transformed T-cells, endothelial cells and vascular smooth muscle cells (Kaur S., Kuznetsova S.A., Pendrak M.L., Romeo M. J., Li Z., Zhang L., and Roberts D.D. "Heparan Sulfate Modification of the Transmembrane Receptor CD47 Is Necessary for Inhibition of T Cell Receptor Signaling by Thrombospondin-1", J Biol Chem 286(17):14991-15002 (2011)). This modification was located distally from the SIRPα binding site, but was required for TSP-1 mediated inhibitory signaling in T-cells.

Since tumor cells may express aberrant glycosylation patterns, it would be desirable to provide anti-CD47 antibodies that bind to CD47 independently of glycosylation. However, whereas known therapeutically relevant anti-CD47 antibodies typically bind effectively to glycosylated CD47, they typically bind poorly (e.g. with a lower binding affinity/higher equilibrium binding constant or reduced maximum binding capacity (Bmax)) to non-glycosylated or deglycosylated CD47.

In one aspect, the antibodies of the present invention bind to glycosylated and non-glycosylated CD47. For instance in one embodiment, binding of the antibody to CD47 is not dependent on glycosylation of CD47. By this it is meant that glycosylation of CD47 is not required for adequate binding of the antibody to CD47, e.g. the antibody shows significant specific binding to CD47 irrespective of the level of glycosylation of CD47. In other words, binding of the antibody to CD47 is at least partially, significantly or substantially independent of glycosylation.

As used herein, "glycosylated" CD47 typically refers to (e.g. human) CD47 that has been N-glycosylated at one or more residues. Human CD47 may be N-glycosylated at one or more (e.g. asparagine) residues at positions N23, N34, N50, N73, N111 and N206 in the amino acid sequence of human CD47 (SEQ ID NO:151). Preferably glycosylated CD47 is glycosylated at least two, three, four, five or six of the above positions.

"Deglycosylated" CD47 refers to a form of CD47 in which one or more glycan chains (e.g. N-glycans) have been removed. Deglycosylation of CD47 may be effected by treatment with an enzyme such as peptide N-glycosidase (PNGase), e.g. PNGase F, which removes N-glycans. The terms "non-glycosylated" and "deglycosylated" are used herein interchangeably. Thus the non-glycosylated or deglycosylated form typically lacks glycan residues at positions N23, N34, N50, N73, N111 and/or N206 in the amino acid sequence of human CD47 (SEQ ID NO:151).

In one embodiment deglycosylation of CD47 does not significantly affect the avidity/affinity and/or maximum binding capacity of the antibody to (human) CD47. The avidity/affinity and the maximum binding capacity (Bmax) of the antibody for glycosylated and deglycosylated forms of CD47 may be determined using standard assays e.g. as set out in the Examples below. The affinity of an antibody for its target epitope is inversely related to its dissociation constant. The dissociation constant may also be directly related to the EC50 value for binding of the antibody to CD47. EC50 is the concentration of antibody which provides half maximal binding, i.e. at which 50% of the binding sites on CD47 are bound. Also, the EC95 value, which is the concentration of antibody which provides 95% maximal binding, is indicative of the concentration of antibody for which 95% of the binding sites on CD47 are bound. Whichever measurement is used, according to embodiments of the present invention it is the relative values obtained for glycosylated and non-glycosylated forms that is important, i.e. an absolute value does not necessarily need to be determined provided that the glycosylated and non-glycosylated forms show reasonably similar results.

Thus in some embodiments, the affinity of the antibody for non-glycosylated and glycosylated forms of CD47 may be compared by determining a ratio of EC50 values or EC95 values for binding of the antibody to each form. Preferably this ratio is less than 5:1, 4:1, 3:1, or 2:1 but may be even higher for the EC95, i.e. may be 25:1, 20:1, 10:1, preferably in a range from 10:1 to 1:10, more preferably 9:1 to 1:9, most preferably 10:1 to 1:10. In other words, the EC50 for binding of the antibody to non-glycosylated CD47 may be no more than 3 or 2 fold higher than the EC50 value for binding of the antibody to glycosylated CD47. The EC95 for binding of the antibody to non-glycosylated CD47 may be no more than 9 or 10 fold higher than the EC95 value for binding of the antibody to glycosylated CD47. In further embodiments, the ratio of EC50 is preferably in the range from 3:1 to 1:3 or 2:1 to 1:2, most preferably 3:1 to 1:3; or the ratio of EC95 is preferably in the range from 10:1 to 1:10, more preferably 9:1 to 1:9, most preferably 10:1 to 1:10. In such embodiments it is considered that deglycosylation does not significantly affect the binding affinity, i.e. binding of the antibody to CD47 is not dependent on glycosylation. In some embodiments, the antibody binds to the non-glycosylated form of CD47 (and preferably to both the non-glycosylated and glycosylated forms of CD47) with an equilibrium binding constant of 80 pM or lower, preferably 70 pM or lower, more preferably 60 pM or lower.

In other embodiments, deglycosylation of CD47 does not significantly reduce the maximum binding capacity of the antibody to CD47. For instance, the maximum binding capacity (i.e. $Bmax_1$) may be determined for binding of the antibody to glycosylated CD47. $Bmax_1$ relates to the level of binding of the antibody to CD47 at excess antibody concentration, i.e. the maximum level of specific binding between the antibody and CD47. $Bmax_1$ is thus a measure of the concentration of available binding sites for the antibody on glycosylated CD47. CD47 may then be deglycosylated (e.g. using PNGase) and the level of binding to CD47 at excess antibody concentration determined once again (at the same concentration of CD47 in the sample). The maximum binding capacity (i.e. $Bmax_2$) of the antibody to deglycosylated CD47 is therefore a measure of the concentration of available binding sites for the antibody on deglycosylated CD47.

In preferred embodiments, the maximum binding capacity (i.e. $Bmax_2$) of the antibody to non-glycosylated CD47 is at least 60%, or at least 70% of the maximum binding capacity (i.e. $Bmax_1$) of the antibody to glycosylated CD47. In such embodiments it is considered that deglycosylation does not significantly affect the maximum binding capacity, i.e. binding of the antibody to CD47 is not dependent on glycosylation.

In further embodiments, the binding properties of the antibody to glycosylated and deglycosylated forms of CD47 may be compared based on a ratio of EC95 values. EC95 is the concentration of antibody at which 95% of the binding sites on CD47 are bound. Thus in some embodiments, the affinity of the antibody for non-glycosylated and glycosylated forms of CD47 may be compared by determining a ratio of EC95 values for binding of the antibody to each form. Preferably this ratio is less than 10:1 or 9:1. In other words, the EC95 for binding of the antibody to non-glycosylated CD47 may be no more than 10 or 9 times higher than the EC95 for binding of the antibody to glycosylated CD47.

<u>Antibody dissociation kinetics</u>

In another aspect of the present invention, anti-CD47 antibodies are provided having a dissociation rate (Koff) from CD47 within a defined range. In particular, it has been demonstrated that anti-CD47 antibodies with a high dissociation rate characterized by a Koff value superior to $1 \times 10^{-3}\ s^{-1}$ detach strongly and rapidly from RBCs but also lose rapidly most of their functional activity on tumor cells, e.g. enhancement of tumor cell phagocytosis. In contrast, anti-CD47 antibodies with a very slow dissociation rate characterized by a Koff value inferior to $1 \times 10^{-4}\ s^{-1}$ detach more slowly from the tumor cells, but will stay stuck on RBCs and may thus have an important sink effect and possibly more side effects.

Therefore, anti-CD47 antibodies with intermediate dissociation kinetics characterized by a Koff value comprised between 1×10^{-4} and $1 \times 10^{-3}\ s^{-1}$ possess an optimal CD47 binding/release equilibrium, i.e. provide a weak sink effect and side effects while maintaining their anti-tumor efficacy. Accordingly, in one aspect the present invention provides an antibody that binds to CD47 and a Koff value for binding to CD47 comprised between $1.0 \times 10^{-4}\ s^{-1}$ to $1.0 \times 10^{-3}\ s^{-1}$. In particular embodiments, the antibody may bind to glycosylated and/or non-glycosylated CD47 with a Koff value in this range. Preferably the antibody has a Koff value for binding to (e.g. glycosylated and/or non-glycosylated) CD47 comprised between $1.0 \times 10^{-4}\ s^{-1}$ to $1.0 \times 10^{-3}\ s^{-1}$, $2.0 \times 10^{-4}\ s^{-1}$ to $1.0 \times 10^{-3}\ s^{-1}$, $2.5 \times 10^{-4}\ s^{-1}$ to $8.0 \times 10^{-4}\ s^{-1}$, $2.5 \times 10^{-4}\ s^{-1}$ to $5.0 \times 10^{-4}\ s^{-1}$, or $3.0 \times 10^{-4}\ s^{-1}$ to $4.5 \times 10^{-4}\ s^{-1}$.

<u>Antibodies</u>

In one aspect, the present invention is directed to mouse, humanized or chimeric monoclonal antibodies that are specifically binding to human CD47, and cell lines that produce such antibodies. Such anti-CD47 antibodies of the invention show various desirable characteristics for cancer therapy (but not limited thereto) such as potent disruption of the CD47-SIRPα interaction regardless of the antibody isotype (and effector functions), a rapid kinetics of dissociation with Koff values in the range of $1.0 \times 10^{-4}\ s^{-1}$ to $1.0 \times 10^{-3}\ s^{-1}$, $2.0 \times 10^{-4}\ s^{-1}$ to $1.0 \times 10^{-3}\ s^{-1}$, $2.5 \times 10^{-4}\ s^{-1}$ to $8.0 \times 10^{-4}\ s^{-1}$, $2.5 \times 10^{-4}\ s^{-1}$ to $5.0 \times 10^{-4}\ s^{-1}$, or $3.0 \times 10^{-4}\ s^{-1}$ to $4.5 \times 10^{-4}\ s^{-1}$ (wherein the benchmark antibodies show lower Koff values between 5.0 to $8.9 \times 10^{-5}\ s^{-1}$). Furthermore, the antibodies of the invention are released rapidly after binding to CD47 on red blood cells (wherein the benchmark antibodies with lower Koff values are more slowly released). Additionally, the anti-CD47 antibodies of the invention enable efficient phagocytosis of CD47-

expressing tumor cells by human monocyte-derived macrophages. Moreover, the anti-CD47 antibodies of the invention show inhibition of tumor growth in xenograft mouse models overexpressing the target CD47. Moreover, said antibodies have at least one of the following characteristics: disrupt the CD47-SIRPα interaction, bind to glycosylated and non-glycosylated CD47 with a ratio of EC50 on deglycosylated CD47 versus glycosylated CD47 not superior to 3.0 and a ratio of EC95 on deglycosylated CD47 versus glycosylated CD47 not superior to 10.0, inhibit the CD47-SIRPα signal transduction, increase phagocytosis of certain CD47 expressing cells, do not cause a significant level of agglutination of cells, and find use in various therapeutic and diagnostic methods. Preferred are antibodies that bind to human CD47 and that are in the IgG4 format with optional mutation(s) (e.g. replacement of amino acids in order to remove T cell epitope(s) even in the CDR region such as CDR2). A preferred embodiment of the invention is a group of anti-CD47 antibodies that additionally show weak red blood cell agglutination activity suggesting additional low toxicity (candidate NOs 19, 33, 20 (including the humanized and further engineered antibodies of candidate 20)) and/or do not induce (or only slightly induce) apoptosis of Jurkat cells (candidate NOs 7, 19, 20 (including the humanized and further engineered antibodies of candidate 20), 22 (including the humanized and further engineered antibodies of candidate 22), 33). Furthermore, a more preferred embodiment of the invention is a group of anti-CD47 antibodies that additionally bind to glycosylated and non-glycosylated CD47 extracellularly or bind to an immunologically active and glycosylated and non-glycosylated fragment thereof. Furthermore, a more preferred embodiment of the invention is a group of anti-CD47 antibodies that bind to the monomer and dimer of CD47 such as human CD47. Furthermore, a more preferred embodiment of the invention is a group of anti-CD47 antibodies that bind to a particular epitope on CD47, i.e. in particular a discontinuous epitope that comprises K59, R63, Y66, T67, H108, T109, T117 and T120 of human CD47 when numbered in accordance with SEQ ID NO: 151 (e.g. candidate 20 and humanized and engineered version thereof). Another example of an embodiment of the invention is a group of anti-CD47 antibodies that bind to a particular epitope on CD47, i.e. in particular a discontinuous epitope that comprises K59, K61, S107, H108, T117, T120 and R121 of human CD47 when numbered in accordance with SEQ ID NO: 151 (e.g. candidate 22 and humanized and engineered version thereof).

Preferred candidates are candidates 7, 14, 15, 19, 20, 22, 26 and 33. Even more preferred candidates are 7, 19, 20, 22, and 33. Even more preferred candidates are 20 and 22. The most preferred candidate is candidate 20 (including the humanized and further engineered antibodies of candidate 20, i.e. candidates 20.1 to 20.30). The CDRs (in KABAT- (Table 2) and IMGT- (Table 3) annotations) and variable regions (Table 1) of exemplary antibodies (referred herein usually by candidate NOs or antibody name) are provided (IMGT annotations preferred). Antibodies of interest include these provided combinations, as well as fusions of the variable regions to appropriate constant regions or fragments of constant regions, e.g. to generate F(ab)' antibodies. Variable regions of interest include at least one CDR sequence of the provided anti-CD47 antibodies, where a CDR may be 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more amino acids. Alternatively, antibodies of interest include a variable region as set forth in the provided antibodies, or pairs of variable regions sequences as set forth herein. These antibodies may be full length antibodies, e.g. having a human immunoglobulin constant region of any isotype, e.g. lgG1, lgG2, lgG3, lgG4, IgA, more preferably IgG4 optionally with mutation(s), e.g. wherein some or all T cell epitope(s) even if in the CDR region are replaced e.g. by silent conservative amino acid(s) (see also experimental part for examples), examples such as the S228P and L235E mutations, i.e. wherein the S228P and L235E mutations are introduced to avoid potential chain exchanges and to decrease the affinity of hIgG4 to Fc gamma receptors, respectively.

Table 1: Selected candidates with candidate no, antibody name, variable region of heavy chain (VH) SEQ ID NOs and variable region of light chain (VL) SEQ ID NOs:

Candidate NO	Antibody name	Variable Region of Heavy chain (herein also referred to as "VH") SEQ ID NO	Variable Region of Light Chain (herein also referred to as "VL") SEQ ID NO
7	VT008-AL5-10G7-10K7	121	122
14	VT008-AL6-14G1-16K1	123	124
15	VT008-AL6-18G1-18K21	125	126
19	VL008-AL17-7G1-7K6	127	128

20	VL008-AL18-14G4-14K1	129	130
22	VL008-AL13-8G5-8K3	131	132
26	VT008-AL6-10G3-10K1	133	134
29	VT008-AL6-20G7-20K7	135	136
30	VT008-AL6-39G2-39K2	137	138
33	VL008-AL17-8G5-8K7	139	140
20.1	H20-VH1-VL1	141	146
20.2	H20-VH1-VL2	141	147
20.3	H20-VH1-VL3	141	148
20.4	H20-VH1-VL4	141	149
20.5	H20-VH1-VL5	141	150
20.6	H20-VH2-VL1	142	146
20.7	H20-VH2-VL2	142	147
20.8	H20-VH2-VL3	142	148
20.9	H20-VH2-VL4	142	149
20.10	H20-VH2-VL5	142	150
20.11	H20-VH3-VL1	143	146
20.12	H20-VH3-VL2	143	147
20.13	H20-VH3-VL3	143	148
20.14	H20-VH3-VL4	143	149
20.15	H20-VH3-VL5	143	150
20.16	H20-VH4-VL1	144	146
20.17	H20-VH4-VL2	144	147
20.18	H20-VH4-VL3	144	148

20.19	H20-VH4-VL4	144	149
20.20	H20-VH4-VL5	144	150
20.21	H20-VH5-VL1	145	146
20.22	H20-VH5-VL2	145	147
20.23	H20-VH5-VL3	145	148
20.24	H20-VH5-VL4	145	149
20.25	H20-VH5-VL5	145	150
20.26	H20-VH2-VL5,Y; h20-H2-L5Y	142	159
20.27	H20-VH3-VL2,Y; h20-H3-L2Y	143	156
20.28	H20-VH3-VL3,Y; h20-H3-L3Y	143	157
20.29	H20-VH4-VL4,Y; h20-H4-L4Y	144	158
20.30	H20-VH4-VL5,Y; h20-H4-L5Y	144	159
20,Y	VL008-AL18-14G4-14K1, Y	129	154
22.1	H22-VH1-VL1	168	172
22.2	H22-VH1-VL2	168	173
22.3	H22-VH1-VL3	168	174
22.4	H22-VH1-VL4	168	175
22.5	H22-VH2-VL1	169	172
22.6	H22-VH2-VL2	169	173
22.7	H22-VH2-VL3	169	174
22.8	H22-VH2-VL4	169	175
22.9	H22-VH3-VL1	170	172
22.10	H22-VH3-VL2	170	173
22.11	H22-VH3-VL3	170	174

22.12	H22-VH3-VL4	170	175
22.13	H22-VH4-VL1	171	172
22.14	H22-VH4-VL2	171	173
22.15	H22-VH4-VL3	171	174
22.16	H22-VH4-VL4	171	175

Table 2: Selected candidates with candidate no, antibody name, CDRs of heavy chain (VH) SEQ ID NOs and CDRs of light chain (VL) SEQ ID NOs (KABAT annotations):

Candidate NO	Antibody name	SEQ ID NO of CDR1 of VH	SEQ ID NO of CDR2 of VH	SEQ ID NO of CDR3 of VH	SEQ ID NO of CDR1 of VL	SEQ ID NO of CDR2 of VL	SEQ ID NO of CDR3 of VL
7	VT008-AL5-10G7-10K7	1	2	3	7	8	9
14	VT008-AL6-14G1-16K1	13	14	15	19	20	21
15	VT008-AL6-18G1-18K21	25	26	27	31	32	33
19	VL008-AL17-7G1-7K6	37	38	39	43	44	45
20	VL008-AL18-14G4-14K1	49	50	51	55	56	57
22	VL008-AL13-8G5-8K3	61	62	63	67	68	69
26	VT008-AL6-10G3-10K1	73	74	75	79	80	81
29	VT008-AL6-20G7-20K7	85	86	87	91	92	93

30	VT008-AL6-39G2-39K2	97	98	99	103	104	105
33	VL008-AL17-8G5-8K7	109	110	111	115	116	117
20, corrected T cell epitope	VL008-AL18-14G4-14K1, corrected T cell epitope	49	50	51	55	152	57

Table 3: Selected candidates with candidate no, antibody name, CDRs of heavy chain (VH) SEQ ID NOs and CDRs of light chain (VL) SEQ ID NOs (IMGT annotations):

Candidate NO	Antibody name	SEQ ID NO of CDR1 of VH	SEQ ID NO of CDR2 of VH	SEQ ID NO of CDR3 of VH	SEQ ID NO of CDR1 of VL	SEQ ID NO of CDR2 of VL	SEQ ID NO of CDR3 of VL
7	VT008-AL5-10G7-10K7	4	5	6	10	11	12
14	VT008-AL6-14G1-16K1	16	17	18	22	23	24
15	VT008-AL6-18G1-18K21	28	29	30	34	35	36
19	VL008-AL17-7G1-7K6	40	41	42	46	47	48
20	VL008-AL18-14G4-14K1	52	53	54	58	59	60
22	VL008-AL13-8G5-8K3	64	65	66	70	71	72
26	VT008-AL6-10G3-10K1	76	77	78	82	83	84
29	VT008-AL6-20G7-	88	89	90	94	95	96

	20K7						
30	VT008-AL6-39G2-39K2	100	101	102	106	107	108
33	VL008-AL17-8G5-8K7	112	113	114	118	119	120
20, corrected T cell epitope	VL008-AL18-14G4-14K1, corrected T cell epitope	52	53	54	58	153	60

In addition to Fabs, smaller antibody fragments and epitope-binding peptides having binding specificity for at least one epitope of CD47 are also contemplated by the present invention and can also be used in the methods of the invention. For example, single chain antibodies can be constructed according to the method of U.S. Pat. No. 4,946,778, which is incorporated herein by reference in its entirety. Single chain antibodies comprise the variable regions of the light and heavy chains joined by a flexible linker moiety. Yet smaller is the antibody fragment known as the single domain antibody, which comprises an isolate VH single domain. Techniques for obtaining a single domain antibody with at least some of the binding specificity of the intact antibody from which they are derived are known in the art. For instance, Ward et al. (Ward S., Güssow D., Griffiths A.D., Jones P.T., and Winter G. "Binding Activities of a Repertoire of Single Immunoglobulin Variable Domains Secreted from *Escherichia coli*," Nature 341:544-46 (1989)), disclose a method for screening to obtain an antibody heavy chain variable region (H single domain antibody) with sufficient affinity for its target epitope to bind thereto in isolated form.

The invention also provides isolated nucleic acids encoding the humanized or chimeric anti-CD47 antibodies, vectors and host cells comprising the nucleic acid, and recombinant techniques for the production of the antibody. Nucleic acids of interest may be at least about 80% identical to the provided nucleic acid sequences, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or identical. Such contiguous sequences may encode a CDR sequence, or may encode a complete variable region. As is known in the art, a variable region sequence may be fused to any appropriate constant region sequence.

For recombinant production of the antibody, the nucleic acid encoding it is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

The anti-CD47 antibody of this invention may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous or homologous polypeptide, which include a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide, an immunoglobulin constant region sequence, and the like. A heterologous signal sequence selected preferably may be one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native antibody signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected.

An "isolated" nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the antibody nucleic acid. An isolated nucleic acid molecule is different than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the antibody where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

Suitable host cells for cloning or expressing the DNA are the prokaryote, yeast, or higher eukaryote cells. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney cells (293 or 293 cells subcloned for growth in suspension culture, Graham F.L. and Smiley J. "Characteristics of a human cell line transformed by DNA from human adenovirus type 5," J. Gen. Virol. 36:59-72 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells; Chinese hamster ovary cells/dhFr- (CHO/dhFr-, Urlaub G. and Chasin L.A. "Isolation of

Chinese hamster cell mutants deficient in dihydrofolate reductase activity," Proc. Natl. Acad. Sci. USA 77(7):4216-20 (1980)); mouse Sertoli cells (TM4, Mather J.P. "Establishment and characterization of two distinct mouse testicular epithelial cell lines", Biol. Reprod. 23(1):243-251 (1980)); monkey kidney cells (CV1, ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor cells (MMT 060562, ATCC CCL51); TR1 cells (Mather J.P., Zhuang L.Z., Perez-Infante V., and Phillips D.M. "Culture of testicular cells in hormone-supplemented serum-free medium", Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; EB66 cells (see e.g. EP 2150275B1); FS4 cells; and a human hepatoma line (Hep G2). Host cells are transformed with the above-described expression or cloning vectors for anti-CD47 antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark R., Thoren-Tolling K., Sjöquist J. "Binding of immunoglobulins to protein A and immunoglobulin levels in mammalian sera", J. Immunol. Meth. 62(1):1-13 (1983)). Protein G is recommended for human γ3 (Guss M., Eliasson M., Olsson A., Uhlén M., Frej A.K., Jörnvall H., Flock J.I., and Lindberg M. "Structure of the IgG-binding regions of streptococcal protein G", EMBO J. 5(1):1567-1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABX™ resin (J.T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin, SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g., from about 0-0.25M salt).

Methods of Use

The humanized or chimeric monoclonal antibodies of the invention can be used in the modulation of phagocytosis, including the methods set forth in International Application US2009/000319, herein specifically incorporated by reference in its entirety. For example, antibody compositions may be administered to increase phagocytosis of cancer cells expressing CD47 and thus are suitable for the treatment of cancer in a subject by administering an effective amount of humanized or chimeric monoclonal antibodies of the invention.

Pharmaceutical compositions for use in the treatment of cancer comprising the humanized or chimeric monoclonal antibodies of the invention and optionally pharmaceutically suitable excipients or carrier are also provided.

The humanized or chimeric monoclonal antibodies of the invention can be used *in vitro* and *in vivo* to monitor the course of CD47 disease therapy. Thus, for example, by measuring the increase or decrease in the number of cells expressing CD47, particularly cancer cells expressing CD47, it can be determined whether a particular therapeutic regimen aimed at ameliorating disease is effective.

In a preferred embodiment, the humanized or chimeric monoclonal antibodies of the invention can be used in treating, delaying the progression of, preventing relapse of or alleviating a symptom of a cancer or other neoplastic condition, as a monotherapy, or in combinations with other anti-cancer agent(s) (combination therapy). As used herein, the terms "cancer" "neoplasm" and "tumor" are interchangeable. Examples of cancer include, without limitation, gastric cancer, breast cancer, lung cancer, ovarian cancer, cervical cancer, prostate cancer, bladder cancer, colorectal cancer, pancreatic cancer, liver cancer, renal cancer, thyroid cancer, brain cancer, head and neck cancer, hematological cancer, carcinoma, melanoma, leiomyoma, leiomyosarcoma, glioma, glioblastoma, etc. The "hematological cancer" refers to a cancer of the blood, and includes leukemia, lymphoma and myeloma among others. Solid tumors include, for example, gastric tumor, breast tumors, lung tumors, ovarian tumors, prostate tumors, bladder tumors, colorectal tumors, pancreatic tumors, liver tumors, kidney tumors, thyroid tumor, brain tumor, head and neck tumors, esophageal tumors and melanoma tumors, etc. Symptoms associated with cancers and other neoplastic disorders include, but are not limited to, inflammation, fever, general malaise, pain, loss of appetite, weight loss, edema, headache, fatigue, rash, anemia, muscle weakness and muscle fatigue.

The combination therapy can include one or more antibodies of the invention co-formulated with, and/or co-administered with, one or more additional therapeutic agents, e.g., chemotherapeutic or anti-neoplastic agents, such as cytokine and growth factor inhibitors, immunosuppressants, anti-inflammatory agents, metabolic inhibitors, enzyme inhibitors, and/or cytotoxic or cytostatic agents. The term "combination" in this context means that the agents are given substantially contemporaneously, either simultaneously or sequentially. Exemplary chemotherapeutic agents include, but are not limited to, aldesleukin, altretamine, amifostine, asparaginase, bleomycin, capecitabine, carboplatin, carmustine, cladribine, cisapride, cisplatin, cyclophosphamide, cytarabine, dacarbazine (DTIC), dactinomycin, docetaxel, doxorubicin, dronabinol, duocarmycin, etoposide, filgrastim, fludarabine, fluorouracil, gemcitabine, granisetron, hydroxyurea, idarubicin, ifosfamide, interferon alpha, irinotecan, lansoprazole, levamisole, leucovorin, megestrol, mesna, methotrexate, metoclopramide, mitomycin, mitotane, mitoxantrone, omeprazole, ondansetron, paclitaxel (Taxol™), pilocarpine, prochloroperazine, saproin, tamoxifen, taxol, topotecan hydrochloride, vinblastine, vincristine and vinorelbine tartrate.

The antibodies of the invention can be combined with an effective dose of an agent that increases patient hematocrit, for example erythropoietin stimulating agents (ESA). Such agents are known and used in the art, including, for example, Aranesp®, Epogen®NF/Procrit®NF, Omontys®, Procrit®, etc.

In other embodiments, the antibodies of the invention can be combined with an effective dose of other antibodies that have been used in treatment of cancer including, without limitation the following FDA approved monoclonal antibodies: rituximab (Rituxan®, CD20: chimeric IgG1), trastuzumab (Herceptin®, HER2: chimeric IgG1), alemtuzumab (Campath®, CD52: humanized IgG1), ibritumomab tiuxetan (Zevalin®, CD20: murine, IgG1, radiolabeled (Yttrium

90), tositumomab-I-131 (Bexxar®: CD20, murine, IgG2a, radiolabeled (Iodine 131)), cetuximab (Erbitux®, EGFR: cjimeric, IgG1), bevacizumab (VEGF: humanized, IgG4), panitumumab (Vectibix®, EGFR: human IgG2), ofatumumab (Arzerra®, CD20: human IgG1), ipilimumab (Ypervoy®, CTLA-4: human IgG1), brentiuximab vedotin (Adectris®, CD30: chimeric, IgG1, drug-conjugate), pertuzumab (Perjecta®, HER2: humanized IgG1, drug conjugate), adotrastuzumab ematansine (Kadcyla®, HER2: humanized, IgG1, drug-conjugate), obinutuzumab (Gazyva®, CD20: humanized and glycol-engineered), nivolumab and pembrolizumab (anti-PD-1s), etc. Trastuzumab targets the HER-2 antigen. This antigen is seen on 25% to 35% of breast cancers and on metastatic gastric cancers. Trastuzumab is approved for the treatment of HER2-overexpressing breast cancers and for HER2-overexpressing metastatic gastric and gastroesophageal junction adenocarcinoma. Cetuximab is used for the treatment of metastatic colorectal cancer, metastatic non-small cell lung cancer and head and neck cancer. Nivolumab and pembrolizumab have been recently approved to treat metastatic melanoma and non-small cell lung cancer. They are tested in clinical trials for lung cancer, renal-cell cancer, lymphoma and mesothelioma. Other cancer drug that are currently tested in clinical trials or researched may also be combined.

Preferred combinations are combinations of a CD47 antibody of the invention and i) an immune check-point inhibitor or ii) an immune modulator reprogramming the anti-tumor activity of macrophages and dendritic cells or iii) an antibody against a tumor associated antigen. Exemplified combinations are herein described for Herceptin® and Erbitux®, wherein the combination with Herceptin® is preferred due to its additive, cooperative, or possibly synergistic effect. Other agents may also be useful to be combined.

The monoclonal antibodies of the invention may be used *in vitro* in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the monoclonal antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize monoclonal antibodies of the invention are flow cytometry, e.g. FACS, MACS, immunohistochemistry, competitive and non-competitive immunoassays in either direct or indirect formats; and the like. Detection of the antigens using the monoclonal antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The monoclonal antibodies of the invention can be bound to many different carriers and used to detect the presence of CD47-expressing cells. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary skill in the art, which find use as tracers in therapeutic methods, for use in diagnostic methods, and the like. For diagnostic purposes a label may be covalently or non-covalently attached to an antibody of the invention or a fragment thereof, including fragments consisting or comprising of CDR sequences. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, and bio-luminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the monoclonal antibodies of the invention, or will be able to ascertain such, using routine experimentation. Furthermore, the binding of these labels to the monoclonal antibodies of the invention can be done using standard techniques common to those of ordinary skill in the art.

In some embodiments the antibody or a fragment thereof is attached to a nanoparticle, e.g. for use in imaging. Useful nanoparticles are those known in the art, for example including without limitation, Raman-silica-gold-nanoparticles (R-Si-Au-NP). The R- Si-Au-NPs consist of a Raman organic molecule, with a narrow-band spectral signature, adsorbed onto a gold core. Because the Raman organic molecule can be changed, each nanoparticle can carry its own signature, thereby allowing multiple nanoparticles to be independently detected simultaneously by multiplexing. The entire nanoparticle is encapsulated in a silica shell to hold the Raman organic molecule on the gold nanocore. Optional polyethylene glycol (PEG)-ylation of R-Si-Au-NPs increases their bioavailability and provides functional "handles" for attaching targeting moieties (see Thakor A.S., Luong R., Paulmurugan R., et al. et al. "The fate and toxicity of raman-active silica-gold nanoparticles in mice", Sci. Transl. Med. 3(79):79ra33 (2011); Jokerst

J.V., Miao Z., zavaleta C., Cheng Z., and Gambhir S.S. "Affibody-functionalized gold-silica nanoparticles for Raman molecular imaging of the epidermal growth factor receptor", Small. 7(5):625-33 (2011); Gao J., Chen K., Miao Z., Ren G., Chen X., Gambhir S.S., Cheng Z. "Affibody-based nanoprobes for HER2-expressing cell and tumor imaging", Biomaterials 32(8):2141-8 (2011); each herein specifically incorporated by reference).

For purposes of the invention, CD47 may be detected by the monoclonal antibodies of the invention when present in biological fluids and on tissues, *in vivo* or *in vitro*. Any sample containing a detectable amount of CD47 can be used. A sample can be a liquid such as urine, saliva, cerebrospinal fluid, blood, serum and the like, or a solid or semi-solid such as tissues, feces, and the like, or, alternatively, a solid tissue such as those commonly used in histological diagnosis.

Another labeling technique which may result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use haptens such as biotin, which reacts with avidin, or dinitrophenol, pyridoxal, or fluorescein, which can react with specific anti-hapten antibodies.

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 predetermined amounts with instructions for performing the diagnostic assay. Where the antibody is labeled with an enzyme, the kit will include substrates and cofactors required by the enzyme (e.g., a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may be included such as stabilizers, buffers (e.g., a block buffer or lysis buffer) and the like. The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

Therapeutic formulations comprising one or more antibodies of the invention are prepared for storage by mixing the antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences, 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. The antibody composition will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of the antibody to be administered will be governed by such considerations, and is the minimum amount necessary to prevent the CD47-associated disease.

The therapeutic dose may be at least about 0.01mg per kg body weight, at least about 0.05mg per kg body weight; at least about 0.1mg per kg body weight, at least about 0.5mg per kg body weight, at least about 1mg per kg body weight, at least about 2.5mg per kg body weight, at least about 5mg per kg body weight, at least about 10mg per kg body weight, and not more than about 100mg per kg body weight with a preference of 0.1 to 20mg per kg body weight. It will be understood by one of skill in the art that such guidelines will be adjusted for the molecular weight of the active agent, e.g. in the use of antibody fragments, or in the use of antibody conjugates. The dosage may also be varied for localized administration, e.g. intranasal, inhalation, etc., or for systemic administration, e.g., i.m., i.p., i.v., and the like.

The antibody need not be, but is optionally formulated with one or more agents that potentiate activity, or that otherwise increase the therapeutic effect. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

Acceptable carriers, excipients, or stabilizers are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyidimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

The active ingredients may also be entrapped in microcapsule prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. Ed. (1980).

The anti-CD47 antibody is administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the anti-CD47 antibody is suitably administered by pulse infusion, particularly with declining doses of the antibody.

For the prevention or treatment of disease, the appropriate dosage of antibody will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibody is administered for preventive purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments.

In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is the anti-CD47 antibody. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and/or dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made without departing from the spirit or scope of the invention.

SEQUENCES

SEQ ID NO: 1
VT008-AL5-10G7; heavy chain; CDR1-Kabat
KYWMH

SEQ ID NO: 2
VT008-AL5-10G7; heavy chain; CDR2-Kabat
EINPSDTYTNYNQKFKG

SEQ ID NO: 3
VT008-AL5-10G7; heavy chain; CDR3-Kabat
VATMVARGFAY

SEQ ID NO: 4
VT008-AL5-10G7; heavy chain; CDR1-IMGT
GYTFTKYW

SEQ ID NO: 5
VT008-AL5-10G7; heavy chain; CDR2-IMGT
INPSDTYT

SEQ ID NO: 6
VT008-AL5-10G7; heavy chain; CDR3-IMGT
ARVATMVARGFAY

SEQ ID NO: 7
VT008-AL5-10K7; light chain; CDR1-Kabat
KASQDVSGAVV

SEQ ID NO: 8
VT008-AL5-10K7; light chain; CDR2-Kabat
LATYRYT

SEQ ID NO: 9
VT008-AL5-10K7; light chain; CDR3-Kabat
QQYYSIPWT

SEQ ID NO: 10
VT008-AL5-10K7; light chain; CDR1-IMGT
QDVSGA

SEQ ID NO: 11
VT008-AL5-10K7; light chain; CDR2-IMGT
LATY

SEQ ID NO: 12
VT008-AL5-10K7; light chain; CDR3-IMGT
QQYYSIPWT

SEQ ID NO: 13
VT008-AL6-14G1; heavy chain; CDR1-Kabat
NYWMY

SEQ ID NO: 14
VT008-AL6-14G1; heavy chain; CDR2-Kabat

WIDPNSGGTKYNEKFKS

SEQ ID NO: 15
VT008-AL6-14G1; heavy chain; CDR3-Kabat
GGYTMDY

SEQ ID NO: 16
VT008-AL6-14G1; heavy chain; CDR1-IMGT
GYTFTNYW

SEQ ID NO: 17
VT008-AL6-14G1; heavy chain; CDR2-IMGT
IDPNSGGT

SEQ ID NO: 18
VT008-AL6-14G1; heavy chain; CDR3-IMGT
ARGGYTMDY

SEQ ID NO: 19
VT008-AL6-16K1; light chain; CDR1-Kabat
RASQSLVHSNGNTYLH

SEQ ID NO: 20
VT008-AL6-16K1; light chain; CDR2-Kabat
KVSNRFS

SEQ ID NO: 21
VT008-AL6-16K1; light chain; CDR3-Kabat
SQSTHVPLT

SEQ ID NO: 22

VT008-AL6-16K1; light chain; CDR1-IMGT
QSLVHSNGNTY

SEQ ID NO: 23
VT008-AL6-16K1; light chain; CDR2-IMGT
KVSN

SEQ ID NO: 24
VT008-AL6-16K1; light chain; CDR3-IMGT
SQSTHVPLT

SEQ ID NO: 25
VT008-AL6-18G1; heavy chain; CDR1-Kabat
NYWIH

SEQ ID NO: 26
VT008-AL6-18G1; heavy chain; CDR2-Kabat
RIDPNTVDAKYNEKFKS

SEQ ID NO: 27
VT008-AL6-18G1; heavy chain; CDR3-Kabat
GGYTMDY

SEQ ID NO: 28
VT008-AL6-18G1; heavy chain; CDR1-IMGT
GYTFINYW

SEQ ID NO: 29
VT008-AL6-18G1; heavy chain; CDR2-IMGT
IDPNTVDA

SEQ ID NO: 30
VT008-AL6-18G1; heavy chain; CDR3-IMGT
SRGGYTMDY

SEQ ID NO: 31
VT008-AL6-18K21; light chain; CDR1-Kabat
RSSQSLVHSNGNTYLH

SEQ ID NO: 32
VT008-AL6-18K21; light chain; CDR2-Kabat
KVSNRFS

SEQ ID NO: 33
VT008-AL6-18K21; light chain; CDR3-Kabat
FQSTHVPWT

SEQ ID NO: 34
VT008-AL6-18K21; light chain; CDR1-IMGT
QSLVHSNGNTY

SEQ ID NO: 35
VT008-AL6-18K21; light chain; CDR2-IMGT
KVSN

SEQ ID NO: 36
VL008-AL6-18K21; light chain; CDR3-IMGT
FQSTHVPWT

SEQ ID NO: 37
VL008-AL17-7G1; heavy chain; CDR1-Kabat
DYYIN

SEQ ID NO: 38
VL008-AL17-7G1; heavy chain; CDR2-Kabat
WIFPGSGLTYYNKKFKG

SEQ ID NO: 39
VL008-AL17-7G1; heavy chain; CDR3-Kabat
PYYGSRWDYAMDY

SEQ ID NO: 40
VL008-AL17-7G1; heavy chain; CDR1-IMGT
VYTFTDYY

SEQ ID NO: 41
VL008-AL17-7G1; heavy chain; CDR2-IMGT
IFPGSGLT

SEQ ID NO: 42
VL008-AL17-7G1; heavy chain; CDR3-IMGT
ARPYYGSRWDYAMDY

SEQ ID NO: 43
VL008-AL17-7K6; light chain; CDR1-Kabat
KSSQSLLNSNNQKNYLA

SEQ ID NO: 44
VL008-AL17-7K6; light chain; CDR2-Kabat
FASTRES

SEQ ID NO: 45
VL008-AL17-7K6; light chain; CDR3-Kabat

QQHYTTPYT

SEQ ID NO: 46
VL008-AL17-7K6; light chain; CDR1-IMGT
QSLLNSNNQKNY

SEQ ID NO: 47
VL008-AL17-7K6; light chain; CDR2-IMGT
FAST

SEQ ID NO: 48
VL008-AL17-7K6; light chain; CDR3-IMGT
QQHYTTPYT

SEQ ID NO: 49
VL008-AL18-14G4; heavy chain; CDR1-Kabat
DYYIN

SEQ ID NO: 50
VL008-AL18-14G4; heavy chain; CDR2-Kabat
RIYPGIGNTYYNKKFKG

SEQ ID NO: 51
VL008-AL18-14G4; heavy chain; CDR3-Kabat
GHYGRGMDY

SEQ ID NO: 52
VL008-AL18-14G4; heavy chain; CDR1-IMGT
GYSFTDYY

SEQ ID NO: 53

VL008-AL18-14G4; heavy chain; CDR2-IMGT
IYPGIGNT

SEQ ID NO: 54
VL008-AL18-14G4; heavy chain; CDR3-IMGT
ARGHYGRGMDY

SEQ ID NO: 55
VL008-AL18-14K1; light chain; CDR1-Kabat
KSSQSLLNSIDQKNYLA

SEQ ID NO: 56
VL008-AL18-14K1; light chain; CDR2-Kabat
FASTKES

SEQ ID NO: 57
VL008-AL18-14K1; light chain; CDR3-Kabat
QQHYSTPWT

SEQ ID NO: 58
VL008-AL18-14K1; light chain; CDR1-IMGT
QSLLNSIDQKNY

SEQ ID NO: 59
VL008-AL18-14K1; light chain; CDR2-IMGT
FAST

SEQ ID NO: 60
VL008-AL18-14K1; light chain; CDR3-IMGT
QQHYSTPWT

SEQ ID NO: 61
VL008-AL13-8G5; heavy chain; CDR1-Kabat
TYWMH

SEQ ID NO: 62
VL008-AL13-8G5; heavy chain; CDR2-Kabat
MIHPNSGTTNYNEKFKS

SEQ ID NO: 63
VL008-AL13-8G5; heavy chain; CDR3-Kabat
SHYYDGHFSY

SEQ ID NO: 64
VL008-AL13-8G5; heavy chain; CDR1-IMGT
GYTFTTYW

SEQ ID NO: 65
VL008-AL13-8G5; heavy chain; CDR2-IMGT
IHPNSGTT

SEQ ID NO: 66
VL008-AL13-8G5; heavy chain; CDR3-IMGT
TRSHYYDGHFSY

SEQ ID NO: 67
VL008-AL13-8K3; light chain; CDR1-Kabat
KSSQSLLNSRTRKNYLA

SEQ ID NO: 68
VL008-AL13-8K3; light chain; CDR2-Kabat
WASTRES

SEQ ID NO: 69
VL008-AL13-8K3; light chain; CDR3-Kabat
KQSYNLWT

SEQ ID NO: 70
VL008-AL13-8K3; light chain; CDR1-IMGT
QSLLNSRTRKNY

SEQ ID NO: 71
VL008-AL13-8K3; light chain; CDR2-IMGT
WAST

SEQ ID NO: 72
VL008-AL13-8K3; light chain; CDR3-IMGT
KQSYNLWT

SEQ ID NO: 73
VT008-AL6-10G3; heavy chain; CDR1-Kabat
NYWIH

SEQ ID NO: 74
VT008-AL6-10G3; heavy chain; CDR2-Kabat
RIDPNSGGTKYNEKFKS

SEQ ID NO: 75
VT008-AL6-10G3; heavy chain; CDR3-Kabat
GGYTMDY

SEQ ID NO: 76
VT008-AL6-10G3; heavy chain; CDR1-IMGT

GYTFTNYW

SEQ ID NO: 77
VT008-AL6-10G3; heavy chain; CDR2-IMGT
IDPNSGGT

SEQ ID NO: 78
VT008-AL6-10G3; heavy chain; CDR3-IMGT
ARGGYTMDY

SEQ ID NO: 79
VT008-AL6-10K1; light chain; CDR1-Kabat
RSSQSLLHSNGNTYLH

SEQ ID NO: 80
VT008-AL6-10K1; light chain; CDR2-Kabat
KVSYRFS

SEQ ID NO: 81
VT008-AL6-10K1; light chain; CDR3-Kabat
FQSTHVPWT

SEQ ID NO: 82
VT008-AL6-10K1; light chain; CDR1-IMGT
QSLLHSNGNTY

SEQ ID NO: 83
VT008-AL6-10K1; light chain; CDR2-IMGT
KVSY

SEQ ID NO: 84

VT008-AL6-10K1; light chain; CDR3-IMGT
FQSTHVPWT

SEQ ID NO: 85
VT008-AL6-20G7; heavy chain; CDR1-Kabat
NYWIY

SEQ ID NO: 86
VT008-AL6-20G7; heavy chain; CDR2-Kabat
YINPRSDDTKYNQKFRD

SEQ ID NO: 87
VT008-AL6-20G7; heavy chain; CDR3-Kabat
GGFTMDF

SEQ ID NO: 88
VT008-AL6-20G7; heavy chain; CDR1-IMGT
GYTFINYW

SEQ ID NO: 89
VT008-AL6-20G7; heavy chain; CDR2-IMGT
INPRSDDT

SEQ ID NO: 90
VT008-AL6-20G7; heavy chain; CDR3-IMGT
ARGGFTMDF

SEQ ID NO: 91
VT008-AL6-20K7; light chain; CDR1-Kabat
RSSQSLLHSNGNTYLH

SEQ ID NO: 92
VT008-AL6-20K7; light chain; CDR2-Kabat
KVSYRFS

SEQ ID NO: 93
VT008-AL6-20K7; light chain; CDR3-Kabat
SQGTHVPYT

SEQ ID NO: 94
VT008-AL6-20K7; light chain; CDR1-IMGT
QSLLHSNGNTY

SEQ ID NO: 95
VT008-AL6-20K7; light chain; CDR2-IMGT
KVSY

SEQ ID NO: 96
VT008-AL6-20K7; light chain; CDR3-IMGT
SQGTHVPYT

SEQ ID NO: 97
VT008-AL6-39G2; heavy chain; CDR1-Kabat
GYNIY

SEQ ID NO: 98
VT008-AL6-39G2; heavy chain; CDR2-Kabat
YIYPYNGISSYNQKFKD

SEQ ID NO: 99
VT008-AL6-39G2; heavy chain; CDR3-Kabat
GGYTMDY

SEQ ID NO: 100
VT008-AL6-39G2; heavy chain; CDR1-IMGT
GYSFTGYN

SEQ ID NO: 101
VT008-AL6-39G2; heavy chain; CDR2-IMGT
IYPYNGIS

SEQ ID NO: 102
VT008-AL6-39G2; heavy chain; CDR3-IMGT
ARGGYTMDY

SEQ ID NO: 103
VT008-AL6-39K2; light chain; CDR1-Kabat
RSSQSLVKSNGNTYLH

SEQ ID NO: 104
VT008-AL6-39K2; light chain; CDR2-Kabat
KVSNRFS

SEQ ID NO: 105
VT008-AL6-39K2; light chain; CDR3-Kabat
SQTTHVPYT

SEQ ID NO: 106
VT008-AL6-39K2; light chain; CDR1-IMGT
QSLVKSNGNTY

SEQ ID NO: 107
VT008-AL6-39K2; light chain; CDR2-IMGT

KVSN

SEQ ID NO: 108
VT008-AL6-39K2; light chain; CDR3-IMGT
SQTTHVPYT

SEQ ID NO: 109
VL008-AL17-8G5; heavy chain; CDR1-Kabat
DYYIN

SEQ ID NO: 110
VL008-AL17-8G5; heavy chain; CDR2-Kabat
WIFPGSGLTYYNKKFKG

SEQ ID NO: 111
VL008-AL17-8G5; heavy chain; CDR3-Kabat
PYYGSRWDYTMDY

SEQ ID NO: 112
VL008-AL17-8G5; heavy chain; CDR1-IMGT
GYTFTDYY

SEQ ID NO: 113
VL008-AL17-8G5; heavy chain; CDR2-IMGT
IFPGSGLT

SEQ ID NO: 114
VL008-AL17-8G5; heavy chain; CDR3-IMGT
ARPYYGSRWDYTMDY

SEQ ID NO: 115

VL008-AL17-8K7; light chain; CDR1-Kabat
KSSQNLLNSNNQKNHLA

SEQ ID NO: 116
VL008-AL17-8K7; light chain; CDR2-Kabat
FASTRES

SEQ ID NO: 117
VL008-AL17-8K7; light chain; CDR3-Kabat
QQHYTTPYT

SEQ ID NO: 118
VL008-AL17-8K7; light chain; CDR1-IMGT
QNLLNSNNQKNH

SEQ ID NO: 119
VL008-AL17-8K7; light chain; CDR2-IMGT
FAST

SEQ ID NO: 120
VL008-AL17-8K7; light chain; CDR3-IMGT
QQHYTTPYT

SEQ ID NO: 121
VT008-AL5-10G7; variable region heavy chain
QVQLQQPGAELVMPGSSVKLSCKTSGYTFTKYWMHWVKRRPGQGLEWIGEINPSDTYT
NYNQKFKGKSTLTVDKSSSTAYMQLSSLTSEDSAVYFCARVATMVARGFAYWGQGTL
VTVSA

SEQ ID NO: 122
VT008-AL5-10K7; variable region light chain

DIVMTQSHKFMSTSVGDRVSITCKASQDVSGAVVWYQEKPGQSPNLLIYLATYRYTGV
PDRFTGSGSGTDFTLTIRSVQAEDMAVYYCQQYYSIPWTFGGGTKLEIK

SEQ ID NO: 123
VT008-AL6-14G1; variable region heavy chain
QVQLQQPGAELVKPGASLRVSCKASGYTFTNYWMYWVRQRPGRGLEWIGWIDPNSGG
TKYNEKFKSKATLTVDKPSSTAYMQLSSLTSEDSAVYNCARGGYTMDYWGQGTSVTV
SS

SEQ ID NO: 124
VT008-AL6-16K1; variable region light chain
DVVMTQTPLSLPVSLGDQASISCRASQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNR
FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPLTFGAGTKLELK

SEQ ID NO: 125
VT008-AL6-18G1; variable region heavy chain
QVQLQQPGAELVKPGTSVKLSCKASGYTFINYWIHWVKQRPGRGLEWIGRIDPNTVDA
KYNEKFKSKATLTVDKPSSIAYMQLSSLTSEDSAVYYCSRGGYTMDYWGQGTSVTVSS

SEQ ID NO: 126
VT008-AL6-18K21; variable region light chain
DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPTLLIYKVSNRF
SGVPDRFSGSGSGTDFTLKISRVEAEDLGIYFCFQSTHVPWTFGGGTKLEIK

SEQ ID NO: 127
VL008-AL17-7G1; variable region heavy chain
QVQLQQSGPELVKPGASVKISCKASVYTFTDYYINWVKQRPGQGLEWVGWIFPGSGLT
YYNKKFKGKATLTVDKSSSTAYMLLSSLTSEDSAVYFCARPYYGSRWDYAMDYWGQG
TSVTVSS

SEQ ID NO: 128

VL008-AL17-7K6; variable region light chain
DIVMTQSPSSLTMSVGQKVTMSCKSSQSLLNSNNQKNYLAWYQQKPGQSPKLLLYFAS
TRESGVPDRFIGSGSGTDFTLTISSVQAEDLADYFCQQHYTTPYTFGGGTKLEIK

SEQ ID NO: 129
VL008-AL18-14G4; variable region heavy chain
QVQLKQSGAELVRPGASVKLSCKASGYSFTDYYINWVKQRPGQGLEWIARIYPGIGNTY
YNKKFKGKATLTAEKSSSTAYMQLNSLTSEDSAVYFCARGHYGRGMDYWGQGTSVTV
SS

SEQ ID NO: 130
VL008-AL18-14K1; variable region light chain
DIVMTQSPSSLAMSVGQKVTMNCKSSQSLLNSIDQKNYLAWYQQKPGQSPKLLVYFAS
TKESGVPDRFIGSGSGTDFTLTISSVQAEDLADYFCQQHYSTPWTFGGGTKLEIK

SEQ ID NO: 131
VL008-AL13-8G5; variable region heavy chain
QVQLQQPGAELVKPGASVKLSCKASGYTFTTYWMHWVKQRPGQGLEWIGMIHPNSGT
TNYNEKFKSKATLTVDKSSSSTYMQLSSLTSEDSAVYYCTRSHYYDGHFSYWGQGTLV
TVSA

SEQ ID NO: 132
VL008-AL13-8K3; variable region light chain
DIVMSQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWAST
RESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCKQSYNLWTFGGGTRLEIK

SEQ ID NO: 133
VT008-AL6-10G3; variable region heavy chain
QVQLQQPGPELVKPGASVKLSCKASGYTFTNYWIHWLNQRPGRGLEWIGRIDPNSGGT
KYNEKFKSKAILTVDKSSSTTYMQLSSLTSEDSAVYYCARGGYTMDYWGQGTSVTVSS

SEQ ID NO: 134
VT008-AL6-10K1; variable region light chain
DVVMPQTPLSLPVSLGDHASISCRSSQSLLHSNGNTYLHWYLQKPGQSPKLLIYKVSYRF
SGVPDRISGSGSGTDFTLKISRVEAEDLGVYFCFQSTHVPWTFGGGTKLEIK

SEQ ID NO: 135
VT008-AL6-20G7; variable region heavy chain
QVQLQQSGTELAKPGASVKLSCKASGYTFINYWIYWVKERPGQVLEWIGYINPRSDDTK
YNQKFRDRATLTADKSSTTAYLQLNSLTNDDSALYYCARGGFTMDFWGQGTSVTVSS

SEQ ID NO: 136
VT008-AL6-20K7; variable region light chain
DVVMTQTPLSLPVSLGDQASISCRSSQSLLHSNGNTYLHWYLQKPGQSPNLLIYKVSYRF
SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQGTHVPYTFGGGTKLEIK

SEQ ID NO: 137
VT008-AL6-39G2; variable region heavy chain
EVQLQQSGPELVKPGASVKISCKASGYSFTGYNIYWVKQSHGNILDWIGYIYPYNGISSY
NQKFKDKATLTVDKSSTTAYMELRSLTSEDSAVYYCARGGYTMDYGGQGTSVTVSS

SEQ ID NO: 138
VT008-AL6-39K2; variable region light chain
DVVMTQTPLSLPVSLGEQASISCRSSQSLVKSNGNTYLHWYLQKPGQSPKLLIYKVSNRF
SGVPDRFSGSGSGTDFTLKISRVEAEDLGLYFCSQTTHVPYTFGGGTKLEIK

SEQ ID NO: 139
VL008-AL17-8G5; variable region heavy chain
LVQLQQSGPELVKPGTSVKISCRSSGYTFTDYYINWVQQRPGQGLEWVGWIFPGSGLTY
YNKKFKGKATLSVDKSSNTAYMLLSSLTSEDSAVYFCARPYYGSRWDYTMDYWGQGT
SVTVSS

SEQ ID NO: 140
VL008-AL17-8K7; variable region light chain
DIVMTQSPSSLTMSVGQKATMSCKSSQNLLNSNNQKNHLAWYQQKPGQSPKLLLYFAS
TRESGVPDRFIGSGSGTDFTLTISSVQAEDLADYFCQQHYTTPYTFGGGTKLEIK

SEQ ID NO: 141
VL008-AL18-14G4; humanized variable region heavy chain; VH1
QVQLLESGAVLARPGTSVKISCKASGYSFTDYYINWVKQRPGQGLEWIGRIYPGIGNTY
YNKKFKGRAKLTAATSASIAYLEFSSLTNEDSAVYYCARGHYGRGMDYWGQGTLVTV
SS

SEQ ID NO: 142
VL008-AL18-14G4; humanized variable region heavy chain; VH2
QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGRIYPGIGN
TYYNKKFKGRVTITRDTSASTAYMELSSLRSEDTAVYYCARGHYGRGMDYWGQGTLV
TVSS

SEQ ID NO: 143
VL008-AL18-14G4; humanized variable region heavy chain; VH3
QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGRIYPGIGN
TYYNKKFKGRVTVTRDTSISTAHMELSSLRSDDTAVYYCARGHYGRGMDYWGQGTAV
TVSS

SEQ ID NO: 144
VL008-AL18-14G4; humanized variable region heavy chain; VH4
QVQLVQSGAEVKKPGSSVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGRIYPGIGNT
YYNKKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGHYGRGMDYWGQGTTVT
VSS

SEQ ID NO: 145
VL008-AL18-14G4; humanized variable region heavy chain; VH5

QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGRIYPGIGN
TYYNKKFKGRVTMTRYTSISTAYMELSRLRSDDTAVYFCARGHYGRGMDYWGQGTTV
TVSS

SEQ ID NO: 146
VL008-AL18-14K1; humanized variable region light chain; VL1
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPPKLLIYFASTK
ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYSTPWTFGGGTKLEIK

SEQ ID NO: 147
VL008-AL18-14K1; humanized variable region light chain; VL2
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPPKLLIYFASTK
ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYSTPWTFGGGTKVEIK

SEQ ID NO: 148
VL008-AL18-14K1; humanized variable region light chain; VL3
EIVLTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKAGQSPKLLIYFASTK
ESGVPDRFSGSGSGTDFTLTIDSLQAEDVAVYYCQQHYSTPWTFGGGTKVEIK

SEQ ID NO: 149
VL008-AL18-14K1; humanized variable region light chain; VL4
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPPKLLIYFASTK
ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQHYSTPWTFGGGAKVEIK

SEQ ID NO: 150
VL008-AL18-14K1; humanized variable region light chain; VL5
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPPKLLIYFASTK
ESGVPDRFSGSGSGTDFTLTISGLQAEDVAVYFCQQHYSTPWTFGGGTKVEIR

SEQ ID NO: 151

CD47 antigen (Rh-related antigen, integrin-associated signal transducer), isoform CRA_b [Homo sapiens], accession: EAW79734.1
MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKW
KFKGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVT
ELTREGETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALL
VAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIAILVI
QVIAYILAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKA
VEEPLNAFKESKGMMNDE

SEQ ID NO: 152
VT008-AL18-14K1; light chain; CDR2-Kabat; CD4+ T cell epitope corrected
YASTKES

SEQ ID NO: 153
VT008-AL18-14K1; light chain; CDR2-IMGT; CD4+ T cell epitope corrected
YAST

SEQ ID NO: 154
VL008-AL18-14K1; variable region light chain; CD4+ T cell epitope corrected
DIVMTQSPSSLAMSVGQKVTMNCKSSQSLLNSIDQKNYLAWYQQKPGQSPKLLVYYAS
TKESGVPDRFIGSGSGTDFTLTISSVQAEDLADYFCQQHYSTPWTFGGGTKLEIK

SEQ ID NO: 155
VL008-AL18-14K1; humanized variable region light chain; VL1; CD4+ T cell epitope corrected
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPPKLLIYYASTK
ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYSTPWTFGGGTKLEIK

SEQ ID NO: 156
VL008-AL18-14K1; humanized variable region light chain; VL2; CD4+ T cell epitope corrected
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPPKLLIYYASTK
ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYSTPWTFGGGTKVEIK

SEQ ID NO: 157

VL008-AL18-14K1; humanized variable region light chain; VL3; CD4+ T cell epitope corrected

EIVLTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKAGQSPKLLIYYASTK
ESGVPDRFSGSGSGTDFTLTIDSLQAEDVAVYYCQQHYSTPWTFGGGTKVEIK

SEQ ID NO: 158

VL008-AL18-14K1; humanized variable region light chain; VL4; CD4+ T cell epitope corrected

DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPPKLLIYYASTK
ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQHYSTPWTFGGGAKVEIK

SEQ ID NO: 159

VL008-AL18-14K1; humanized variable region light chain; VL5; CD4+ T cell epitope corrected

DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPPKLLIYYASTK
ESGVPDRFSGSGSGTDFTLTISGLQAEDVAVYFCQQHYSTPWTFGGGTKVEIR

SEQ ID NO: 160

hu-sCD47-6His; extracellular domain of human CD47 antigen with 6His-tag

MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKW
KFKGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVT
ELTREGETIIELKYRVVSWFSPASSSGSSSHHHHHH

SEQ ID NO: 161

hu-IgG4 S228P; human constant region heavy chain IgG4 S228P mutant

GCTAGCACCAAGGGCCCCTCTGTGTTTCCTCTGGCCCCTTGCTCCCGGTCCACCTCCG
AATCTACAGCCGCTCTGGGCTGCCTCGTGAAAGACTACTTCCCCGAGCCTGTGACAG
TGTCCTGGAACTCTGGCGCCCTGACCAGCGGAGTGCATACCTTCCCTGCTGTGCTGC
AGTCCTCCGGCCTGTACTCCCTGTCCTCCGTCGTGACAGTGCCCTCCAGCTCTCTGGG
CACCAAGACCTATACCTGCAACGTGGACCACAAGCCCTCCAACACCAAGGTGGACA
AGAGAGTGGAATCTAAGTACGGCCCTCCCTGCCCCCCTTGTCCTGCCCCTGAATTTC
TGGGCGGACCCTCCGTGTTCCTGTTCCCCCCAAAGCCTAAGGACACCCTGATGATCT

CCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGATGTGTCTCAGGAAGATCCCGAG
GTGCAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCATAATGCCAAGACCAAGCC
TCGGGAAGAACAGTTCAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCA
CCAGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGGCCTGC
CCAGCTCCATCGAAAAGACCATCTCCAAGGCCAAGGGCCAGCCCCGGGAACCCCAG
GTGTACACACTGCCTCCATCCCAGGAAGAGATGACCAAGAACCAGGTGTCCCTGAC
CTGTCTCGTGAAGGGATTCTACCCCTCCGATATCGCCGTGGAATGGGAGTCCAACGG
CCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCCTT
CTTCCTGTACTCTCGCCTGACCGTGGACAAGTCCCGGTGGCAGGAAGGCAACGTGTT
CTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTC
TCTGTCCCTGGGCAAG

SEQ ID NO: 162
huIgG4_S228P; human constant region heavy chain IgG4 S228P mutant
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 163
hu-IgG4 S228P-L235E; human constant region heavy chain IgG4 S228P-L235E mutant
GCTAGCACCAAGGGCCCCTCTGTGTTTCCTCTGGCCCCTTGCTCCCGGTCCACCTCCG
AATCTACAGCCGCTCTGGGCTGCCTCGTGAAAGACTACTTCCCCGAGCCTGTGACAG
TGTCCTGGAACTCTGGCGCCCTGACCAGCGGAGTGCATACCTTCCCTGCTGTGCTGC
AGTCCTCCGGCCTGTACTCCCTGTCCTCCGTCGTGACAGTGCCCTCCAGCTCTCTGGG
CACCAAGACCTATACCTGCAACGTGGACCACAAGCCCTCCAACACCAAGGTGGACA
AGAGAGTGGAATCTAAGTACGGCCCTCCCTGCCCCCCTTGTCCTGCCCCTGAATTTG
AAGGCGGACCCTCCGTGTTCCTGTTCCCCCCAAAGCCTAAGGACACCCTGATGATCT
CCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGATGTGTCTCAGGAAGATCCCGAG

GTGCAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCATAATGCCAAGACCAAGCC
TCGGGAAGAACAGTTCAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCA
CCAGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGGCCTGC
CCAGCTCCATCGAAAAGACCATCTCCAAGGCCAAGGGCCAGCCCCGGGAACCCCAG
GTGTACACACTGCCTCCATCCCAGGAAGAGATGACCAAGAACCAGGTGTCCCTGAC
CTGTCTCGTGAAGGGATTCTACCCCTCCGATATCGCCGTGGAATGGGAGTCCAACGG
CCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCCTT
CTTCCTGTACTCTCGCCTGACCGTGGACAAGTCCCGGTGGCAGGAAGGCAACGTGTT
CTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTC
TCTGTCCCTGGGCAAG

SEQ ID NO: 164
huIgG4_S228P-L235E; human constant region heavy chain IgG4 S228P-L235E mutant
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 165
h20-H2-L5Y; heavy chain in huIgG4_S228P format
QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGRIYPGIGN
TYYNKKFKGRVTITRDTSASTAYMELSSLRSEDTAVYYCARGHYGRGMDYWGQGTLV
TVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVD
KSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG

SEQ ID NO: 166
h20-H2-L5Y; heavy chain in hu-IgG4_S228P-L325E format
QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGRIYPGIGN
TYYNKKFKGRVTITRDTSASTAYMELSSLRSEDTAVYYCARGHYGRGMDYWGQGTLV
TVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVD
KSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG

SEQ ID NO: 167
h20-H2-L5Y; light chain
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPPKLLIYYASTK
ESGVPDRFSGSGSGTDFTLTISGLQAEDVAVYFCQQHYSTPWTFGGGTKVEIRRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 168
VL008-AL13-8G5; humanized variable region heavy chain; VH1m
QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWMHWVHQAPGQRLEWMGMIHPNSG
TTNYNQKFQGRVTITVDKSASTAYMELSSLRSEDTAVYYCTRSHYYDGHFSYWGQGTL
VTVSS

SEQ ID NO: 169
VL008-AL13-8G5; humanized variable region heavy chain; VH2m
QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWMHWVRQAPGQGLEWMGMIHPNSG
TTNYNQKFQGRVTMTVDKSASTAYMELSSLRSEDSAVYYCTRSHYYDGHFSYWGQGT
LVTVSS

SEQ ID NO: 170

VL008-AL13-8G5; humanized variable region heavy chain; VH3
QVQLQESGAEVKKPGASVKVSCKASGYTFTTYWMHWVRQAPGQGLEWMGMIHPNSG
TTNYNEKFKSRVTMTRDTSTSTVYMELSSLRSEDTAVYYCTRSHYYDGHFSYWGQGTL
VTVSS

SEQ ID NO: 171
VL008-AL13-8G5; humanized variable region heavy chain; VH4
QVQLVQSGAEVKKPGASVKVSCKGSGYTFTTYWMHWVRQAPGQGLEWMGMIHPNSG
TTNYNEKFKSRVTLTRDTSISTAYMELSRLTSDDTAVYYCTRSHYYDGHFSYWGQGTM
VTVSS

SEQ ID NO: 172
VL008-AL13-8K3; humanized variable region light chain; VL1
DIVMTQSPGSLAVSLGERATFNCKSSQSLLNSRTRKNYLAWYQQKPGQPPKLLIYWAST
RESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCKQSYNLWTFGGGTKVEVK

SEQ ID NO: 173
VL008-AL13-8K3; humanized variable region light chain; VL2
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSRTRKNYLAWYQQKPGQPPKLLIYWAST
RESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCKQSYNLWTFGGGTKLEIK

SEQ ID NO: 174
VL008-AL13-8K3; humanized variable region light chain; VL3
EIVLTQSPDSLAVSLGERATINCKSSQSLLNSRTRKNYLAWYQQKAGQSPKLLIYWASTR
ESGVPDRFSGSGSGTDFTLTIDSLQAEDVAVYYCKQSYNLWTFGGGTKVEIK

SEQ ID NO: 175
VL008-AL13-8K3; humanized variable region light chain; VL4
DIVMTQSPDSLPVSLGERATINCKSSQSLLNSRTRKNYLAWYQQKPGQPPKLLIYWAST
RESGVPDRFTGSGSGTDFTLTISALQAEDVAVYYCKQSYNLWTFGQGTRLEIK

SEQ ID NO: 176
Constant region of human kappa light chain
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

EXAMPLES

I. Generation of mouse anti-CD47 antibodies

1. Immunization of mice

In order to generate mouse antibodies against human CD47 (hCD47) and potentially cross-reacting with mouse CD47 (mCD47), NMRI wild type or CD47-KO (C57BL6) mice were immunized as follows with a protocol of comprising 4 DNA injections at 2 week intervals followed by two final boosts at one week intervals with CD47 DNA or CHO cells transfected with a CD47 expression vector:

- (5) WT mice: Prime humanCD47 DNA + Boost humanCD47 DNA
- (3) CD47 -/- mice: Prime humanCD47 DNA + Boost murineCD47 CHO
- (3) CD47 -/- mice: Prime murineCD47 CHO + Boost murineCD47 CHO

Animal sacrifice was carried out according to serum screening results. The presence and the titer of IgG binding to human or mouse CD47 was monitored in the sera of immunized animals by using an ELISA (coating of hCD47-humanIgG1 fusion protein, hCD47-Fc, or of mCD47-humanIgG1 fusion protein, mCD47-Fc) and by flow cytometry on stable CHO cells expressing hCD47 or mCD47 (obtained after transfection with a vector encoding hCD47 or mCD47, followed by selection of stable CD47-expressing clones, not shown). Animals displaying strong anti-CD47 IgG titers were sacrificed. Their spleens and lymph nodes were extracted and the mononuclear cells (MNCs) were purified and frozen.

2. Single B cell screening using the ISAAC technology and generation of recombinant anti-CD47 antibodies

The ISAAC technology, described in WO2009/017226, is a unique method for detecting individual antibody-secreting cells using microarray chips, which enables the analysis of live cells on a single-cell basis and offers a rapid, efficient and high-throughput (up to 234,000 individual cells) system for identifying and recovering the relevant cells.

An array of single live cells was prepared by applying mouse MNCs, previously purified from spleen and lymph nodes of immunized mice, to a microarray chip. The chip surface was previously coated with the target antigen (hCD47-humanIgG1 fusion protein, hCD47-Fc) and the anti-CD47 mouse antibodies secreted by an antibody secreting cell were trapped by the CD47 coated on the surface around the well. After washings, the presence of mouse IgG bound to the immobilized CD47 was detected by an anti-mouse IgG antibody coupled to Cy3 and fluorescence microscopy. Binding of the antigen to the specific antibodies formed distinct circular spots, which were easily distinguishable from nonspecific signals. CD47-specific antibody-secreting single cells were then retrieved by micromanipulation, mRNA was recovered from single cells and cDNA sequences coding the variable regions of the heavy (VH) and light (VL) chain of IgG were amplified by RT-PCR. The VH and VL sequences were then cloned in expression vectors containing a mouse gamma-2a constant region (Fcγ2a) and a kappa or lambda constant region, respectively. After co-transfection of the H and L chain expression vectors in CHO cells, the recombinant antibody was purified on a protein A column from the cell supernatants and tested for confirmation of CD47 recognition and specificity by ELISA on plates coated with hCD47-Fc or mCD47-Fc and by flow cytometry on CHO cells transfected or not with hCD47 or mCD47.

In total, 55 antibodies were identified, out of which 34 (belonging to 18 different germline families) were produced and purified. From those 34 antibodies, 19 recognized human CD47 only, 1 antibody recognized mouse CD47 only and 14 antibodies recognized both human and mouse CD47.

II. Sequences of the anti-CD47 candidates

Tables 1 to 3 (*supra*) reference the amino acid sequences of the 10 selected candidates. In the listed sequences, the CDRs are identified according to the Kabat and IMGT annotation.

III. *In vitro* characterization of recombinant mouse anti-CD47 antibodies

1. CD47 binding assay by ELISA

All antibodies were tested for their capacity to bind to recombinant hCD47-Fc and mCD47-Fc coated on ELISA plates. Among the 34 purified antibodies tested, 19 recognized hCD47 only, 1 antibody recognized mCD47 only and 14 antibodies recognized both human and mouse CD47 (data not shown).

2. CD47 binding assay by flow cytometry on CD47-transfected CHO cells and cross-reactivity with mouse and cynomolgus CD47.

The capacity of the identified mouse anti-CD47 antibodies to recognize the cell membrane expressed human CD47 as well as CD47 protein from other species was further analyzed by flow cytometry by using CHO cells stably expressing the CD47 antigen of human, mouse or non-human primate (cynomolgus monkey) origin. The expression of the species-specific CD47 on CHO cell surface was validated by using staining with appropriate anti-CD47 antibodies and flow cytometry on non-fixed cells. The antibody B6H12 (mouse IgG1, Abcam) was used to confirm the expression of human and cynomolgus CD47, MIAP301 (rat IgG2a, BD Biosciences) to check the expression of mouse CD47, and MEM122 (mouse IgM, abcam) for the expression of monkey CD47.

Anti-CD47 antibodies and isotype control antibodies were incubated at various concentrations from 7.3µg/mL to 3.3ng/mL with CHO cells expressing different CD47 species at +4°C for 30 minutes. After 2 washings, the presence of antibody bound to cell membrane CD47 was revealed by incubation with a PE-coupled anti-mouse or anti-human IgG antibody depending on the isotype of the primary antibody and analysis on an Accuri-C6 flow cytometer (BD Biosciences). The differences between the mean fluorescence intensity (MFI) obtained for each of the antibody concentration and the intensity obtained in absence of primary antibody (delta-MFI), were calculated and plotted against the concentrations of antibodies. Negative control antibodies of appropriate isotype (mIgG1, mIgG2a, hIgG1, hIgG4) that did not recognize CD47 were tested under the same conditions to measure background antibody staining (non-specific staining).

Different anti-hCD47 benchmark antibodies were also tested in parallel to our candidates. Those included, the mouse B6H12, the chimeric 5F9 (c5F9) and the humanized 5F9 (hu5F9; vh2-vl2) antibodies form Stanford University (WO2011/143624); the mouse 2A1 antibody and its humanized variant AB06.12 from Inhibrx (US2013/0224188; WO2014/123580); the mouse 5A3M3 antibody from Novimmune (WO2014/087248); and the mouse VxP037-01LC1 antibody from Frazier et al. (WO2014/093678).

The results obtained for 10 mouse anti-CD47 antibodies are summarized in the Table 4 below, and compared with the results obtained with the benchmark antibodies. All 10 mouse anti-CD47 candidates bound strongly to CHO cells expressing the human and cynomolgus CD47, but not to the non-transfected CHO cells. Moreover, 5 of the candidates (candidate 14, 15, 26, 29 and 30) also strongly bound to CHO cells expressing the mouse CD47, while the 5 other antibodies did not. These results showed that 5 antibodies specifically recognized CD47 of human and cynomolgus origin (candidates 7, 19, 20, 22 and 33) and that 5 other antibodies also cross-reacted against the CD47 of mouse origin (candidates 14, 15, 26, 29, 30), suggesting the recognition of different epitope(s) by those antibodies.

As reported, the 2A1 and B6H12 benchmarks recognized the hCD47 and cross-reacted with cynoCD47 but not with mCD47, while the VxP037-01LC1 antibody cross-reacted against both cynomolgus and mouse CD47, as well as possibly against hamster CD47 expressed on wild type CHO cells.

Table 4: Summary of cross-reactivity of anti-CD47 antibodies

Antibody	CHO	CHO-hCD47	CHO-mCD47	CHO-cynoCD47
B6H12	-	++	-	+++
c5F9	-	+++	-	+++
hu5F9	-	+++	-	+++
5A3M3	-	++	-	++
2A1	-	+++	-	+++
AB06.12	-	+++	-	+++
VxP037-01LC1	++	+++	+++	+++

Candidate 7	-	+++	-	+++
Candidate 14	+	+++	+++	+++
Candidate 15	+	+++	+++	+++
Candidate 26	+/-	+++	+++	+++
Candidate 19	-	+++	-	+++
Candidate 33	-	+++	-	+++
Candidate 20	+/-	+++	+/-	+++
Candidate 22	+/-	+++	-	+++
Candidate 29	-	+++	+++	+++
Candidate 30	+/-	+++	+++	+++

- ; +/- ; + ; ++ ; +++ ranging from “no” detection to “strong” detection

3. Inhibition of CD47/SIRPα interaction by ELISA

The antibodies were tested for their ability to disrupt the CD47-SIRPα interaction by ELISA. The day before the experiment, hCD47-hIgG1 fusion protein was coated on the bottom of 96 well plates and incubated overnight at 4°C. The wells were then washed and saturated for two hours at room temperature. After washing steps, the antigen (hSIRPα-6HIS fusion protein, Gentaur) as well as the antibodies to be tested were added in each well and incubated 1 hour at room temperature. After washings, the presence or not of the interacting SIRPα was detected by an HRP-conjugated anti-6His secondary antibody (Bethyl) and peroxidase substrate. All 10 selected antibodies were found to inhibit the binding of hSIRPα to hCD47 (data not shown).

4. Inhibition of CD47/SIRPα interaction by flow cytometry on CHO cells

The eight best candidates were then further selected according to their binding profile by ELISA and on CD47-transfected CHO cells (candidates 7, 14, 15, 19, 20, 22, 26, 33) and were tested for their capacity to inhibit the binding of human SIRPα (hSIRPα) to hCD47 expressed on CHO cells. Human CD47-transfected cells (3×10^5 cells/well of 96-well plate) were first incubated at +4°C for 30 minutes with serial dilutions of anti-CD47 antibodies or isotype control antibody (in mIgG2a or mIgG1 format). The cells were then washed and incubated with

10µg/mL of His-Tagged hSIRPα (His-hSIRPα, Gentaur) for 30 minutes at +4°C. After washings, the binding of His-hSIRPα to CHO cells was revealed by incubation with a rabbit anti-His antibody (Bethyl), followed by a FITC-conjugated goat anti-rabbit IgG (BD Biosciences) and flow cytometry analysis on an Accuri-C6 flow cytometer (BD Biosciences).

The percentage of inhibition of hSIRPα binding to hCD47 was calculated as follows:

% inhibition = (1-(MFI_wAb – MFI_wohSIRPα)/(MFI_whSIRPα – MFI_wohSIRPα))x100, where MFI_wAb is the Mean Intensity Fluorescence (MFI) obtained with the hCD47-CHO cells incubated with the tested antibody and hSIRPα; MFI_wohSIRPα is the MFI obtained in the absence of hSIRPα (100% hSIPRα binding inhibition); and MFI_whSIRPα is the fluorescence with hSIRPα but without pre-incubation with antibody (0% SIRPα binding inhibition). The percentage inhibition was plotted against antibody concentration and the IC50 values were calculated using a nonlinear regression analysis model of the GraphPad Prism software.

The results presented in the Figure 1 and Table 5 below show that the 8 selected candidates (numbers 7, 14, 15, 19, 20, 22, 26, and 33) strongly inhibited the binding of hSIRPα on hCD47 expressed on CHO cells, with IC50 values ranging from 0.36 to 1.10 µg/mL (2.4 to 7.3 nM). The 8 anti-CD47 candidates were superior to the B6H12 and 5A3M3 antibodies and were similar to the 2A1 and VxP037-01LC1 antibodies.

Table 5: IC50 values of anti-CD47 antibodies in the CD47/SIRPα inhibition assay with CD47-transfected CHO cells and flow cytometry analysis (mean values of n independent experiments)

Antibody (mIgG format)	Mean IC50 (µg/mL)	n
B6H12	4.920	7
2A1	0.739	7
VxP037-01LC1	0.759	6
5A3M3	4.798	4
Candidate 7	0.657	6
Candidate 14	0.737	5
Candidate 15	0.605	5
Candidate 19	0.858	5

Candidate 20	0.440	2
Candidate 22	0.482	2
Candidate 26	0.359	4
Candidate 33	1.108	4

5. Binding of anti-CD47 antibodies on hCD47 expressed by Raji cells

The eight further selected candidates (numbers 7, 14, 15, 19, 20, 22, 26, 33) were tested for their capacity to bind to hCD47 expressed on the Raji human B lymphoma cell line. Raji cells (ATCC-CLL-86; 2 x 10^5 cells/well of 96-well plate) were first incubated at +4°C for 30 minutes with serial dilutions of anti-CD47 antibodies or control isotype antibody (in mIgG2a or mIgG1 format). The cells were then washed and incubated for 30 minutes at +4°C with a PE-conjugated F(ab)'2 goat anti-mouse IgG antibody (1/100 dilution, Beckman Coulter). After washings, the fluorescence of the cells was analyzed by flow cytometry on an Accuri-C6 flow cytometer (BD Biosciences).

The delta of MFI, representing the difference between the intensity measured with the tested antibody and the intensity in the absence of antibody, was calculated for each antibody concentration and the values were plotted against the antibody concentrations. The EC50 values were then calculated by using a nonlinear regression analysis model of the GraphPad Prism software.

The results presented in the Figure 2 and Table 6 below, show that the 8 candidates (numbers 7, 14, 15, 19, 20, 22, 26, and 33) strongly bound to hCD47 expressed on Raji cells, with EC50 values ranging from 0.03 to 0.17 µg/mL (0.2 to 1.1 nM). The 8 anti-CD47 candidates were superior to the B6H12 and 5A3M3 antibodies and were similar to the 2A1 and VxP037-01LC1 antibodies from the competitors.

Table 6: EC50 values of anti-CD47 antibodies in the CD47 binding assay with Raji cells and flow cytometry analysis (mean values of n independent experiments)

Antibody (mIgG format)	Mean EC50 (µg/mL)	n
B6H12	2.930	2

2A1	0.058	3
VxP037-01LC1	0.074	2
5A3M3	0.301	1
Candidate 7	0.058	2
Candidate 14	0.055	2
Candidate 15	0.031	2
Candidate 19	0.165	2
Candidate 20	0.096	3
Candidate 22	0.136	2
Candidate 26	0.070	2
Candidate 33	0.112	2

6. Inhibition of CD47/SIRPα interaction by flow cytometry on Raji cells

Six of the 8 further selected candidates (numbers 14, 15, 19, 20, 22, 33) were tested for their capacity to inhibit the binding of hSIRPα to hCD47 expressed on human B lymphoma Raji cells. To this end, Raji cells (ATCC-CCL-86; 2 x 10^5 cells/well of 96-well plate) were first incubated at +4°C for 30 minutes with serial dilutions of anti-CD47 antibodies or isotype control antibody (chimeric anti-CD47 candidates produced in hIgG1 or hIgG4 format). The cells were then washed and incubated with 2.5µg/mL of His-Tagged hSIRPα (His-hSIRPα, Gentaur) for 30 minutes at +4°C. After washings, the binding of His-hSIRPα to Raji cells was revealed by incubation with a mouse anti-His antibody (1/1000 dilution; Qiagen), followed by a PE-conjugated goat anti-mouse IgG (1/100 dilution; Beckman Coulter) and flow cytometry analysis on an Accuri-C6 flow cytometer (BD biosciences).

The percentage of inhibition of hSIRPα binding to hCD47 was calculated as follows: % inhibition = (1-(MFI_wAb – MFI_wohSIRPα)/(MFI_whSIRPα – MFI_wohSIRPα))x100, where MFI_wAb is the Mean Intensity Fluorescence (MFI) obtained with the Raji cells incubated with the tested antibody and hSIRPα; MFI_wohSIRPα is the MFI obtained in the absence of hSIRPα (100% hSIPRα binding inhibition); and MFI_whSIRPα is the florescence with hSIRPα but without pre-incubation with antibody (0% SIRPα binding inhibition). The percent inhibition was plotted against antibody concentration and the IC50 values were calculated by using a nonlinear regression analysis model of the GraphPad Prism software.

The results presented in the Figure 3 and Table 7 below show that the 6 selected candidates (numbers 14, 15, 19, 20, 22 and 33) strongly inhibited the binding of hSIRPα on hCD47 expressed on Raji cells, with IC50 values ranging from 0.021 to 0.369 µg/mL (0.14 to 2.46 nM). The 6 anti-CD47 candidates were similar or superior to the chimeric B6H12 and to the chimeric 5F9 antibodies, as well as to the humanized 5F9 and AB06.12 antibodies.

Table 7: IC50 values of anti-CD47 antibodies in the hCD47/hSIRPα inhibition assay with Raji cells and flow cytometry analysis (mean values of n independent experiments)

Antibody (hIgG format)	Mean IC50 (µg/mL)	n
Chimeric B6H12	0.400	1
Chimeric c5F9	0.209	5
Humanized Hu5F9	0.109	3
Humanized AB06.12	0.082	4
Candidate 14	0.021	2
Candidate 15	0.021	2
Candidate 19	0.369	3
Candidate 20	0.110	6
Candidate 22	0.233	4
Candidate 33	0.110	3

7. Phagocytosis of Raji cells by human macrophages

CD47 is considered as a “don’t eat me” signal that prevents the phagocytosis of CD47-expressing cells by interacting with SIRPα expressed on cells with phagocytosis activity such as macrophages. In order to evaluate the ability of the best candidates to increase the phagocytosis of tumor cells by macrophages, human B lymphoma Raji cells were first loaded with 5(6)-Carboxyfluorescein N-hydroxysuccinimidyl ester (CFSE, 2.5 µM, Abcam) and then incubated with different dilutions of anti-CD47 antibodies or control antibody (mIgG1 format). The cells were then washed and placed in the presence of adherent macrophages previously differentiated from human peripheral blood monocytes in 24-well plates in the presence of 10 µg/mL M-CSF

(Peprotech) for 9 days. After 4 hours incubation at +37°C, the cell mixtures were firmly washed with cold PBS and the adherent macrophages were fixed with PFA. The fixed cells were examined under an Axiovert 40FL fluorescent microscope (Zeiss) equipped with an HB050 lamp. Pictures of the green fluorescence (Raji cells) and bright-field (macrophages) were monitored with a digital AxioCam camera and the number of Raji cells (green fluorescent) and macrophages were counted per field by using the ZEN lite Image Analysis software. A phagocytic index was then calculated as the number of Raji cells ingested per 100 macrophages.

The results presented in the Figure 4 and Table 8 below, show that the 8 selected candidates (numbers 7, 14, 15, 19, 20, 22, 26 and 33) enhanced the phagocytosis of Raji cells by human macrophages. At a sub-optimal concentration (0.125 µg/mL), the candidates were superior to the B6H12 and 5A3M3 antibodies, and similar to the 2A1 and VxP037-01LC1 antibodies.

Table 8: Phagocytic index of anti-CD47 antibodies (at 0.125 µg/mL) in the Raji phagocytic assay with human macrophages

Antibody (mIgG1 format)	Mean	SD	n
B6H12	33.1	31.3	7
2A1	112.8	39.9	5
VxP037-01LC1	88.4	28.9	4
5A3M3	29.9	4.4	2
Candidate 7	123.6	14.9	3
Candidate 14	78.9	39.3	5
Candidate 15	74.4	35.6	4
Candidate 19	73.9	16.8	3
Candidate 20	118.6	36.6	4
Candidate 22	105.8	55.5	4
Candidate 26	85.3	15.1	2
Candidate 33	71.4	27.7	3

8. Red blood cell agglutination assay

The 8 pre-selected candidates were tested for their capacity for inducing the agglutination of human red blood cells (RBCs) and were compared with benchmark antibodies.

In the first series of experiments, RBCs purified from human peripheral blood were incubated with serial dilution of anti-CD47 antibodies or control antibodies for 1 hour at 37°C in U-bottom 96 plates. After incubation, evidence of RBC agglutination was demonstrated by the appearance of a haze as opposed to a punctate red pellet of non-agglutinated RBCs. The area of the RBC pellets was measured and the hemagglutination index was calculated by the ratio of the area of RBCs at different antibody concentrations versus the area of RBCs without antibody, essentially as described in WO2014/123580. Hemagglutination indexes were plotted against the antibody concentrations, as shown in Figure 5. The candidates 14 and 15, and to a lesser extent candidate 26, were found to induce a strong agglutination of purified RBCs. Of note, these 3 antibodies belong to the same clone family and cross-react with mCD47. In contrast, candidates 19, 20 and 33 did not induce agglutination of purified RBCs when tested in concentrations ranging from 0.01 nM to 240 nM (~1.5 ng/mL to 36 µg/mL), as also observed with the 2A1 antibody. Candidates 7 and 22 were found to induce weak agglutination for a narrow window of concentration as also observed with the antibodies VxP037-01LC1 and B6H12. Antibody 5A3M3 induced agglutination of purified RBCs only at high concentrations.

In a second set of experiments, the 8 selected candidates were tested for their capacity to induce the agglutination of human RBCs by using unpurified RBCs from whole peripheral blood, essentially as described in WO2014/087248. To this end, human peripheral blood (1/5 final dilution) was incubated with serial dilutions of anti-CD47 antibodies or control antibodies overnight at 37°C in flat-bottomed 96-well plates. After incubation, the plates were gently agitated, tilted at about 30° and allowed to rest for 10 minutes. The evidence of RBC agglutination was demonstrated by the appearance of a clumped deposit in the form of a crescent at the bottom around the inferior border of the well. Different formats of the anti-CD47 candidates were tested and compared with benchmark antibodies. Candidate 20 in mIgG1 or hIgG1 chimeric format did not induce RBC agglutination in the range of concentrations tested from 0.023 to 50µg/mL (~0.15 to 330nM), as also observed for the 2A1 and AB06.12 (humanized 2A1) antibodies. Weak RBC agglutination was observed for concentrations higher than 16 µg/mL for both candidate 20 and AB06.12 antibody in hIgG4 format. In contrast, the chimeric 5F9 (c5F9) and humanized 5F9 (hu5F9, vh2/vl2 variant) induced stronger agglutination when tested in both hIgG1 and hIgG4 format. Chimeric B6H12 induced RBC agglutination in hIgG1 format but more weakly in hIgG4 isotype.

In this assay with whole blood, candidates 19, 22 and 33 were found to induce RBC agglutination at concentrations higher than 5 μg/mL. This observation was independent of the tested format (mIgG or chimeric hIgG). Finally, candidates 14 and 15, and to a lesser extent candidates 7 and 26 induced strong agglutination of unpurified RBCs.

RBC agglutination assay data are summarized in Table 9 below.

Table 9: RBC agglutination activity of anti-CD47 antibodies

Antibody	RBC agglutination with				
	Purified RBC	Whole Blood			
	mIgG or hIgG	mIgG1	mIgG2a	hIgG1	hIgG4
B6H12	+ / - (m, h)	+++	+++	+++	+/++
c5F9	Nd	nd	nd	+++	+++
hu5F9	Nd	nd	nd	+++	+++
5A3M3	+++ (m1)	+/-	nd	nd	nd
2A1	- (m1)	-	nd	nd	nd
AB06.12	Nd	nd	nd	-	+/-
VxP037-01LC1	+ (m1)	+	nd	nd	nd
Candidate 7	++ (m1, m2a)	++	++	nd	++
Candidate14	++++ (m, h)	++++	++++	++++	++++
Candidate 15	+++ (m, h)	++++	++++	++++	+++
Candidate 26	++ (m2a)	nd	++	nd	++
Candidate 19	- (m1, m2a)	+++	++	+++	+/+++
Candidate 33	-	+++	+++	+++	+++
Candidate 20	-	-	-	-	+/-
Candidate 22	+/++	+++	+++	+++	+++

nd: not done; m: mIgG; m1: mIgG1; m2a: mIgG2a; h: hIgG

+/-, weak agglutination observed at 50 μg/mL antibody only; +, agglutination at 50 μg/mL antibody; ++, agglutination for concentrations ≥16.7 μg/mL; +++, agglutination for concentrations ≥5.6 μg/mL

9. Affinity of the best mouse anti-CD47 candidates

The kinetic constants (Kon & Koff) and the dissociation constant (KD) of the mouse anti-CD47 candidates were further measured by surface plasmon resonance (SPR) on Biacore T200 (GE Healthcare) using a soluble preparation of human CD47 extracellular domain tagged with a 6His-tag (SEQ ID NO: 160) (hCD47-his protein) and compared to benchmarks. Briefly, the binding affinity between antibodies and hCD47-his was measured using the single-cycle kinetics protocol of the Biacore T200 instrument at +20°C. An anti-human or anti-mouse IgG (Fc) was first immobilized on the surface of Serie S Sensor Chips CM5 using the human or mouse antibody capture and amine coupling kits, following the manufacturer's instructions (GE Healthcare). This resulted in approximately 10,000 response units (RU) immobilized on the surface. The mouse, chimerized or humanized antibodies to be tested were then captured onto the appropriate anti-mouse or anti-human IgG surface in all flow cells except flow cell 1 used as control, at concentration and contact time adjusted so that ~300 RU were captured on the surface. Binding kinetics were studied by passing increasing concentrations of hCD47-his in a series of five 10 minute injections through all flow cells at a rate of 30 µL/min. Three-fold dilutions were used up to a maximum concentration of 270 nM. Following the final injection, buffer was passed across each flow cell for 60 minutes (mouse) or 30 minutes (human) to monitor the dissociation of bound antigen. Regeneration of the binding surface was carried out at the end of the cycle by flowing 3 M $MgCl_2$ for 60 s at 20 µL/min followed by 10 mM glycine-HCl, pH 1.7 for 180 s at 10 µl/min (for human) or by flowing 10 mM glycine-HCl, ph 1.7 for 360 s at 10 µl/min (for mouse). A second cycle with the same antibodies but no antigen was run as a control. Kinetics binding constants were estimated by non-linear fitting of the sensogram data to the 1:1 binding model provided by the Biacore T200 evaluation software. Results are shown in Table 10 below. All candidates displayed an affinity in the nM range with a KD of between 0.9 to 4.2 nM, except for candidate 22 which showed a weaker affinity, with a KD of 15.7 nM. As shown in Figure 6, all pre-selected candidates except candidate 33 differed from the B6H12, 2A1 and VxP037-01LC1 antibodies by displaying more rapid kinetics of dissociation, with Koff values in the range of 3.4×10^{-3} to 1.8×10^{-4} s^{-1}, as compared to 5.0 to 8.9×10^{-5} s^{-1} for B6H12, 2A1 and VxP037-01LC1.

Table 10: Kinetics and affinity constants of anti-CD47 antibodies by Biacore (mean values of independent experiments)

Antibody	KD (M)	Kon (1/Ms)	Koff (1/s)	n
B6H12	5.14×10^{-9}	1.29×10^{4}	6.59×10^{-5}	7
VxP037-01LC1	2.56×10^{-10}	3.54×10^{5}	8.87×10^{-5}	3
2A1	1.27×10^{-10}	4.01×10^{5}	4.99×10^{-5}	4
Candidate7	1.94×10^{-9}	1.90×10^{5}	3.70×10^{-4}	2
Candidate 14	3.85×10^{-9}	8.74×10^{5}	3.37×10^{-3}	2
Candidate 15	4.24×10^{-9}	5.65×10^{5}	2.39×10^{-3}	2
Candidate 19	2.25×10^{-9}	7.90×10^{4}	1.76×10^{-4}	4
Candidate 20	4.16×10^{-9}	1.01×10^{5}	4.18×10^{-4}	4
Candidate 22	1.57×10^{8}	2.07×10^{4}	3.25×10^{-4}	2
Candidate 26	1.87×10^{-9}	2.00×10^{5}	3.72×10^{-4}	2
Candidate 33	8.98×10^{-10}	7.46×10^{4}	6.70×10^{-5}	2

10. Release of anti-CD47 antibodies after binding to human RBCs or to tumor cells, and phagocytosis of tumor cells by anti-CD47 antibodies according to their Koff value

CD47 is expressed on circulating blood cells, in particular on RBCs, and represents an important source of sink effect that may significantly impact the pharmacokinetics and the efficacy of a therapeutic anti-CD47 antibody by lowering the amount of free antibody available for the targeted cells such as tumor cells. Moreover, an important and prolonged binding of anti-CD47 antibodies to RBCs may also increase the risk of toxicity such as anemia. The kinetics of binding versus release of an antibody from its antigen is mostly dependent on its kinetics of association and dissociation that can be quantified by measuring the Kon and Koff values, respectively, for example by SPR as carried out for the anti-CD47 antibodies of this invention. In order to evaluate *in vitro* the impact of the dissociation kinetics of anti-CD47 antibodies on their release after binding to RBCs, purified human RBCs were incubated with 1 µg/mL of mouse anti-CD47 candidates 14, 19, 20 or 22, or with 1 µg/mL mouse anti-CD47 antibody 2A1 or B6H12. These anti-CD47 candidates were selected because they possessed Koff values superior to 1×10^{-4} s^{-1} or even superior to 1×10^{-3} s^{-1} for candidate 14 (see Table 10). Conversely, the Koff value of the anti-CD47 2A1 and B6H12 benchmarks was inferior to 1×10^{-4} s^{-1} (Table 10). After 30 minutes incubation with antibodies at +4°C, RBCs were washed, resuspended in antibody-free PBS/BSA/EDTA buffer and incubated either at +37°C for 6 hours or 24 hours. At different incubation times, RBCs were washed in PBS/BSA/EDTA buffer and incubated with a

PE-conjugated goat anti-mouse IgG at +4°C. The binding of the mouse anti-CD47 antibodies on RBCs was further analyzed by flow cytometry. For each antibody, the mean fluorescence intensity (MFI) obtained by gating on viable RBCs after 6 hours (T+6h) or 24 hours (T+24h) was recorded and compared to the MFI obtained on RBCs analyzed just before the incubation periods at +37°C (T0). The results were expressed as the percentage of decrease of the MFI recorded after T+6h or T+24h incubation at +37°C compared to the MFI recorded at T0 before the incubation steps.

As shown in Figure 20, the MFI of the RBCs stained with the candidates 14, 19, 20 and 22 was already significantly reduced after 6 hours and even more strongly reduced after 24 hours incubation at +37°C. These results indicated that the candidates 14, 19, 20 and 22 progressively detached from the RBCs after having bound the membrane-expressed CD47 antigen. In contrast, after 6 or 24 hours of incubation at +37°C, the MFI of RBCs stained with the antibody 2A1 or B6H12 was not modified or only weakly, indicating that the antibody 2A1 and B6H12 were strongly stuck on RBCs and were not or only slowly released in the extracellular medium.

Of note, while an agglutination was observed with certain antibodies (candidates 14 and 22, antibody B6H12), this did not strongly impact the analysis of single RBCs by flow cytometry.

These results suggest that anti-CD47 antibodies such as candidates 14, 19, 20 and 22 that share a dissociation kinetic with a high Koff value superior to 1 x 10^{-4} s^{-1} will be more rapidly released from RBCs to reach the target cells. Thus anti-CD47 antibodies such as candidate 14, 19, 20 and 22 will be more available for reaching the CD47 expressed on the targeted tumor cells compared to antibodies with slow dissociation kinetics such as 2A1 or B6H12 that possess a Koff value inferior to 1 x 10^{-4} s^{-1}.

Conversely, an antibody with a high dissociation rate from cell-expressed CD47 may be less efficient to enhance tumor cell phagocytosis than antibodies with a slower dissociation rate. To address this point, an experiment, similar to the binding/release experiment performed above on RBCs, was carried out to measure the *in vitro* release of the anti-CD47 antibodies by tumor cells and its effect on tumor cell phagocytosis. To this end, human Raji lymphoma cells were incubated with the mouse anti-CD47 candidates 14, 19, 20 or 22, or with the mouse anti-CD47 antibodies B6H12 or 2A1 at 1 µg/mL for 30 minutes at +4°C, then washed and incubated again in antibody-free medium for 24 hours at +37°C. Cells were then collected after the 24 hour incubation times (T+24h) washed in PBS and fixed with 4% PFA. As positive control of staining, cells were also fixed with 4% PFA just before being incubated for 24 hours (time T0). After the last incubation time, fixed cells were stained by a PE-conjugated goat anti-mouse IgG antibody and analyzed by flow cytometry. For each antibody, the mean fluorescence intensity (MFI) obtained after 24 hour (T+24h) incubation was recorded and compared to the MFI obtained on Raji cells analyzed just before the incubation periods at +37°C (T0). The results were expressed as the percentage of decrease of the MFI of the cells recorded after T+24h incubation at +37°C compared to the MFI recorded at T0 before the incubation steps.

As shown in Figure 21A an important decrease of the MFI of candidate 14 was observed already after 24 hours incubation of the Raji cells at 37°C, while a weaker diminution was observed for the candidates 19, 20 and 22 as well as for the antibody 2A1, but not for B6H12. These results show that anti-CD47 antibodies with very rapid dissociation kinetics such as candidate 14 possessing a Koff value superior to 1 x 10^{-3} s^{-1}, will detach more quickly and more strongly from Raji tumor cells, and may thus be less efficient to enhance tumor cell phagocytosis than anti-CD47 antibodies with a slower dissociation rate such as candidate 19, 20 and 22 possessing a Koff value inferior to 1 x 10^{-3} s^{-1}.

To address this point, Raji cells were first labelled with CFSE dye and then stained with the anti-CD47 antibodies at 0.1 or 1 µg/mL for 30 minutes at +4°C. Raji cells were then washed and incubated for 24 hours at +37°C in antibody-free medium as described above. After the 24 hour incubation period (T+24h), the Raji cells were tested in a phagocytosis assay with hMDM by using a flow cytometry phagocytosis assay, essentially as described by Tseng et al. (Tseng D., Volkmer J-P., Willingham S.B., Contreras-Trujillo H., et al. "Anti-CD47 antibody-mediated phagocytosis of cancer by macrophages primes an effective antitumor T-cell response", PNAS 110(27):11103-08 (2013)). The target Raji tumor cells, previously stained with CFSE dye before being stained by the anti-CD47 antibodies, were incubated with hMDM that have been previously labelled with Far-Red dye. Following 60 minutes of incubation at 37°C, the cells were collected, analyzed by flow cytometry and the CFSE and Far-Red fluorescence were monitored. The population of cells that appeared double-stained by CFSE and Far-Red corresponded to the target tumor cells that had been phagocytosed by the macrophages. As positive control of phagocytosis (time T0), the same experiment was carried out with the same preparation of hMDM labelled with Far-Red and incubated with CFSE-labelled Raji cells stained with the different anti-CD47 antibodies at 0.1 or 1 µg/mL as above, but Raji cells were not incubated for 24 hours at +37°C. This corresponded to the time T0 of reference before incubation in antibody-free medium. The percentage of cells phagocytosed was calculated from the percentage of cells double-stained by CFSE and Far-Red in each condition. The percentage of phagocytosis specific for each antibody was calculated by subtracting the percentage of phagocytosis obtained with Raji cells treated in the same conditions with an irrelevant antibody. The results were finally expressed as the percentage of decrease of the specific phagocytosis obtained at T+24h compared to the specific phagocytosis measured at T0 for each tested antibody, by applying the formula: % decrease phagocytosis = (1 – (% of specific phagocytosis at T+24h with antibody A / % of specific phagocytosis at T0 with antibody A)) x 100.

As shown in Figure 21B, a very strong decrease of phagocytosis was observed for Raji cells stained with anti-CD47 candidate 14, for which about 60% decrease of phagocytosis was observed after staining with 1 µg/mL candidate 14 and even up to 100% decrease was observed on Raji cells stained with 0.1 µg/mL. In contrast, only a weak decrease of phagocytosis was observed for the Raji cells stained with the candidates 19, 20 and 22 as for benchmarks 2A1 and B6H12.

Overall, these results show that anti-CD47 antibodies with a high dissociation rate characterized by a Koff value superior to 1 x 10^{-3} s^{-1} (such as candidate 14) will detach strongly and rapidly from RBCs but will also lose rapidly most of their functional activity on tumor cells, i.d. enhancement of tumor cell phagocytosis. Thus, such antibodies may have lower sink/side effect but also weaker anti-tumor efficacy. At the opposite, anti-CD47 antibodies with very slow dissociation rate characterized by a Koff value inferior to 1 x 10^{-4} s^{-1} (such as 2A1 and B6H12), will detach more slowly from the tumor cells, but will stay stuck on RBCs and may thus have important sink effect and possibly more side effects. Therefore, anti-CD47 antibodies with intermediate dissociation kinetics characterized by a Koff value comprised between 1 x 10^{-4} and 1 x 10^{-3} s^{-1} (such as candidate 19, 20 or 22) will possess optimal CD47 binding/release equilibrium to accommodate with a weak sink effect while maintaining their anti-tumor efficacy.

11. Apoptosis assay with Jurkat cells

Among the 8 candidates tested, candidates 14 and 15 were found to induce significant apoptosis of human Jurkat cells (T cell leukemia cells), and candidate 26 also induced weak apoptosis (data not shown), whereas candidate 20 and 22 did not induce apoptosis. These data suggest that this family of antibodies recognized an epitope on hCD47 that delivered signaling activity into CD47-expressing cells following CD47 engagement. This type of agonistic antibodies may induce off-target effects *in vivo*.

12. Summary of *in vitro* characterization

In summary, 34 anti-CD47 antibodies have been produced, purified and evaluated *in vitro* for specificity and functionality. Eight candidates (numbers 7, 14, 15, 19, 20, 22, 26, 33) were preselected based on their potency in binding and functional assays (inhibition of hCD47/hSIRPα interaction, induction of phagocytosis), and then fully characterized in assays measuring their affinity and RBC agglutination activity.

All 8 candidates strongly recognized both hCD47 and cynoCD47, and bound hCD47 with an affinity in the range of 0.9 to 15.7nM. All 8 candidates strongly inhibited hCD47/hSIRPα interaction and were capable of inducing phagocytosis of Raji lymphoma cells by human macrophages.

Three antibodies (candidates 14, 15, 26) belonging to the same clone family, also cross-reacted with mCD47, and were found to induce significant apoptosis of Jurkat tumor cells and to induce strong RBC agglutination. These candidates may induce potential toxicity when injected *in vivo*.

Candidate 20 (unique in its clone family) had the best profile, with strong functional activity and a very weak but detectable RBC agglutination, indicating weak, if any, potential toxicity.

Candidates 19 and 33 (same clone family), as well as candidates 7 and 22 (unique in their respective clone families) also displayed strong functional activities with some but acceptable RBC agglutination depending on the IgG format tested.

Candidate 19, 20 and 22 with Koff values between 1×10^{-4} and 1×10^{-3} s^{-1} also displayed rapid release once bound to human RBCs but kept efficient functional activity as measured by the phagocytosis of tumor cells by human macrophages.

IV. *In vivo* characterization of selected mouse anti-CD47 candidates

Selected candidates 19, 20 and 22, specific to hCD47, were tested *in vivo* in mouse tumor xenograft models. Candidates 14 and 15 were also tested *in vivo* in first studies as mCD47 cross-reacting anti-CD47 antibodies.

1. Human lymphoma Raji model

Anti-CD47 candidates 14, 19, 20 and 22 were evaluated *in vivo* in the human Non-Hodgkin's Lymphoma xenograft model with NOG mice (n=8 per group) engrafted with Raji cells (1.5 million cells injected subcutaneously in the right flank). Ten days after cell transplant, tumors were palpable and the antibody therapy (10 mg/kg/dose, intraperitoneal injection, 3 times a week up to 8 weeks) was started for the four anti-CD47 candidates in parallel with a negative control antibody and the anti-CD47 benchmark antibodies 2A1, B6H12 and VpX037-01LC1. All antibodies were in the mIgG1 isotype format. Tumor growth was monitored every 2 days and mouse survival was recorded. The four candidates 14, 19, 20 and 22 delayed the growth of Raji cells, as did the benchmarks B6H12 and 2A1, but not the VxP037-01LC1 antibody that cross-reacts strongly with mouse CD47 (Figure 9A). As shown in Figure 9B, candidates 20 and 22 were also found to induce significant protection of the mice when compared to the control group, as did the benchmarks B6H12 and 2A1, but not VxP037-01LC1.

These results indicated that the four candidates tested (candidates 14, 19, 20 and 22) were able to control growth of the human NHL Raji tumor cells in immunocompromised mice, as previously reported for reference anti-CD47 antibodies, such as B6H12. Candidates 20 and 22 appeared more potent than candidates 14 and 19 for prolonging mice survival.

2. Human A2780 ovary xenograft model

Anti-CD47 candidate 20 was further evaluated *in vivo* in the human A2780 ovarian xenograft model either alone or in combination with the anti-EGFR antibody Cetuximab (Erbitux®) or the anti-Her2 antibody Trastuzumab (Herceptin®). A2780 cells strongly expressed CD47 as detected by the binding of candidate 20, as well as Her2 as detected by binding of Herceptin® (data not shown). In contrast, A2780 cells expressed only low levels of EGFR *in vitro* as measured by staining with Erbitux® (data not shown). NOG mice (n=4 per group) were engrafted with Luciferase-transfected A2780 (A2780/Luc) cells (10 million cells injected intraperitoneally). One day after the cell transplant, antibody therapy (10 mg/kg/dose, intraperitoneal injection, 3 times a week up to 5 weeks) was begun with anti-CD47 candidate 20 alone or in combination with Herceptin® or Erbitux®.

Bioluminescence intensity (BLI) was monitored every 5 days from both ventral and dorsal positions. Results presented in Figure 10 showed that treatment with the anti-CD47 candidate 20 alone delayed the growth of A2780 tumor cells. A delay in tumor growth was also observed with Herceptin® alone, but not with Erbitux® alone. Furthermore, the combination of anti-CD47 candidate 20 in combination with Herceptin® or Erbitux® also delayed the growth of A2780 cells.

These results suggest that candidate 20 was capable of controlling the growth of A2780 ovarian tumor cells *in vivo* in NOG immunocompromised mice when used as single agent, and can show cooperative activity in combination with anti-Her-2 antibody (Herceptin®) or anti-EGFR antibody (Erbitux®).

3. Human A549 lung xenograft model

Anti-CD47 candidate 20 was also evaluated in vivo in the human A549 lung adenocarcinoma xenograft model (Steiner P., Joynes C., Bassi R., Wang S., Tonra J.R., et al. "Tumor Growth Inhibition with Cetuximab and Chemotherapy in Non–Small Cell Lung Cancer Xenografts Expressing Wild-type and Mutated Epidermal Growth Factor Receptor", Clin Cancer Res 13(5):1542-51(2007); Kellar A., Egan C., and Morris D. "Preclinical Murine Models for Lung Cancer: Clinical Trial Applications", BioMed Res Int 2015, ID 621324 (2015)) alone or in combination with the anti-EGFR antibody Cetuximab (Erbitux®) or the anti-Her2 antibody Trastuzumab (Herceptin®). In vitro, A549 cells (ATCC-CCL-185) strongly expressed CD47 as detected by the binding of candidate 20, as well as EGFR as measured by staining with Erbitux®, and, with a weaker intensity, Her2 as detected with Herceptin® by flow cytometry (data not shown). NOG mice (n=4 per group) were engrafted with A549 cells (10 million cells injected subcutaneously). Ten days after the cell transplant, once the tumor was palpable (volume > $30mm^3$), the antibody therapy (10 mg/kg/dose, intraperitoneal injection, 3 times a week up to 10 weeks) was started for the anti-CD47 candidate 20 alone or in combination with Herceptin® or Erbitux®.

As shown in Figure 11, the anti-CD47 candidate 20 alone, as well as Erbitux® and Herceptin®, were able to delay the growth of A549 tumor cells as quantified by measurement of the tumor volume (Figure 11A) and were able to significantly protect the mice when compared to the vehicle group (Figure 11B). The combination of anti-CD47 candidate 20 with Herceptin® resulted in a more important delay of the A549 tumor cell growth (Figure 11A) and to a stronger protection of the mice in the survival analysis (Figure 11B).

These results suggest that the anti-CD47 candidate 20, when used as single agent or in combination with Herceptin®, was capable to delay the growth of the A549 lung adenocarcinoma *in vivo* in NOG mice.

V. Humanization of anti-CD47 candidate 20

1. Humanized anti-CD47 candidates 20

Mouse candidate 20 was selected as the first lead for humanization. Five VH and 5 VL variants were generated by CDR engraftment into human acceptor frameworks. The sequences were analyzed for removal of potential T-Cell epitopes with MHC class II high affinity using *in silico* algorithms, as well as for the presence of post-translational modifications such as Fv glycosylation and deamidation. The amino acid sequences of the 5 VH variants (VH1 to VH5) aligned with the sequence of the mouse VH sequence of candidate 20 (VH0), are presented in Figure 7. The amino acid sequences of the 5 VL variants (VL1 to VL5) aligned with the sequence of the mouse VL sequence of candidate 20 (VL0), are presented in Figure 8.

The 5 humanized VH variants were cloned in an expression vector containing a human Fcγ4-S228P backbone (SEQ ID NO: 162) and the 5 humanized VL variants were cloned into an expression vector containing a human Kappa backbone (SEQ ID NO: 176), in order to generate hIgG4/kappa humanized variants. The mutation S228P was introduced in the Fc fragment of all hIgG4 antibodies to avoid potential chain exchanges that have been observed with hIgG4 (Angal S., King D.J., Bodmer M.W., Turner A., Lawson A.D., Roberts G., Pedley D., and Adair J.R. "A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody", Mol Immunol 30(1):105-8 (1993)). Twenty-five hIgG4/kappa variants were produced and purified from CHO cells co-transfected with one of the 5 VH and one of the 5 VL vectors.

The resulting antibodies were named h20-VHx-VLx, where h20 designated humanized candidate 20, VHx designated the Heavy chain variable domain with the humanized VH variant x (VH1 to VH5), and Lx designated the Light chain variable domain with the humanized VL variant x (VL1 to VL5). The 25 purified antibodies were tested for their binding activity and specificity on human Raji lymphoma cells and CHO cells transfected or not with hCD47 or mCD47, for their functional activity in inhibiting the binding of hSIRPα on Raji cells and for their capacity to induce human RBC agglutination in whole blood agglutination assay. The kinetics (Kon and Koff) and affinity (KD) constants were also measured by SPR on Biacore. In all assays, the 25 humanized variants were compared with a hIgG4 chimeric candidate 20 generated by cloning of the mouse VH0 and VL0 sequences into the appropriate vectors containing a human Fcγ4-S228P and human Kappa backbone.

All results obtained in the different assays are summarized in Table 12 and Table 13 below.

Table 12: Characteristics of humanized variants of candidate 20

Candidate No	Antibody (hIgG4)	CD47 Binding (EC50 µg/mL)	CD47/SIRPα inhibition (IC50 µg/mL)	Phagocytosis Raji / HDMC	RBC agglutination (3 donors)
20	Chim. 20	0.150	0.138	++	+/-
20.1	h20-VH1-VL1	0.193	0.161	++	++
20.2	h20-VH1-VL2	0.218	0.216	++	++
20.3	h20-VH1-VL3	0.184	0.228	++	++/+++
20.4	h20-VH1-VL4	0.248	0.261	++	-
20.5	h20-VH1-VL5	0.206	0.111	++	+/++
20.6	h20-VH2-VL1	0.177	0.098	++	+/++
20.7	h20-VH2-VL2	0.156	0.076	Nd	+
20.8	h20-VH2-VL3	0.184	0.079	Nd	+
20.9	h20-VH2-VL4	0.173	0.123	Nd	+
20.10	h20-VH2-VL5	0.191	0.051	Nd	-
20.11	h20-VH3-VL1	0.145	0.118	++	+/-
20.12	h20-VH3-VL2	0.153	0.160	Nd	+/-

20.13	h20-VH3-VL3	0.147	0.173	Nd	+/-
20.14	h20-VH3-VL4	0.143	0.135	Nd	+
20.15	h20-VH3-VL5	0.143	0.113	Nd	+
20.16	h20-VH4-VL1	0.113	0.100	++	+/-
20.17	h20-VH4-VL2	0.124	0.086	Nd	+/-
20.18	h20-VH4-VL3	0.164	0.142	Nd	+/-
20.19	h20-VH4-VL4	0.139	0.192	Nd	-
20.20	h20-VH4-VL5	0.136	0.095	Nd	+/-
20.21	h20-VH5-VL1	0.190	0.169	++	+
20.22	h20-VH5-VL2	0.189	0.174	Nd	+/++
20.23	h20-VH5-VL3	0.190	0.169	Nd	+/-
20.24	h20-VH5-VL4	0.177	0.133	Nd	+/++
20.25	h20-VH5-VL5	0.159	0.112	Nd	+/-

nd: not done

For RBC agglutination: +/-, weak agglutination observed at 50 µg/mL antibody only; +, agglutination at 50 µg/mL antibody; ++, agglutination for concentrations ≥16.7 µg/mL; +++, agglutination for concentrations ≥5.6 µg/mL

Table 13: Kinetics and affinity constants of humanized anti-CD47 antibodies of candidate 20 by Biacore (mean values of independent experiments)

Candidate No	Antibody (hIgG4)	mean Kon (1/Ms) x10^3	mean Koff (1/s) x 10^{-4}	mean KD (nM)
20	Chim 20	169.33	5.05	3.22
20.1	h20-H1-L1	118.40	6.34	5.85
20.2	h20-H1-L2	119.50	6.91	6.45
20.3	h20-H1-L3	136.50	11.05	7.82
20.4	h20-H1-L4	130.95	8.39	7.33
20.5	h20-H1-L5	126.30	7.72	7.58
20.6	h20-H2-L1	197.50	5.95	3.53
20.7	h20-H2-L2	193.00	6.27	3.67
20.8	h20-H2-L3	189.00	6.60	3.77
20.9	h20-H2-L4	189.00	6.28	3.65

20.10	h20-H2-L5	219.00	6.32	3.34
20.11	h20-H3-L1	222.00	5.61	2.79
20.12	h20-H3-L2	246.00	5.60	2.49
20.13	h20-H3-L3	213.50	5.94	3.05
20.14	h20-H3-L4	216.50	5.81	3.02
20.15	h20-H3-L5	242.00	5.28	2.36
20.16	h20-H4-L1	175.00	6.19	3.86
20.17	h20-H4-L2	167.50	6.34	4.18
20.18	h20-H4-L3	180.00	7.33	4.37
20.19	h20-H4-L4	179.00	6.85	4.15
20.20	h20-H4-L5	192.00	6.24	3.5
20.21	h20-H5-L1	115.90	9.79	9.2
20.22	h20-H5-L2	142.00	11.65	8.55
20.23	h20-H5-L3	104.20	8.41	9.93
20.24	h20-H5-L4	103.25	10.21	10.99
20.25	h20-H5-L5	126.40	10.86	9.69

All humanized variants with the VH1 and VH5 sequences had a lower affinity (higher KD) than the other variants and lower than that of the chimeric candidate 20. The other variants showed Kon, Koff and KD constants similar to the ones of the chimeric candidate 20. None of the variants showed a significant increased affinity to hCD47 as compared to chimeric candidate 20. The Koff values of all humanized variants were observed to be in the range of 5.3 x 10^{-4} to 11.7 x 10^{-4} s^{-1}.

All 25 variants bound strongly to CD47 expressed on Raji cells with an EC50 similar to the EC50 of the chimeric candidate 20. They also specifically recognized hCD47 but not mCD47 expressed on CHO cells, like the chimeric candidate 20 (not shown).

All 25 variants strongly inhibited the binding of hSIRPα to CD47 expressed on Raji cells, with an IC50 in the same range than the IC50 of chimeric candidate 20. Variants with the VH2 sequence had an IC50 lower than the other variants, as well as the chimeric candidate 20.

All tested hIgG4 variants were capable of enhancing the phagocytosis of Raji cells by human macrophages in a similar manner as the chimeric hIgG4 candidate 20.

Some variants of the VH1 family (h20-VH1-VL1, h20-VH1-VL2, h20-VH1-VL3, h20-VH1-VL5), variant h20-VH2-VL1 and variants h20-VH5-VL2 and h20-VH5-VL4 displayed a tendency to induce more RBC agglutination than the other variants, as well as than the chimeric candidate 20.

2. Optimization of the humanized antibodies and lead selection

Analysis of potential T cell epitopes within the VH and VL sequences of the humanized variants has revealed the presence of a high affinity CD4+ T cell epitope (LIYFASTKESGV) within the CDR2 domain of the 5 VL variants (VL1 to VL5). This potential T cell epitope, also present within the CDR2 of the mouse VL0 sequence, had not been removed in the 5 first humanized VL variants to avoid any loss of affinity and/or activity of the first humanized candidates. A single point mutation F to Y at position 56 (F56Y) of the VL sequence (amino acid numbering without the signal peptide) was however sufficient to remove the immunogenicity of this potential T cell epitope (LIY<u>F</u>ASTKESGV to LIY<u>Y</u>ASTKESGV). The F56Y mutation was therefore introduced into the humanized VL1 to VL5 sequences. These new sequences were designated as VL1-F56Y to VL5-F56Y and the corresponding humanized antibodies produced by combining one of the VH1 to VH5 variants with one of the VL1-F56Y to VL5-F56Y variants were designated as h20-Hx-LxY. Five humanized variants (candidates 20.26 (h20-H2-L5Y), 20.27 (h20-H3-L2Y), 20.28 (h20-H3-L3Y), 20.29 (h20-H4-L4Y), 20.30 (h20-H4-L5Y)) were selected and produced in a human IgG4 format containing the S228P mutation (SEQ ID NO: 162) known to stabilized the hIgG4 protein (Angal S et al. Mol Immunol, 1993). Purified antibodies were further characterized *in vitro* for their binding activity to hCD47 on Raji cells, for their potency to inhibit hCD47/hSIRPα interaction on Raji cells and for their human RBC agglutination activity using whole blood. The 5 humanized Hx-LxY variants were also compared to the chimeric candidate 20 produced in a hIgG4-S228P format, as well as to the AB06.12 and Hu5F9 humanized antibodies cloned in a hIgG4-S228P.

As shown in Figures 12 and 13, the five F56Y-mutated variants bound to Raji cells (Figure 12) and inhibited the binding of hSIRPα to Raji cells (Figure 13) with a similar efficiency to their non-mutated counterparts and similar to the chimeric candidate 20. The five F56Y-mutated humanized variants had slightly higher RBC agglutination activity in the whole blood assay than the chimeric candidate 20, but still lower to the Hu5F9 benchmark (Table14).

Finally, the five F56Y-mutated humanized variants of candidate 20 were found to display strong recognition of hCD47 on Raji cells and strong inhibition of hSIRPα binding to hCD47 on Raji cells, similar to the AB06.12 and Hu5F9 antibodies (Figure 14). The Table 14 below summarizes the results obtained in the different assays.

Table 14: Characteristics of humanized h20-Hx-LxY variants of candidate 20 (representative of 2 independent experiments)

Antibody (hIgG4-S228P)	CD47 binding (EC50, ng/mL)	CD47/SIRPα Inhibition (IC50, ng/mL)	RBC agglutination (4 donors)
h20-H2-L5Y, candidate 20.26	97.5	112.6	++ ; + ; ++ ; +
h20-H3-L2Y, candidate 20.27	85.4	101.1	+ ; + ; ++ ; +/-
h20-H3-L3Y, candidate 20.28	116.7	169.9	+ ; + ; ++ ; +
h20-H4-L4Y, candidate 20.29	146.6	180.7	+ ; + ; ++ ; +/-
h20-H4-L5Y, candidate 20.30	105.1	149.5	++ ; + ; ++ ; ++
Chimeric Candidate 20	75.1	110.2	+ ; +/- ; + ; +/-
Hu5F9	80.0	122.8	+++ ; + ; +++ ; +++
AB06.12	83.1	97.2	+ ; +/- ; +/- ; -

+/-, weak agglutination observed at 50 µg/mL antibody only; +, agglutination at 50 µg/mL antibody; ++, agglutination for concentrations ≥16.7 µg/mL; +++, agglutination for concentrations ≥5.6 µg/mL

The kinetics (Kon and Koff) and affinity (KD) constants of the 5 new humanized h20-Hx-LxY variants were determined by SPR on Biacore. Results in Table 15 show that the 5 humanized variants with the VL CDR2-F56Y mutation have conserved an affinity of 2.0 to 2.8 nM, similar to the candidate 20 and similar to the corresponding humanized variants without the VL CDR2-F56Y mutation (Table 15).

Table 15: Kinetics and affinity constants of anti-CD47 humanized variants of candidate 20 with VL CDR2-F56Y mutation, as measured by Biacore (mean values of 2 independent experiments)

Antibody (hIgG4 format)	mean Kon (1/Ms) x 10^3	mean Koff (1/s) x 10^{-4}	mean KD (nM)	mean Rmax (RU)
h20-H2-L5Y, candidate 20.26	306.5	6.99	2.32	124.8
h20-H3-L2Y, candidate 20.27	292.0	5.84	2.06	106.6
h20-H3-L3Y, candidate 20.28	264.0	5.73	2.32	119.9
h20-H4-L4Y, candidate 20.29	305.5	7.61	2.61	102.4
h20-H4-L5Y, candidate 20.30	254.5	6.93	2.82	111.6
Chimeric Candidate 20	225.0	5.43	2.48	144.6
Hu5F9-G4P	101.4	0.75	0.73	85.5

The 5 humanized variants with the VL CDR2-F56Y mutation have also conserved a rapid dissociation kinetics, with a Koff value in the range of 5.73 x 10^{-4} to 7.61 x 10^{-4} s-1, as observed for the mouse and candidate 20 (Table 13 and 15).

3. Humanization of anti-CD47 candidate 22

Mouse candidate 22 was also selected for humanization. Four VH and 4 VL variants were generated by CDR engraftment into human acceptor frameworks, essentially as described for mouse candidate 20. The sequences were analyzed for removal of potential T-Cell epitopes with MHC class II high affinity using *in silico* algorithms, as well as for the presence of post-translational modifications such as Fv glycosylation and deamidation.

The 2 humanized VH variants h22-VH3 and h22-VH4 were cloned in an expression vector containing a human Fcγ4-S228P backbone (SEQ ID NO: 162) and the 4 humanized h22-VL variants were cloned into an expression vector containing a human Kappa backbone (SEQ ID NO: 176), in order to generate hIgG4/kappa humanized variants. Eight of the hIgG4/kappa variants were produced and purified from CHO cells co-transfected with one of the h22-VH3 or h22-VH4 and one of the 4 h22-VL vectors. The resulting antibodies were called h22-VHx-VLx, where h22 designated humanized candidate 22, VHx designated the Heavy chain variable domain with the humanized VH variant x (VH3 to VH4), and Lx designated the Light chain variable domain with the humanized VL variant x (VL1 to VL4). The kinetics (Kon and Koff) and affinity (KD) constants of the 8 purified antibodies were measured by SPR on Biacore. The 8 purified antibodies were also tested for their binding activity and specificity on human Raji lymphoma cells, for their functional activity in inhibiting the binding of hSIRPα on Raji cells and for their capacity to induce human RBC agglutination in whole blood. In all assays, the 8 humanized variants were compared with a hIgG4 chimeric candidate 22 generated by cloning of the mouse VH and VL sequences of candidate 22 into the appropriate vectors containing a human Fcγ4-S228P and human Kappa backbone. For RBC agglutination, the 8 humanized variants were also compared to the Hu5F9 antibody.

All results obtained in the different assays are summarized in Table 16 and Table 17 below.

Table 16: Characteristics of humanized variants of candidate 22

Candidate No	Antibody (hIgG4)	CD47 Binding (EC50 µg/mL)	CD47/SIRPα inhibition (IC50 µg/mL)	RBC agglutination (2 donors)
22	Chim. 22	0.122	0.140	++
22.9	h22-VH3-VL1	0.101	0.075	+++
22.10	h22-VH3-VL2	0.141	0.109	+++
22.11	h22-VH3-VL3	0.139	0.096	+++/++++
22.12	h22-VH3-VL4	0.108	0.116	++++
22.13	h22-VH4-VL1	0.070	0.119	+++
22.14	h22-VH4-VL2	0.099	0.093	+++
22.15	h22-VH4-VL3	0.124	0.098	+++
22.16	h22-VH4-VL4	0.184	0.116	++++
-	Hu5F9	nt	nt	++/+++

nt; not tested;

+/-, weak agglutination observed at 50 µg/mL antibody only; +, agglutination at 50 µg/mL antibody; ++, agglutination for concentrations ≥16.7 µg/mL; +++, agglutination for concentrations ≥5.6 µg/mL; ++++, agglutination for concentrations ≥1.9 µg/mL

Table 17: Kinetics and affinity constants of humanized anti-CD47 antibodies of candidate 22 by Biacore (mean values of 2 independent experiments)

Candidate No	Antibody (hIgG4)	mean Kon (1/Ms) x10^3	mean Koff (1/s) x 10^{-4}	mean KD (nM)
22	Chim. 22	31.15	3.81	12.20
22.9	h22-VH3-VL1	24.65	3.18	12.95
22.10	h22-VH3-VL2	28.75	3.15	11.05
22.11	h22-VH3-VL3	28.35	3.06	10.91
22.12	h22-VH3-VL4	25.25	2.52	10.00
22.13	h22-VH4-VL1	25.60	3.37	13.20
22.14	h22-VH4-VL2	31.25	3.25	10.44
22.15	h22-VH4-VL3	29.10	3.51	12.10
22.16	h22-VH4-VL4	28.15	2.63	9.37

All 8 humanized variants with the h22-VH3 or h22-VH4 sequences combined with the h22-VL1 to h22-VL4 sequences showed Kon, Koff and KD constants similar to the ones of the chimeric candidate 22. None of the variants showed a significant increased affinity to hCD47 as compared to chimeric candidate 22. The Koff values of all humanized variants from candidate 22 were observed to be in the range of 2.5 x 10^{-4} to 3.8 x 10^{-4} s^{-1}.

All 8 variants bound strongly to CD47 expressed on Raji cells with an EC50 similar to the EC50 of the chimeric candidate 22.

All 8 variants strongly inhibited the binding of hSIRPα to CD47 expressed on Raji cells, with an IC50 in the same range than the IC50 of chimeric candidate 22.

All 8 variants displayed a tendency to induce slightly more RBC agglutination than the chimeric candidate 22. Among them, 5 had similar RBC agglutination propensity than Hu5F9 benchmark.

VI. Epitope mapping, dimerization & glycosylation

1. Epitope mapping

The epitope recognized on hCD47 antigen by the mouse anti-CD47 candidates 20 and 22 was determined by using the high resolution method developed by CovalX (Bich C., Scott M., Panagiotidis A., Wenzel R. J., Nazabal A., Zenobi R. "Characterization of antibody-antigen interactions: Comparison between surface plasmon resonance measurements and hih-mass matrix-assisted laser desorption/ionization mass spectromrtry", Analytical Biochem 375:35-45, (2008)). The anti-CD47 antibodies were complexed with a soluble preparation of human CD47 extracellular domain tagged with a 6His-tag (SEQ ID NO: 160).

The anti-CD47 antibody/hCD47 complexes then were incubated with deuterated cross-linkers and subjected to multi-enzymatic cleavage. After enrichment of the cross-linked peptides, the samples were analyzed by high resolution mass spectrometry (nLC-Orbitrap MS) and the data generated were analyzed using XQuest and Stavrox software. The MALDI ToF MS analysis has been performed using CovalX's HM4 interaction module with a standard nitrogen laser and focusing on different mass ranges from 0 to 1500 kDa.

As depicted in Figure 15, the chemical cross-linking analysis showed that the epitope on hCD47 recognized by the chimeric candidate 20 was discontinuous and comprised the amino-acid residues K59, R63, Y66, T67, H108, T109, T117 and T120 (SEQ ID 151) or the amino-acid residues xxK_{59}xxxR_{63}xx$Y_{66}T_{67}$xx--------xx$H_{108}T_{109}$xxxxxxxT_{117}xxT_{120}xx of hCD47 (SEQ ID 151) where "x" is for any amino acid residue (numbering according to SEQ ID NO: 151).

A similar analysis showed that the epitope on hCD47 recognized by the chimeric candidate 22 (Figure 16) was also discontinuous and comprised K59, K61, S107, H108, T117, T120 and R121 of hCD47 (SEQ ID NO: 151) or the amino-acid residues xxK_{59}xK_{61}xxxxxx---------xxxxxxxx$S_{107}H_{108}$xxxxxxxxT_{117}xx$T_{120}R_{121}$xx of hCD47 (SEQ ID 151) where "x" is for any amino acid residue (numbering according to SEQ ID NO: 151).

Using the same method, the epitope on hCD47 recognized by AB06.12 was also determined and found to be different from candidate 20 or 22 (data not shown).

2. Dimerization of CD47

The High Mass MALDI ToF analysis and SEC-HPLC analysis of the soluble hCD47 (extracellular IgV domain) preparation showed the presence of monomer, dimer and multimers of hCD47, naturally formed *in vitro*. To further investigate the capability of the candidates 20 and 22 to recognize the dimer of hCD47, a mixture of anti-CD47 antibody/soluble hCD47 was submitted to cross-linking using CovalX's K200 MALDI MS analysis kit with an excess of antigen. Data suggest that Candidate 20 may bind both the dimer (60.8%) and the monomer of hCD47 (39,2%), while it cannot be excluded that complexes of one antibody with 2 monomers are detected (Table 18). Similarly, the candidate 22 may have formed cross-link with the hCD47 dimer (3,8%) and monomer (5%) (Table 18). Further experiments confirmed that candidates 20 and 22 both bind the human CD47 monomer and the human CD47 dimer that have been purified by SEC-HPLC (see Figures 18 and 19). Analysis of the soluble hCD47 by SE-HPLC showed heterogeneity in the protein preparation, with the presence of putative CD47 monomers and dimers (Figure 17). The monomer and dimer fractions were further purified by semi preparative SE-HPLC using Superdex 200 Increase 10/300 GL from GE healthcare. SDS-PAGE analysis in non-reducing conditions further demonstrated the purity of the 2 fractions, with a large band at ~30kDa observed for the CD47 monomer fraction corresponding to the expected sizes of glycosylated hCD47, and a major band slightly above 50kDa observed for the CD47 dimer fraction (Figure 18). The purified CD47 monomer and dimer fractions were then coated on ELISA plates and the anti-CD47 antibodies were tested for their capacity to recognize the 2 fractions. As shown in Figure 19, candidates 20 and 22 were capable to bind both the hCD47 monomer and dimer with similar efficacy.

Table 18: Analysis of soluble hCD47 in mixture with candidate 20, candidate 22 and after cross-link between soluble hCD47 and candidate 20 and candidate 22

Species	MW (kDa)	% area
Candidate 20	149.981	0
Candidate 20 and one hCD47	176.412	39.2
Candidate 20 and two hCD47	202.491	60.8
Candidate 22	150.150	91.2
Candidate 22 and one hCD47	176.750	5.0
Candidate 22 and two hCD47	201.135	3.8

3. <u>Recognition of deglycosylated hCD47</u>

It is known since more than 40 years (see Meezan E., Wu H. C., Black P. H., Robbins P. W. “Comparative studies on the carbohydrate-containing membrane components of normal and virus-transformed mouse fibroblasts. II Separation of glycoproteins and glycopeptides by

Saphadex chromatography", Biochemistry 8 (6):2518-24 (1969)), that the mechanisms of glycosylation can be modified in tumor cells, leading to the expression of proteins with altered glycosylation patterns (for more recent reviews, see also Pinho S.S. and Reis C.A. "Glycosylation in cancer: mechanisms and clinical implications", Nat Rev Cancer 15: 540-55 (2015)). CD47 is a highly glycosylated transmembrane protein with 6 N-glycosylation sites at positions N23, N34, N50, N73, N111, N206 (Lindberg F.P., Gresham H.D., Schwarz E., and Brown E.J. "Molecular cloning of integrin-associated protein: An immunoglobulin family member with multiple membrane-spanning domains implicated in $\alpha_v\beta_3$-dependent ligand binding", J Cell Biol 123: 485-96 (1993)).

As the CD47 glycosylation pattern is subjected to be modified in tumor cells, a key aspect to assess was whether therapeutic antibodies are dependent on the CD47 glycosylation level for their binding to the target. To address this point, soluble hCD47 was treated with PNGase to remove the N-glycosylations (Maley F., Trimble R. B., Tarentino A. L., and Plummer T. H. "Characterization of glycoproteins and their associated oligosaccharides through the use of endoglycosidases", Anal. Biochem 180:195-204 (1989)), then candidates 20 or 22 were cross-linked using DSS and K200 during 180min at room temperature to generate anti-CD47 antibody/deglycosylated-hCD47 cross-linked complexes. These complexes were used to monitor the nature of these non-covalent interactions according to the method described by CovalX (Bich C., Scott M., Panagiotidis A., Wenzel R. J., Nazabal A., Zenobi R. "Characterization of antibody-antigen interactions: Comparison between surface plasmon resonance measurements and hih-mass matrix-assisted laser desorption/ionization mass spectromrtry", Analytical Biochem 375:35-45 (2008)). Upon monitoring the uncomplexed and complexed molecules by MALDI-ToF, we successfully identified the peaks corresponding to the soluble antibodies added in excess (148.944kDa for candidate 20 and 149.173kDa for 22), as well as of the antibody-CD47 complexes (respectively 174.407 kDa and 163.440kDa), but failed to identify a peak corresponding to the uncomplexed deglycosylated CD47 (15.901kDa; see Table 19). These data suggest that all the available deglycosylated hCD47 was complexed with either candidate 20 or with candidate 22, supporting the notion that antibodies 20 and 22 are capable to recognize both glycosylated and N-deglycosylated hCD47.

Table 19: Mass spectrometry analyses of soluble deglycosylated hCD47 cross-linked with either candidate 20 or candidate 22

Species	MW (kDa)	% area
Candidate 20	148.944	84.8
Candidate 20 and monomeric deglycosylated-hCD47	174.407	15.2
monomeric deglycosylated-hCD47	15.901	0
Candidate 22	149.173	92.8
Candidate 22 and monomeric hCD47	163.440	7.2
monomeric deglycosylated-hCD47	15.901	0

To confirm and quantify the recognition of deglycosylated hCD47 by candidates 20 and 22 as observed with the CovalX approach, the antibodies were tested by ELISA on coated hCD47 and deglycosylated-hCD47 preparations. The successful removal of N-glycans from hCD47 was confirmed by Size Exclusion Chromatography analysis (not shown). The anti-CD47 candidates 20 and 22 were tested in a chimeric hu-IgG4 format in parallel to the humanized variant h20-H2L5Y also in hu-IgG4 isotype. The antibodies were tested along with the AB06.12 and Hu5F9 humanized antibodies generated in a hu-IgG4 format to allow the same recognition efficiency by ELISA. As shown in Figure 22A and Tables 20, 21 and 22, although Hu5F9 and AB06.12 displayed slightly lower maximum binding, all antibodies strongly bound with similar profiles and EC50, EC95, equilibrium binding constant and maximum specific binding (Bmax) values to the glycosylated CD47 ($Bmax_1$). In contrast, the binding of Hu5F9 and of AB06.12 to N-deglycosylated CD47 was strongly reduced when compared to the binding observed for the chimeric candidates 20, 22, and for the humanized variant h20-H2L5Y (Figure 22B, Tables 20, 21 and 22). Indeed, the chimeric candidates 20, 22, and the humanized antibody h20-H2L5Y bound on deglycosylated CD47 with an EC50 value that was less than 3.0 fold the EC50 value on glycosylated CD47 and/or with an EC95 value that was less than 10.0 fold the EC95 value measured on glycosylated CD47. In contrast the EC50 and EC95 values of AB06.12 and Hu5F9 were more than 3.0 and 10.0 fold superior to the EC50 and EC95 values measured on glycosylated CD47, respectively (Table 20). Moreover, the equilibrium binding constant values of the chimeric candidates 20, 22, and of the humanized antibody h20-H2L5Y were ≤ 60 pM on both glycosylated and on N-deglycosylated CD47, while the equilibrium binding constant values of AB06.12 and Hu5F9 were in the range of 90-130 pM on N-deglycosylated CD47 (Table 21). Finally, the chimeric candidates 20, 22, and the humanized antibody h20-H2L5Y bound both glycosylated and N-deglycosylated CD47 with a maximum specific binding (Bmax) value ≥ 1.9 OD on glycosylated ($Bmax_1$) and on deglycosylated ($Bmax_2$) CD47, while the $Bmax_2$ values of AB06.12 and Hu5F9 were < 1.5 OD on N-deglycosylated CD47 (Table 22).

These results further demonstrate that recognition of hCD47 by candidates 20, 22 and by the humanized h20-H2L5Y antibody does not depend on the N-glycosylation level of CD47. Conversely, Hu5F9 and AB06.12 antibodies significantly lose their binding capacity on N-deglycosylated CD47. As the glycosylation level and nature of cell surface proteins can be modified on tumor cells, it is possible that the glycosylation of the CD47 antigen can be also modified when expressed or overexpressed by tumor cells. Of note, the glycosylation pattern of CD47 does not seem to be necessary for its interaction with SIRPα (Subramanian S., Boder E.T., and Discher D.E. "Phylogenetic divergence of CD47 interactions with human signal regulatory protein α reveals locus of species specificity." J. Biolog. Chem. 282(3):1805-18 (2007)), suggesting that aberrant glycosylation will not alter the CD47/SIRPα interaction. Therefore, cancer cells overexpressing CD47 will protect themselves from phagocytosis independently on their glycosylation pattern. Conversely, the N-glycosylation pattern of hCD47 may affect antibody binding and significantly lower therapeutic efficacy. As a consequence, we expect a broader anti-tumor effect by using anti-CD47 therapeutic antibodies such as 20, 22 and preferably h20-H2L5Y, whose binding to hCD47 are not dependent on its glycosylation level.

Table 20: Characteristics of binding on glycosylated-hCD47 compared to N-Deglycosylated hCD47 of anti-CD47 chimeric candidates 20 and 22, humanized h20-H2L5Y antibody and benchmark antibodies, as measured by ELISA. The EC50 and EC95 values were calculated by using the non-linear regression / log(antibody) versus response (four parameters) model of the GraphPad Prism software.

Antibody (hu-IgG4)	Binding to Glycosylated hCD47-His by ELISA		Binding to N-Deglycosylated hCD47-His by ELISA		Ratio EC Deglycosylated / EC Glycosylated	
	EC50 (pM)	**EC95 (pM)**	**EC50 (pM)**	**EC95 (pM)**	**EC50 ratio**	**EC95 ratio**
Chimeric Candidate 20	28.0	667.0	47.4	3335.0	1.7	5.0
h20-H2L5Y	44.0	1334.0	96.0	11339.0	2.2	8.5
Chimeric Candidate 22	38.7	4669.0	42.0	6670.0	1.1	1.4
AB06.12	75.4	2054.4	427.5	214782.0	5.7	107.4
Hu5F9	54.7	1961.0	253.5	54027.0	4.6	27.6

Table 21: Equilibrium binding constant on Glycosylated-hCD47 compared to N-Deglycosylated hCD47 of anti-CD47 chimeric candidates 20 and 22, humanized h20-H2L5Y antibody and benchmark antibodies, as measured by ELISA. The equilibrium binding constant values were calculated by using the binding saturation / one-site binding model of the GraphPad Prism software (mean +/- SD of triplicates).

Antibody (hu-IgG4)	Equilibrium binding constant on Glycosylated hCD47 (pM)		Equilibrium binding constant on Deglycosylated hCD47 (pM)	
	mean	SD	mean	SD
Chimeric Candidate 20	27.3	3.3	38.8	4.6
h20-H2L5Y	43.6	7.2	55.7	10.5
Chimeric Candidate 22	34.4	6.7	31.8	5.9
AB06.12	67.0	6.8	93.6	28.6
Hu5F9	49.0	4.9	129.0	43.2

Table 22: Maximum specific binding (Bmax) on Glycosylated-hCD47 ($Bmax_1$) compared to N-Deglycosylated ($Bmax_2$) hCD47 of anti-CD47 chimeric candidates 20 and 22, humanized h20-

H2L5Y antibody and benchmark antibodies, as measured by ELISA. The Bmax values were calculated by using the binding saturation / one-site binding model of the GraphPad Prism software (mean +/- SD of triplicates).

Antibody (hu-IgG4)	$Bmax_1$ on Glycosylated hCD47 (OD value)		$Bmax_2$ on Deglycosylated hCD47 (OD value)	
	mean	SD	mean	SD
Chimeric Candidate 20	2.90	0.07	2.20	0.05
h20-H2L5Y	2.85	0.09	1.97	0.07
Chimeric Candidate 22	2.85	0.11	2.57	0.09
AB06.12	2.48	0.05	0.97	0.06
Hu5F9	2.66	0.05	1.40	0.10

VII. *In vivo* efficacy of humanized anti-CD47 antibody h20-H2L5Y

The humanized variant h20-H2L5Y was evaluated in a hu-IgG4 format (h20-H2L5Y-G4) as single agent and in combination with anti-Her2 Trastuzumab (Herceptin®) in the A549 lung adenocarcinoma xenograft model as previously described. NOG mice (n=8 per group) were engrafted with A549 cells (10 million cells injected subcutaneously). The antibody therapy was started fourteen days after the cell transplant, once the tumor volume was about 100 mm^3. The anti-CD47 antibody h20-H2L5Y and Herceptin® were injected alone or in combination at the dose of 10 mg/kg/dose by intraperitoneal injection, 3 times a week for up to 10 weeks. As shown in Figure 23 the combination of the anti-CD47 antibody h20-H2L5Y-G4 with Herceptin® significantly prolong the survival of treated-mice when compared to the control group of animals injected with the vehicle ($p<0.005$) and to the group of animals treated with Herceptin® alone ($p<0.05$). Thus, these results show an increased efficacy of the combination of humanized anti-CD47 h20-H2L5Y antibody with Herceptin® for controlling the growth of the A549 lung adenocarcinoma *in vivo* in NOG mice, and confirm the results previously obtained with the mouse anti-CD47 candidate 20.

The humanized h20-H2L5Y antibody was also evaluated in a mouse xenograft model of Her-2 positive gastric cancer, as single agent and in combination with anti-Her2 Trastuzumab (Herceptin®) that is approved for the treatment of Her2-overexpressing metastatic gastric cancers. For this study, the human NCI-N87 cell line (ATCC®- CRL-5822) was selected for xenograft as a Her-2 positive gastric tumor responding to Herceptin® treatment (Matsui Y., Inomata M., Tojigamori M., Sonoda K., Shiraishi N., Kitano S. "Suppression of tumor growth in human gastric cancer with HER2 overexpression by an anti-HER2 antibody in a murine model", Int. J. Oncol. 27(3):681-5 (2005)). *In vitro*, these cells very strongly expressed Her-2 as detected by the binding of Herceptin® and strongly expressed CD47 as measured by staining with h20-H2L5Y antibody and flow cytometry analysis (data not shown). The studies were carried out in NOG mice, and the humanized h20-H2L5Y antibody was tested in a hu-IgG4-S228P-L235E format (h20-H2L5Y-G4PE). For this purpose, the humanized antibody h20-H2L5Y was further produced in a hu-IgG4 format carrying the S228P and L235E mutations following cloning of the h20-VH2 variant in an expression vector containing a human Fcγ4-S228P-L235E backbone (SEQ ID NO: 164). The L235E mutation has been shown to strongly reduce the affinity of hu-IgG4 to Fcγ receptors (FcγRs) and therefore abrogates the Fc effector functions such as ADCC or ADCP (Alegre ML, Collins AM, Pulito VL, Brosius RA, Olson WC, et al. "Effect of a single amino acid mutation on the activating and immunosuppressive properties of a "humanized" OKT3 monoclonal antibody", J. Immunol. 148(11): 3461-68 (1992); Reddy MP, Kinney CAS., Chaikin MA, Fishman-Lobell J, Tsui P, et al. "Elimination of Fc Receptor-Dependent Effector Functions of a Modified IgG4 Monoclonal Antibody to Human CD4", J. Immunol. 164:1925-33 (2000)). The L235E mutation will therefore reduce the risk of side effects such as phagocytosis or lysis of opsonized normal cells (such as RBCs, platelets, etc). NOG mice (n=8 per group) were engrafted with NCI-N87 cells (10 million cells injected subcutaneously) and the antibody therapy was started seven days after the cell transplant, once the tumor volume was about $100mm^3$. The anti-CD47 antibody h20-H2L5Y-G4PE was injected alone or in combination at 10mg/kg/dose, whereas Herceptin® was injected alone or in combination at the dose of 2.5mg/kg/dose. Antibodies were administered by intraperitoneal injection, 3 times a week for up to 5 weeks. As shown in Figure 24, a delay of the NCI-N87 growth was observed in the group of mice treated with the anti-CD47 antibody h20-H2L5Y-G4PE alone, and a stronger delay of the

NCI-N87 growth was observed in mice treated with the combination of Herceptin® with the anti-CD47 h20-H2L5Y-G4PE antibody (Figure 24). Similar results were obtained when the study was carried out in NOD/SCID mice (data not shown). This reduction of the NCI-N87 tumor growth in the group of mice treated with the combination of Herceptin® and anti-CD47 h20-H2L5Y-G4PE was statistically higher when compared to the group of mice treated with Herceptin® alone ($p<0.05$, Student's t-test), thus demonstrating the cooperative effect between anti-CD47 h20-H2L5Y antibody and Herceptin® for controlling the proliferation of NCI-N87 tumor cells *in vivo*, and potentially of other Her-2 positive gastric tumors.

Further aspects:

1. An antibody that is selected from the group of antibodies consisting of:
   (a) A group 1 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 1-3 (Kabat annotation) or SEQ ID NOs: 4-6 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 7-9 (Kabat annotation) or SEQ ID NOs: 10-12 (IMGT annotation); or
   (b) A group 2 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 13-15 (Kabat annotation) or SEQ ID NOs: 16-18 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 19-21 (Kabat annotation) or SEQ ID NOs: 22-24 (IMGT annotation); or
   (c) A group 3 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 25-27 (Kabat annotation) or SEQ ID NOs: 28-30 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 31-33 (Kabat annotation) or SEQ ID NOs: 34-36 (IMGT annotation); or
   (d) A group 4 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 37-39 (Kabat annotation) or SEQ ID NOs: 40-42 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 43-45 (Kabat annotation) or SEQ ID NOs: 46-48 (IMGT annotation); or
   (e) A group 5 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 49-51 (Kabat annotation) or SEQ ID NOs: 52-54

(IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 55-57 (Kabat annotation) or SEQ ID NOs: 58-60 (IMGT annotation); or

(f) A group 6 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 61-63 (Kabat annotation) or SEQ ID NOs: 64-66 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 67-69 (Kabat annotation) or SEQ ID NOs: 70-72 (IMGT annotation); or

(g) A group 7 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 73-75 (Kabat annotation) or SEQ ID NOs: 76-78 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 79-81 (Kabat annotation) or SEQ ID NOs: 82-84 (IMGT annotation); or

(h) A group 8 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 85-87 (Kabat annotation) or SEQ ID NOs: 88-90 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 91-93 (Kabat annotation) or SEQ ID NOs: 94-96 (IMGT annotation); or

(i) A group 9 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 97-99 (Kabat annotation) or SEQ ID NOs: 100-102 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 103-105 (Kabat annotation) or SEQ ID NOs: 106-108 (IMGT annotation); or

(j) A group 10 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 109-111 (Kabat annotation) or SEQ ID NOs: 112-114 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 115-117 (Kabat annotation) or SEQ ID NOs: 118-120 (IMGT annotation);

(k) A group 11 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 49-51 (Kabat annotation) or SEQ ID NOs: 52-54 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 55, 152, 57 (Kabat annotation) or SEQ ID NOs: 58, 153, 60 (IMGT annotation);

(l) and wherein the group of antibodies that are preferred are selected from the group of antibodies consisting of the group 5 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 49-51 (Kabat annotation) or SEQ ID NOs: 52-54 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 55-57 (Kabat annotation) or SEQ ID NOs: 58-60 (IMGT annotation), the group 6 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 61-63 (Kabat annotation) or SEQ ID NOs: 64-66 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 67-69 (Kabat annotation) or SEQ ID NOs: 70-72 (IMGT annotation); and the group 11 of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 49-51 (Kabat annotation) or SEQ ID NOs: 52-54 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 55, 152, 57 (Kabat annotation) or SEQ ID NOs: 58, 153, 60 (IMGT annotation) are preferred.

2. The antibody of aspect 1, wherein said antibody is selected from the group of antibodies consisting of:
   (a) The group 1 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 121 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 122; or
   (b) The group 2 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 123 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 124; or
   (c) The group 3 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 125 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 126; or
   (d) The group 4 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 127 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 128; or
   (e) The group 5 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 129 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 130; or

(f) The group 6 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 131 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 132; or

(g) The group 7 of antibodies that comprise a VH havingan amino acid sequence set forth in SEQ ID NO: 133 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 134; or

(h) The group 8 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 135 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 136; or

(i) The group 9 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 137 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 138; or

(j) The group 10 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 139 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 140; or

(k) The group 11 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 129 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 154;

(l) and wherein the group of antibodies that are preferred are selected from the group of antibodies consisting of the group 5 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 129 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 130, the group 6 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 131 and/or a VL having an amino acid sequence set forth in SEQ ID NO: 132, and the group 11 of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 129, and/or a VL having an amino acid sequence set forth in SEQ ID NO: 154.

3. The antibody of any one of aspects 1-2, wherein the antibody is a full length chimeric, mouse or humanized antibody.

4. The antibody of aspect 3, wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 141 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 142 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 143 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 144 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 145 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 168 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 172-175; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 169 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 172-175; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 170 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 172-175; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 171 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 172-175.

5. The antibody of any one of aspects 1-4, wherein the antibody has a Koff value of about 1.0 x 10^{-4} s^{-1} (1/s) or more.

6. The antibody of any of aspects 1-5, having a human IgG1, IgG2, IgG3, IgG4 or IgA constant region, preferably a human IgG4 with optionally the mutation S228P (SEQ ID NO: 162) and/or with optionally the mutations S228P-L235E (SEQ ID NO: 164).

7. The antibody of any of aspects 1-5, wherein the antibody is an antibody fragment, and wherein the antibody fragment is optionally an $F(ab')_2$ or an Fab fragment.

8. The antibody of any of aspects 1-7, wherein the antibody is a bispecific antibody.

9. The antibody of any one of aspects 1-8, wherein the antibody binds specifically to human CD47 and does not activate CD47 upon binding.

10. The antibody of any one of aspects 1-9, wherein the antibody binds to a discontinuous epitope on CD47, preferably human CD47.

11. The antibody of aspect 10, wherein the discontinuous epitope comprises amino acid residues K59, R63, Y66, T67, H108, T109, T117 and T120 (SEQ ID 151); or the discontinuous epitope comprises amino acid residues K59, K61, S107, H108, T117, T120 and R121 of hCD47 (SEQ ID NO: 151).

12. The antibody of any one of aspects 1-11, wherein the antibody binds the CD47 monomer and CD47 dimer, preferably the human CD47 monomer and dimer, respectively.

13. The antibody of any one of aspects 1-12, wherein the antibody binds the glycosylated and deglycosylated forms of CD47, preferably the human CD47 glycosylated and deglycosylated forms, respectively.

14. A polynucleotide encoding an antibody set forth in any one of aspects 1-13.

15. A cell that produces an antibody set forth in any one of aspects 1-13.

16. A pharmaceutical composition comprising an antibody set forth in any one of aspect 1-13, optionally further comprising a pharmaceutical acceptable excipient.

17. An antibody of any of aspects 1-13 or a pharmaceutical composition of aspect 16, for use in the treatment of a disease in a subject.

18. The antibody or composition for use of aspect 17, for use in the treatment of cancer.

19. The antibody or composition for use of aspect 17 or aspect 18, for use as a monotherapy.

20. The antibody or composition for use of aspect 17 or aspect 18, for use in combination therapy, preferably a combination with a Her-2 inhibitor or an anti-Her-2 antibody or an EGFR inhibitor or an anti-EGFR antibody.

21. The antibody or composition for use according to aspects 17-20, wherein the subject is human and/or a non-human animal.

22. A method of detecting the presence of CD47 in a biological sample or tissue, the method comprising (i) contacting said sample or tissue with the antibody set forth in any one of aspects 1-13, and (ii) determining the presence of antibody bound to said tissue or sample.

23. A process for producing an antibody of any one of aspects 1-13, comprising culturing the cell of aspect 15 so that the nucleic acid of aspect 14 is expressed, and optionally recovering the antibody from the cell culture.

24. A method of treating or preventing cancer in a subject, comprising administering to a subject having or at risk of developing cancer an effective amount of an antibody of aspects 1-13.

Further embodiments are defined in the following numbered paragraphs:

1. An antibody that binds to glycosylated and non-glycosylated CD47, wherein binding of the antibody to CD47 is not dependent on the glycosylation of CD47.
2. The antibody of paragraph 1, wherein the antibody is suitable for use as a therapeutic antibody.
3. The antibody of paragraph 1 or paragraph 2, wherein the antibody binds glycosylated and deglycosylated forms of human CD47.

4. The antibody of paragraph 3, wherein the glycosylated form of human CD47 comprises one or more N-glycosylated residues at positions N23, N34, N50, N73, N111 and/or N206 in the amino acid sequence of human CD47 (SEQ ID NO:151).
5. The antibody of any preceding paragraph, wherein the deglycosylated form of human CD47 comprises glycosylated human CD47 that has been treated with a peptide N-glycosidase (PNGase) for removal of N-glycosylations.
6. The antibody of any preceding paragraph, wherein (i) a ratio of EC50s of binding of the antibody to non-glycosylated versus glycosylated forms of CD47 is less than 4, 3, 2, 1, 0.5, 0.25, preferably in a range from 4 to 1, more preferably 3 to 1, most preferably 2 to 1; and/or (ii) a ratio of EC95s of binding of the antibody to non-glycosylated versus glycosylated forms of CD47 is less than 25, 20, 10, 1, 0.5 or 0.25, preferably in a range from 10 to 1, more preferably 9 to 1, most preferably 9 to 1.
7. The antibody of any preceding paragraph, wherein the antibody binds to each of glycosylated and non-glycosylated CD47 with an equilibrium binding constant of 80 pM or lower, preferably 70 pM or lower, more preferably 60pM or lower.
8. The antibody of any preceding paragraph, wherein a maximum binding capacity (Bmax2) of the antibody to non-glycosylated CD47 is at least 60% of a maximum binding capacity (Bmax1) of the antibody to glycosylated CD47.
9. The antibody of any preceding paragraph, wherein the antibody has a Koff value for binding to glycosylated and/or non-glycosylated CD47 of about 1.0 x 10-4 s-1 (1/s) or more.
10. The antibody of paragraph 9, wherein the antibody has a Koff value for binding to glycosylated and/or non-glycosylated CD47 of from 1.0 x 10-4 s-1 to 1.0 x 10-3 s-1.
11. The antibody of paragraph 10, wherein the antibody has a Koff value for binding to glycosylated and/or non-glycosylated CD47 of from 2.5 x 10-4 s-1 to 8.0 x 10-4 s-1.
12. An antibody that binds to CD47, wherein the antibody has a Koff value for binding to CD47 of from 1.0 x 10-4 s-1 to 1.0 x 10-3 s-1.
13. An antibody that is selected from the group of antibodies consisting of (i) a group of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 49-51 (Kabat annotation) or SEQ ID NOs: 52-54 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 55-57 (Kabat annotation) or SEQ ID NOs: 58-60 (IMGT annotation), (ii) a group of antibodies that comprise a heavy

chain having each of the CDR sequences set forth in SEQ ID NOs: 61-63 (Kabat annotation) or SEQ ID NOs: 64-66 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 67-69 (Kabat annotation) or SEQ ID NOs: 70-72 (IMGT annotation); and (iii) a group of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 49-51 (Kabat annotation) or SEQ ID NOs: 52-54 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 55, 152, 57 (Kabat annotation) or SEQ ID NOs: 58, 153, 60 (IMGT annotation).

14. The antibody of paragraph 13, wherein said antibody is selected from the group of antibodies consisting of:
    (a) a group of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 129 and a VL having an amino acid sequence set forth in SEQ ID NO: 130; or
    (b) a group of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 131 and a VL having an amino acid sequence set forth in SEQ ID NO: 132; or
    (c) a group of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 129 and a VL having an amino acid sequence set forth in SEQ ID NO: 154.
15. The antibody of any one of paragraphs 13-14, wherein the antibody is a full length chimeric, mouse or humanized antibody.
16. The antibody of paragraph 15, wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 141 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 142 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 143 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid

sequence set forth in SEQ ID NO: 144 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 145 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159 or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 168 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 172-175; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 169 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 172-175; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 170 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 172-175; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 171 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 172-175.

17. The antibody of any one of paragraphs 13-16, wherein the antibody has a Koff value between 1.0 x 10-4 s^{-1} to 1.0 x 10^{-3} s^{-1}.
18. The antibody of any of paragraphs 13-17, having a human IgG4 constant region with optionally the mutation S228P (SEQ ID NO: 162) or with optionally the mutations S228P-L235E (SEQ ID NO: 164).
19. The antibody of any of paragraphs 13-18, wherein the antibody is an antibody fragment, and wherein the antibody fragment is optionally an $F(ab')_2$ or an Fab fragment.
20. The antibody of any of paragraphs 13-19, wherein the antibody is a bispecific antibody.
21. The antibody of any one of paragraphs 13-20, wherein the antibody binds specifically to CD47 and disrupts the CD47-SIRPα interaction, preferably the antibody binds specifically to human CD47 and disrupts the human CD47-SIRPα interaction, respectively.
22. The antibody of any one of paragraphs 13-21, wherein the antibody binds to a discontinuous epitope on CD47, preferably human CD47.

23. The antibody of paragraph 22, wherein the discontinuous epitope comprises amino acid residues K59, R63, Y66, T67, H108, T109, T117 and T120 of human CD47 (SEQ ID 151); or the discontinuous epitope comprises amino acid residues K59, K61, S107, H108, T117, T120 and R121 of human CD47 (SEQ ID NO: 151).
24. The antibody of any one of paragraphs 13-23, wherein the antibody binds the CD47 monomer and CD47 dimer, preferably the human CD47 monomer and dimer, respectively.
25. The antibody of any one of paragraphs 13-24, wherein the antibody binds the glycosylated and deglycosylated forms of CD47, preferably the human CD47 glycosylated and deglycosylated forms, respectively.
26. A polynucleotide encoding an antibody set forth in any one of paragraphs 1-24.
27. A cell that produces an antibody set forth in any one of paragraphs 1-24.
28. A pharmaceutical composition comprising an antibody set forth in any one of paragraph 1-24, optionally further comprising a pharmaceutical acceptable excipient.
29. An antibody of any of paragraphs 1-24 or a pharmaceutical composition of paragraph 28, for use in the treatment of a disease in a subject.
30. The antibody or composition for use of paragraph 29, for use in the treatment of cancer.
31. The antibody or composition for use of paragraph 30, for use as a monotherapy.
32. The antibody or composition for use of paragraph 30, for use in combination therapy, preferably a combination with a Her-2 inhibitor or an anti-Her2 antibody or an EGFR inhibitor or an anti-EGFR antibody.
33. The antibody or composition for use according to paragraphs 29-32, wherein the subject is human or a non-human animal.
34. A method of detecting the presence of CD47 in a biological sample or tissue, the method comprising (i) contacting said sample or tissue with the antibody set forth in any one of paragraphs 1-24, and (ii) determining the presence of antibody bound to said tissue or sample.
35. A process for producing an antibody of any one of paragraphs 1-24, comprising culturing the cell of paragraph 27 so that the nucleic acid of paragraph 26 is expressed, and optionally recovering the antibody from the cell culture.

36. A method of treating or preventing cancer in a subject, comprising administering to a subject having or at risk of developing cancer an effective amount of an antibody of paragraphs 1-24.

CLAIMS

1. An antibody that binds to glycosylated and non-glycosylated CD47, wherein binding of the antibody to CD47 is not dependent on the glycosylation of CD47.

2. The antibody of claim 1, wherein the antibody is suitable for use as a therapeutic antibody.

3. The antibody of claim 1 or claim 2, wherein the antibody binds glycosylated and deglycosylated forms of human CD47.

4. The antibody of claim 3, wherein the glycosylated form of human CD47 comprises one or more N-glycosylated residues at positions N23, N34, N50, N73, N111 and/or N206 in the amino acid sequence of human CD47 (SEQ ID NO:151).

5. The antibody of any preceding claim, wherein the deglycosylated form of human CD47 comprises glycosylated human CD47 that has been treated with a peptide N-glycosidase (PNGase) for removal of N-glycosylations.

6. The antibody of any preceding claim, wherein (i) a ratio of EC50s of binding of the antibody to non-glycosylated versus glycosylated forms of CD47 is less than 4:1, 3:1, 2:1, 1:2, 1:3 or 1:4, preferably in a range from 4:1 to 1:4, more preferably 3:1 to 1:3, most preferably 2:1 to 1:2; and/or (ii) a ratio of EC95s of binding of the antibody to non-glycosylated versus glycosylated forms of CD47 is less than 25:1, 20:1 or 10:1, 1:10, 1:20 or 1:25, preferably in a range from 10:1 to 1:10, more preferably 9:1 to 1:9, most preferably 10:1 to 1:10.

7. The antibody of any preceding claim, wherein the antibody binds to each of glycosylated and non-glycosylated CD47 with an equilibrium binding constant of 80 pM or lower, preferably 70 pM or lower, more preferably 60pM or lower.

8. The antibody of any preceding claim, wherein a maximum binding capacity ($Bmax_2$) of the antibody to non-glycosylated CD47 is at least 60% of a maximum binding capacity ($Bmax_1$) of the antibody to glycosylated CD47.

9. The antibody of any preceding claim, wherein the antibody has a Koff value for binding to glycosylated and/or non-glycosylated CD47 of about 1.0×10^{-4} s^{-1} (1/s) or more.

10. The antibody of claim 9, wherein the antibody has a Koff value for binding to glycosylated and/or non-glycosylated CD47 of from 1.0×10^{-4} s^{-1} to 1.0×10^{-3} s^{-1}.

11. The antibody of claim 10, wherein the antibody has a Koff value for binding to glycosylated and/or non-glycosylated CD47 of from 2.5×10^{-4} s^{-1} to 8.0×10^{-4} s^{-1}.

12. An antibody that binds to CD47, wherein the antibody has a Koff value for binding to CD47 of from 1.0×10^{-4} s^{-1} to 1.0×10^{-3} s^{-1}.

13. The antibody of any preceding claim, wherein the antibody is a full length chimeric, mouse or humanized antibody.

14. The antibody of any of claims 1 to 12, wherein the antibody is an antibody fragment, and wherein the antibody fragment is optionally an $F(ab')_2$ or an Fab fragment.

15. The antibody of any preceding claim, wherein the antibody is a bispecific antibody.

16. The antibody of any preceding claim, wherein the antibody binds specifically to glycosylated and non-glycosylated CD47 and disrupts the CD47-SIRPα interaction, preferably the antibody binds specifically to glycosylated and non-glycosylated human CD47 and disrupts the human CD47-SIRPα interaction, respectively.

17. The antibody of any preceding claim, having a human IgG4 with optionally the mutation S228P (SEQ ID NO: 162) or with optionally the double mutation S228P-L235E (SEQ ID NO: 164)

18. The antibody of any preceding claim, wherein the antibody binds to a discontinuous epitope on CD47, preferably human CD47.

19. The antibody of claim 18, wherein the discontinuous epitope comprises amino acid residues K59, R63, Y66, T67, H108, T109, T117 and T120 of human CD47 (SEQ ID 151); or the discontinuous epitope comprises amino acid residues K59, K61, S107, H108, T117, T120 and R121 of human CD47 (SEQ ID NO: 151).

20. The antibody of any preceding claim, wherein the antibody binds the CD47 monomer and CD47 dimer, preferably the human CD47 monomer and dimer, respectively.

21. The antibody of any preceding claim, wherein the antibody is selected from the group of antibodies consisting of (i) a group of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 49-51 (Kabat annotation) or SEQ ID NOs: 52-54 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 55-57 (Kabat annotation) or SEQ ID NOs: 58-60 (IMGT annotation), (ii) a group of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 61-63 (Kabat annotation) or SEQ ID NOs: 64-66 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 67-69 (Kabat annotation) or SEQ ID NOs: 70-72 (IMGT annotation); and (iii) a group of antibodies that comprise a heavy chain having each of the CDR sequences set forth in SEQ ID NOs: 49-51 (Kabat annotation) or SEQ ID NOs:52-54 (IMGT annotation) and a light chain having each of the CDR sequences set forth in SEQ ID NOs: 55, 152, 57 (Kabat annotation) or SEQ ID NOs: 58, 153, 60 (IMGT annotation).

22. The antibody of claim 20, wherein said antibody is selected from the group of antibodies consisting of:

(a) a group of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 129 and a VL having an amino acid sequence set forth in SEQ ID NO: 130; or
(b) a group of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 131 and a VL having an amino acid sequence set forth in SEQ ID NO: 132; or
(c) a group of antibodies that comprise a VH having an amino acid sequence set forth in SEQ ID NO: 129 and a VL having an amino acid sequence set forth in SEQ IDNO: 154.

23. The antibody of any of claims 1 to 20, which is a humanized antibody comprising a VH having an amino acid sequence set forth in SEQ ID NO: 141 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 142 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 143 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 144 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs: 155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 145 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 146-150 and/or SEQ ID NOs:155-159; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 168 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 172-175; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 169 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 172-175; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 170 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 172-175; or wherein the humanized antibody comprises a VH having an amino acid sequence set forth in SEQ ID NO: 171 and a VL that is selected from the group of VLs consisting of amino acid sequences set forth in SEQ ID NOs: 172-175.

24. A polynucleotide encoding an antibody set forth in any preceding claim.

25. A cell that produces an antibody set forth in any one of claims 1 to 23.

26. A pharmaceutical composition comprising an antibody set forth in any one of claims 1-23, optionally further comprising a pharmaceutical acceptable excipient.

27. An antibody of any of claims 1-23 or a pharmaceutical composition of claim 26, for use in the treatment of a disease in a subject.

28. The antibody or composition for use of claim 27, for use in the treatment of cancer.

29. The antibody or composition for use of claim 28, wherein the cancer is gastric, lung, ovarian or breast cancer, preferably gastric cancer.

30. The antibody or composition for use of any of claims 27 to 29, for use as a monotherapy.

31. The antibody or composition for use of any of claims 27 to 29, for use in combination therapy, preferably a combination with a Her-2 inhibitor or an anti-Her2 antibody or an EGFR inhibitor or an anti-EGFR antibody.

32. The antibody or composition for use according to claims 27-31, wherein the subject is a human or a non-human animal.

Figure 1

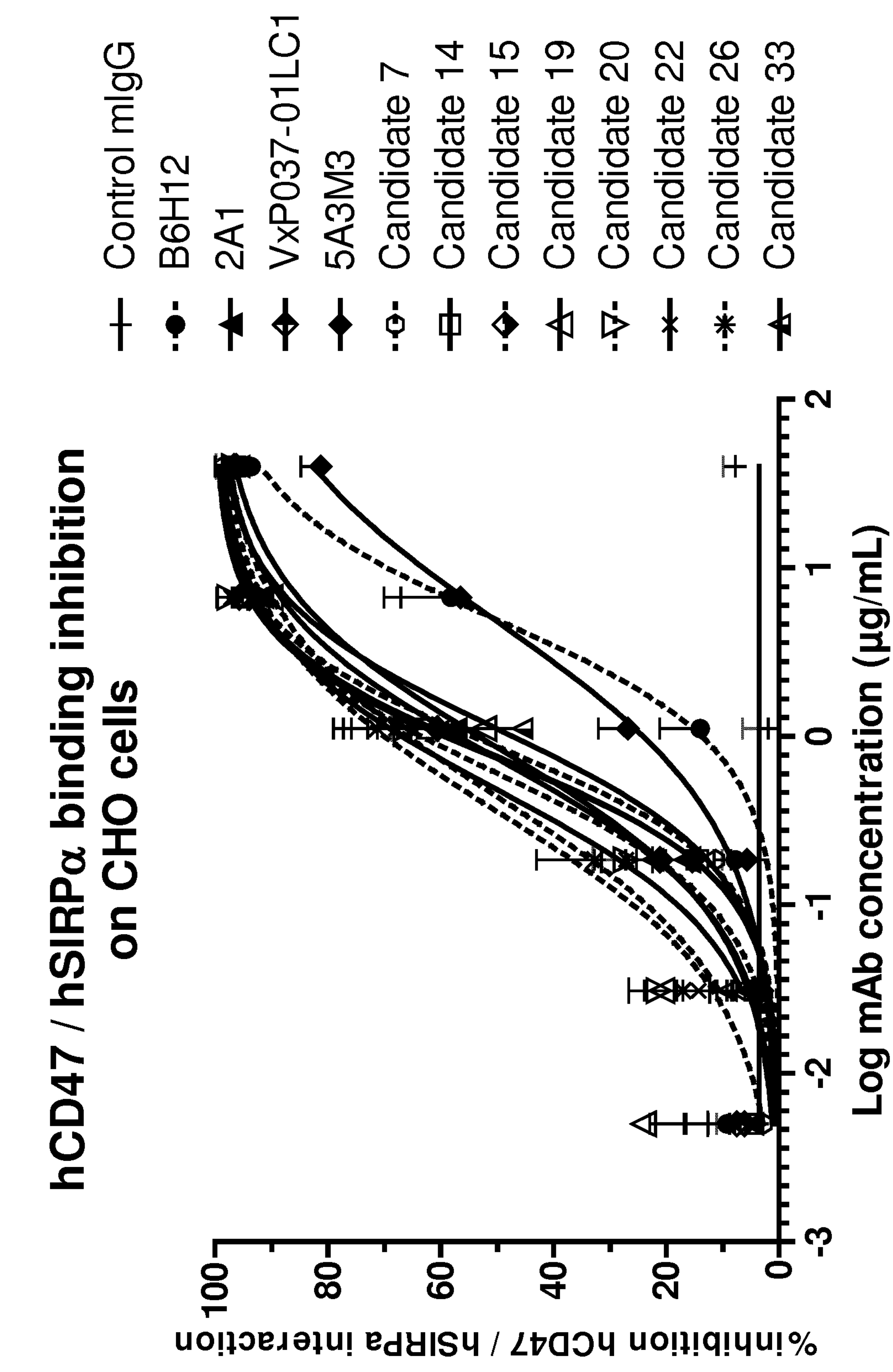

hCD47 / hSIRPα binding inhibition on CHO cells
%inhibition hCD47 / hSIRPa interaction
Log mAb concentration (µg/mL)
100
80
60
40
20
0
-3
-2
-1
0
1
2
Control mIgG
B6H12
2A1
VxP037-01LC1
5A3M3
Candidate 7
Candidate 14
Candidate 15
Candidate 19
Candidate 20
Candidate 22
Candidate 26
Candidate 33

Figure 2

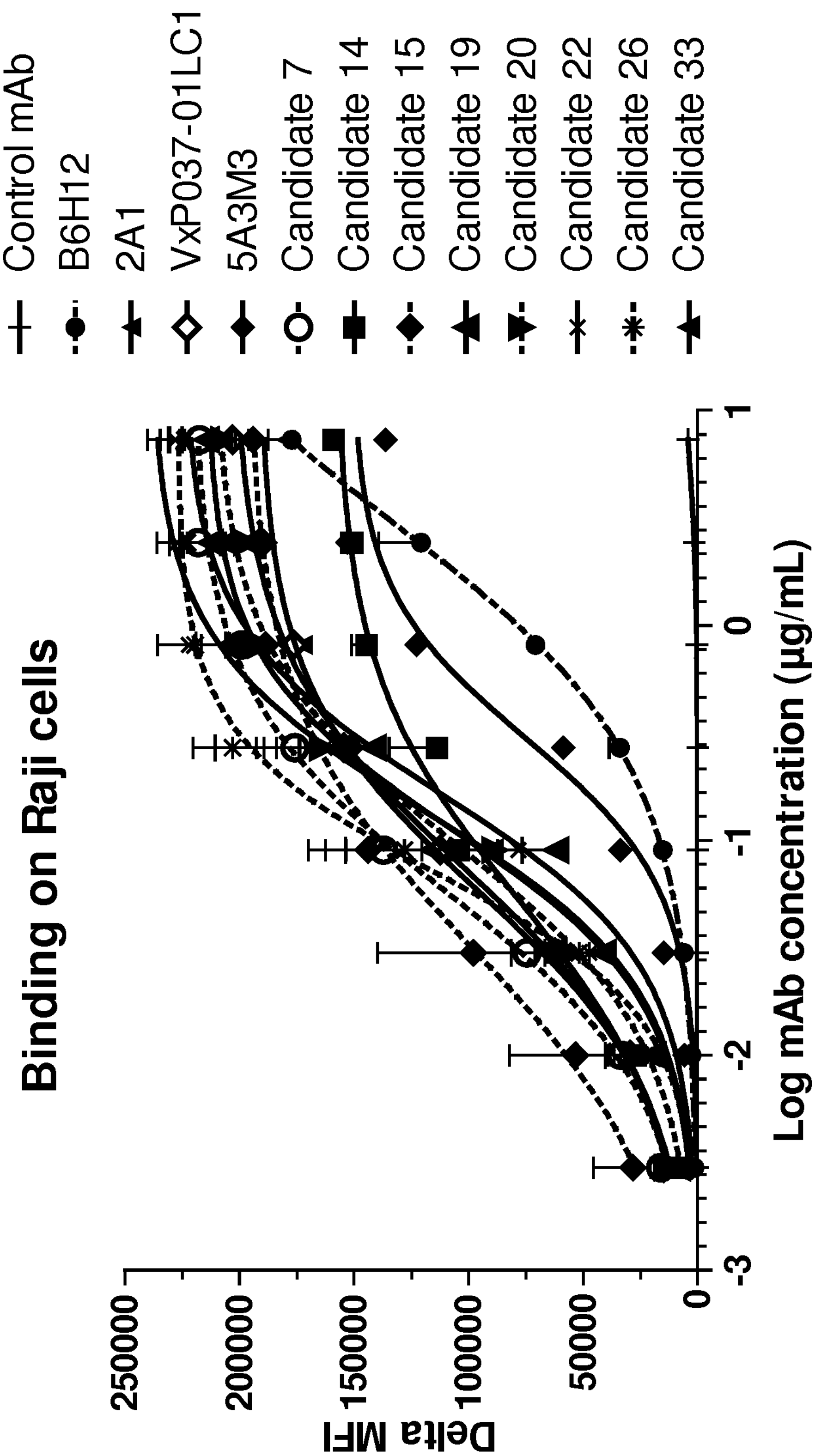

Binding on Raji cells
Delta MFI
250000
200000
150000
100000
50000
0
-3
-2
-1
0
1
Log mAb concentration (µg/mL)
Control mAb
B6H12
2A1
VxP037-01LC1
5A3M3
Candidate 7
Candidate 14
Candidate 15
Candidate 19
Candidate 20
Candidate 22
Candidate 26
Candidate 33

Figure 3

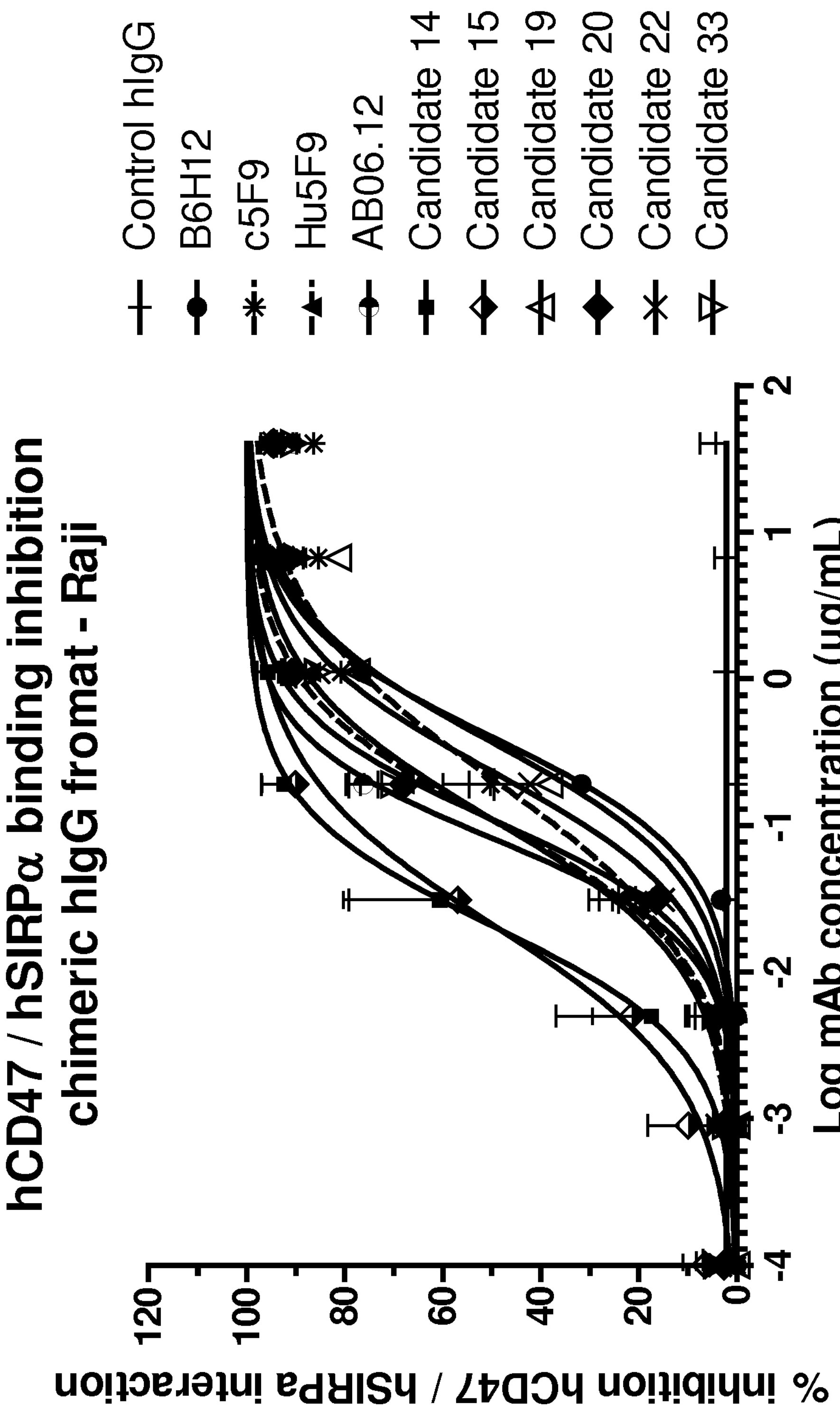

hCD47 / hSIRPα binding inhibition
chimeric hIgG fromat - Raji
% inhibition hCD47 / hSIRPa interaction
120
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2
Log mAb concentration (µg/mL)
Control hIgG
B6H12
c5F9
Hu5F9
AB06.12
Candidate 14
Candidate 15
Candidate 19
Candidate 20
Candidate 22
Candidate 33

Figure 4

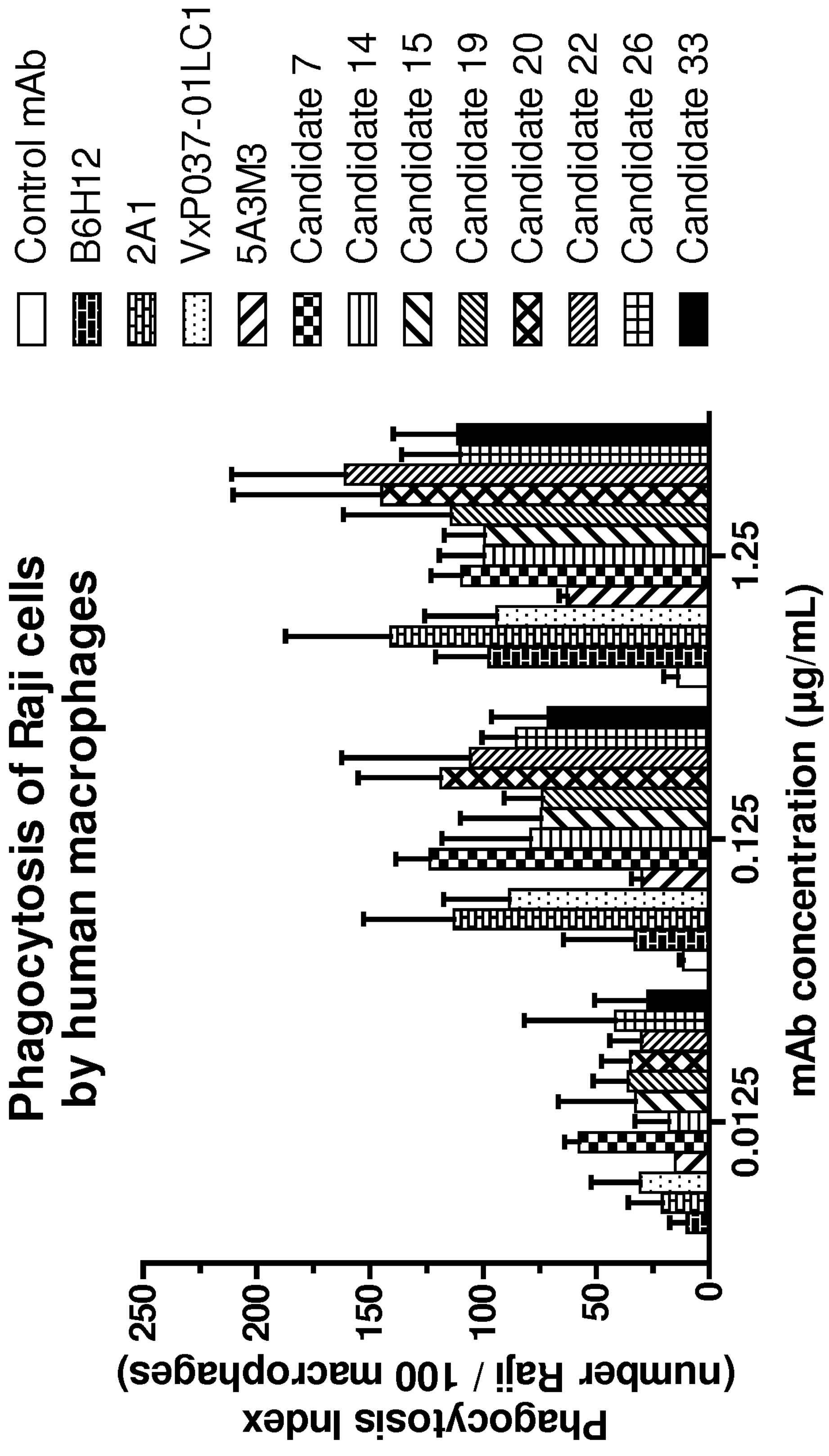

Phagocytosis of Raji cells
by human macrophages
Phagocytosis Index
(number Raji / 100 macrophages)
250
200
150
100
50
0
0.0125
0.125
1.25
mAb concentration (µg/mL)
Control mAb
B6H12
2A1
VxP037-01LC1
5A3M3
Candidate 7
Candidate 14
Candidate 15
Candidate 19
Candidate 20
Candidate 22
Candidate 26
Candidate 33

Figure 5

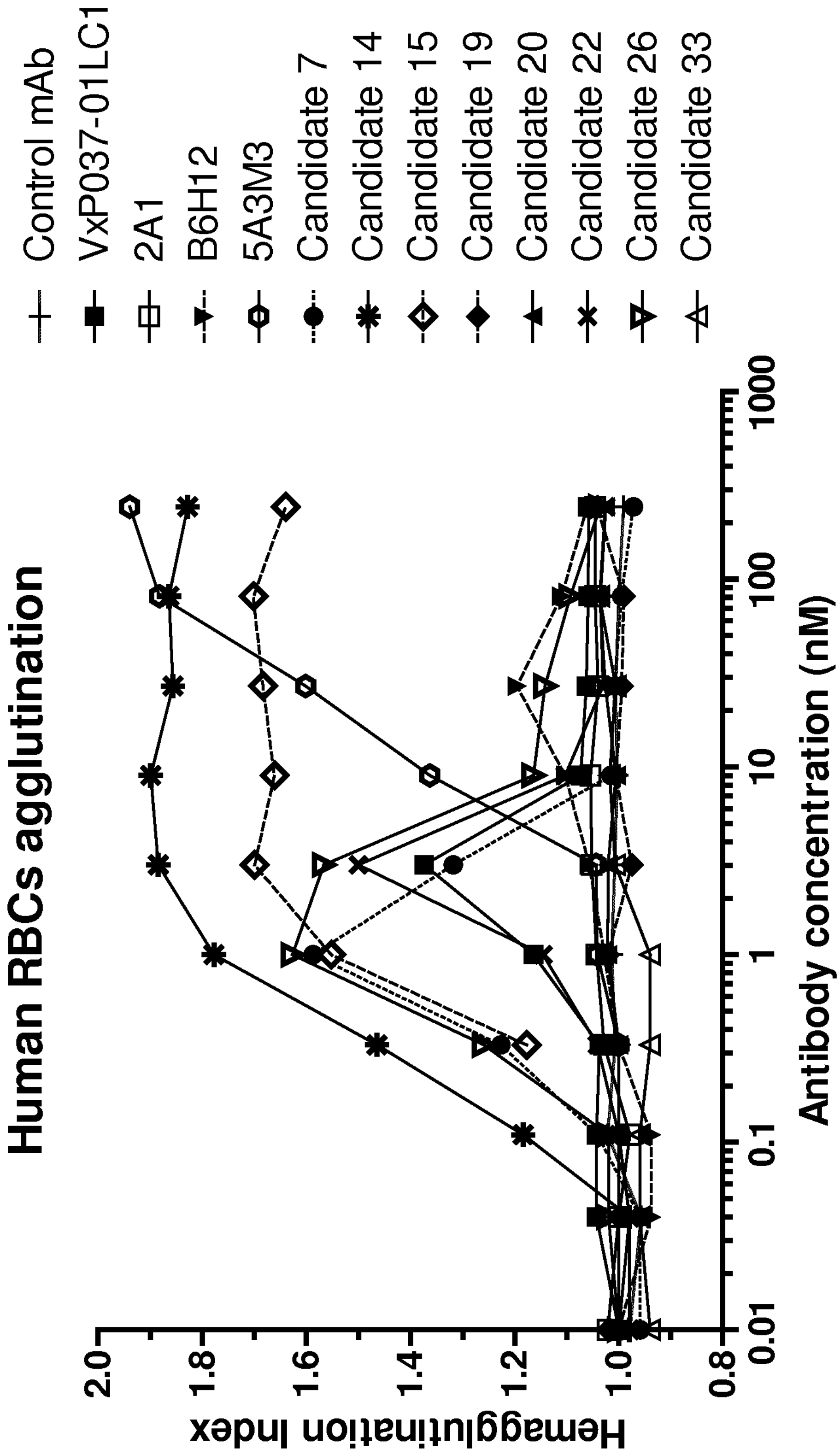

Human RBCs agglutination
Hemagglutination Index
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.01
0.1
1
10
100
1000
Antibody concentration (nM)
Control mAb
VxP037-01LC1
2A1
B6H12
5A3M3
Candidate 7
Candidate 14
Candidate 15
Candidate 19
Candidate 20
Candidate 22
Candidate 26
Candidate 33

Figure 6

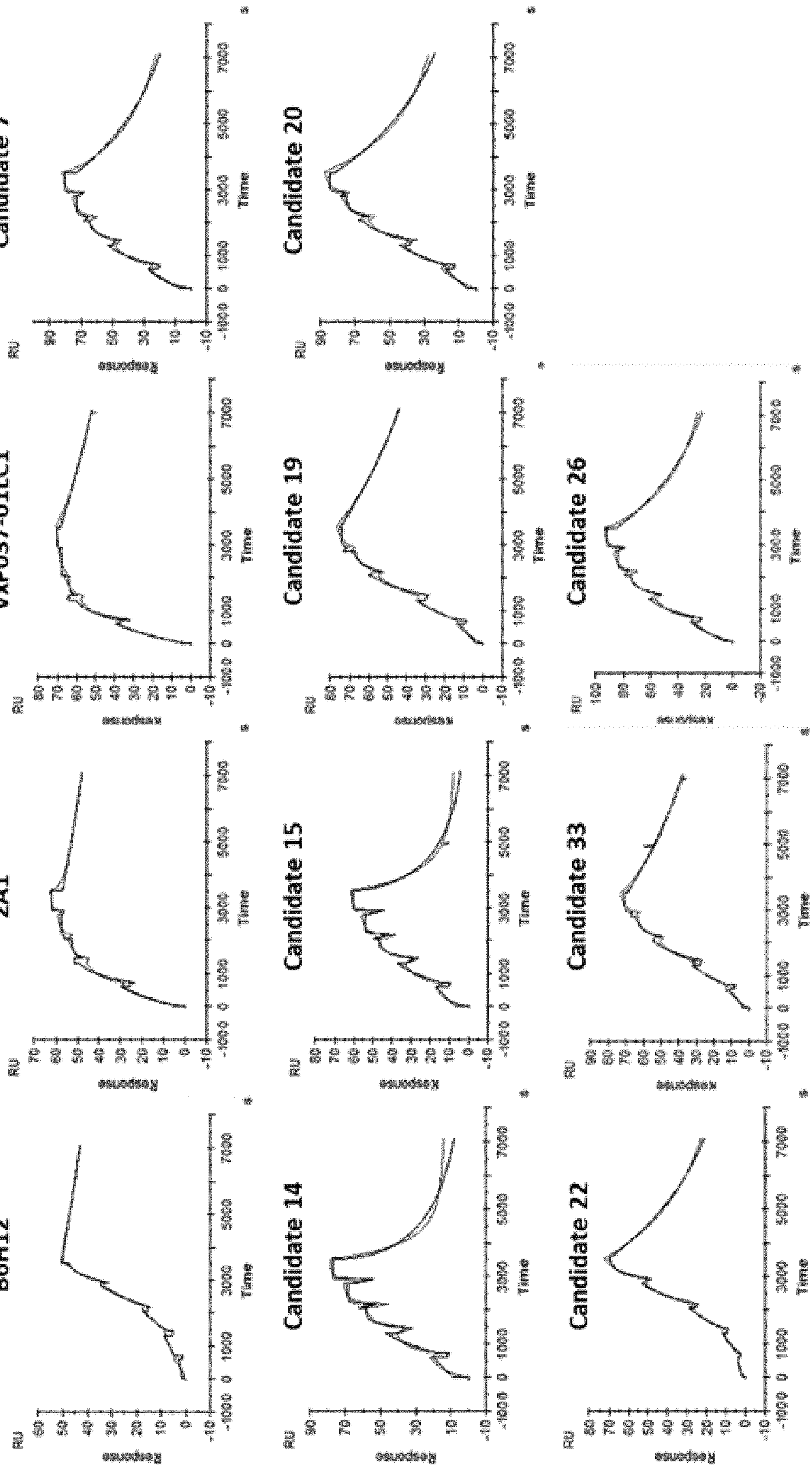

B6H12
2A1
VxP037-01LC1
Candidate 7
Candidate 14
Candidate 15
Candidate 19
Candidate 20
Candidate 22
Candidate 33
Candidate 26
Response
RU
Time
s

Figure 7

```
                1                                                50
VH0     (1)     QVQLKQSGAELVRPGASVKLSCKASGYSFTDYYINWVKQRPGQGLEWIAR
VH1     (1)     QVQLLESGAVLARPGTSVKISCKASGYSFTDYYINWVKQRPGQGLEWIGR
VH2     (1)     QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGR
VH3     (1)     QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGR
VH4     (1)     QVQLVQSGAEVKKPGSSVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGR
VH5     (1)     QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGR

                51                                              100
VH0     (51)    IYPGIGNTYYNKKFKGKATLTAEKSSSTAYMQLNSLTSEDSAVYFCARGH
VH1     (51)    IYPGIGNTYYNKKFKGRAKLTAATSASIAYLEFSSLTNEDSAVYYCARGH
VH2     (51)    IYPGIGNTYYNKKFKGRVTITRDTSASTAYMELSSLRSEDTAVYYCARGH
VH3     (51)    IYPGIGNTYYNKKFKGRVTVTRDTSISTAHMELSSLRSDDTAVYYCARGH
VH4     (51)    IYPGIGNTYYNKKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGH
VH5     (51)    IYPGIGNTYYNKKFKGRVTMTRYTSISTAYMELSRLRSDDTAVYFCARGH

                101             118
VH0     (101)   YGRGMDYWGQGTSVTVSS
VH1     (101)   YGRGMDYWGQGTLVTVSS
VH2     (101)   YGRGMDYWGQGTLVTVSS
VH3     (101)   YGRGMDYWGQGTAVTVSS
VH4     (101)   YGRGMDYWGQGTTVTVSS
VH5     (101)   YGRGMDYWGQGTTVTVSS
```

Figure 8

```
                1                                                50
VL0      (1)    DIVMTQSPSSLAMSVGQKVTMNCKSSQSLLNSIDQKNYLAWYQQKPGQSP
VL1      (1)    DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPP
VL2      (1)    DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPP
VL3      (1)    EIVLTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKAGQSP
VL4      (1)    DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPP
VL5      (1)    DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPP

                51                                              100
VL0     (51)    KLLVYFASTKESGVPDRFIGSGSGTDFTLTISSVQAEDLADYFCQQHYST
VL1     (51)    KLLIYFASTKESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYST
VL2     (51)    KLLIYFASTKESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYST
VL3     (51)    KLLIYFASTKESGVPDRFSGSGSGTDFTLTIDSLQAEDVAVYYCQQHYST
VL4     (51)    KLLIYFASTKESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQHYST
VL5     (51)    KLLIYFASTKESGVPDRFSGSGSGTDFTLTISGLQAEDVAVYFCQQHYST

                101       113
VL0    (101)    PWTFGGGTKLEIK
VL1    (101)    PWTFGGGTKLEIK
VL2    (101)    PWTFGGGTKVEIK
VL3    (101)    PWTFGGGTKVEIK
VL4    (101)    PWTFGGGAKVEIK
VL5    (101)    PWTFGGGTKVEIR
```

Figure 9A

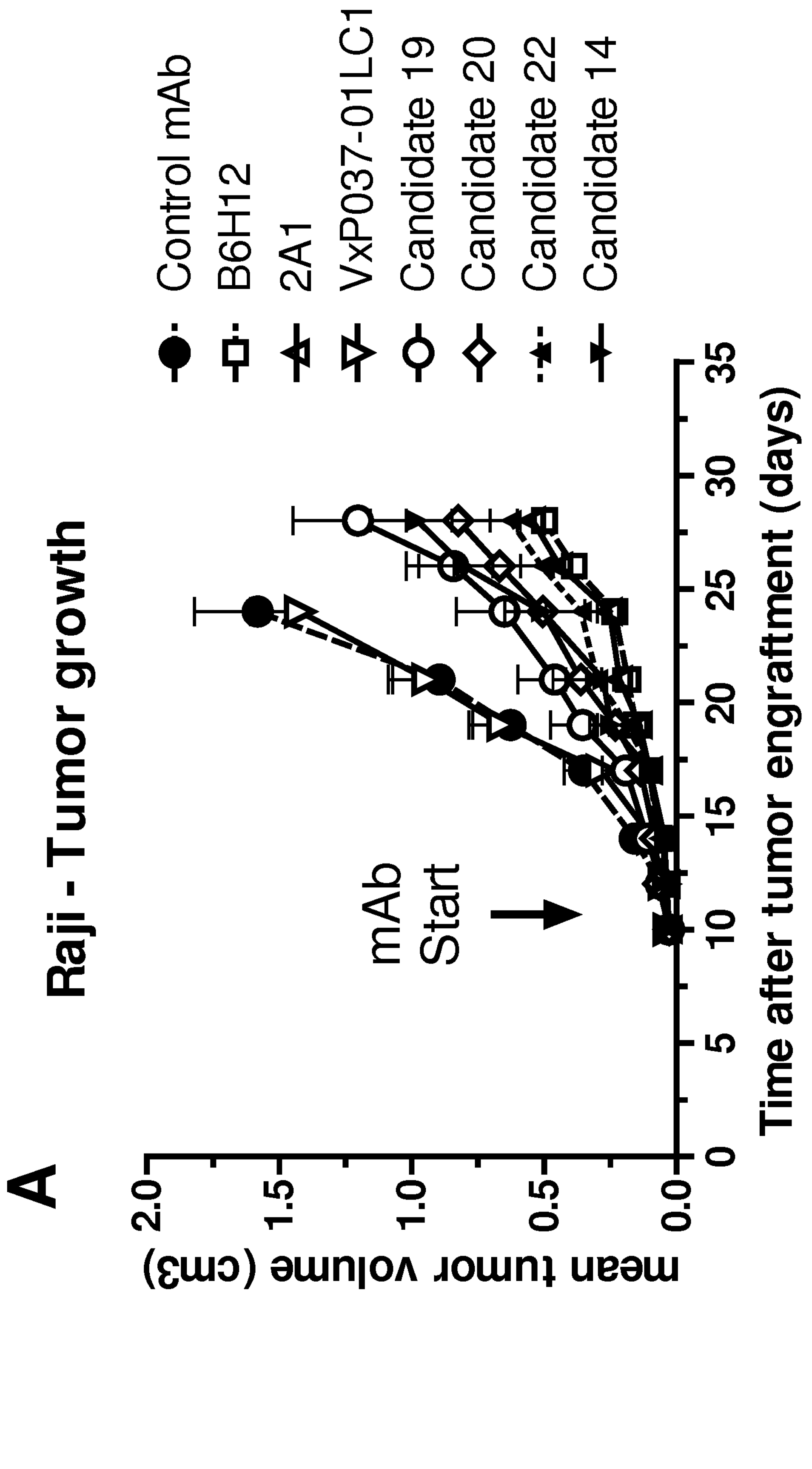
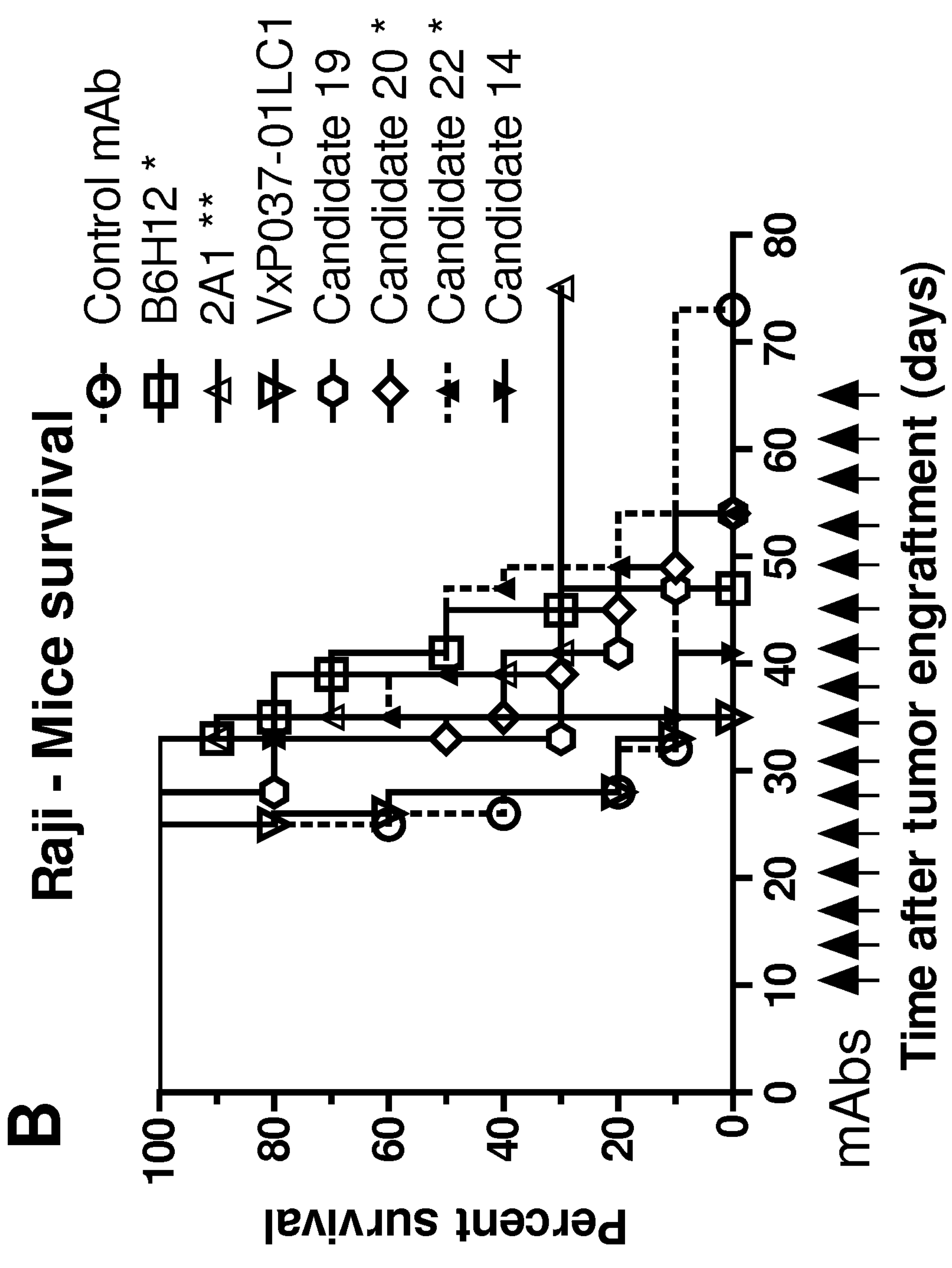

A
Raji - Tumor growth
mean tumor volume (cm3)
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
35
Time after tumor engraftment (days)
mAb
Start
Control mAb
B6H12
2A1
VxP037-01LC1
Candidate 19
Candidate 20
Candidate 22
Candidate 14

Figure 9B


B
Raji - Mice survival
Percent survival
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
mAbs
Time after tumor engraftment (days)
Control mAb
B6H12 *
2A1 **
VxP037-01LC1
Candidate 19
Candidate 20 *
Candidate 22 *
Candidate 14

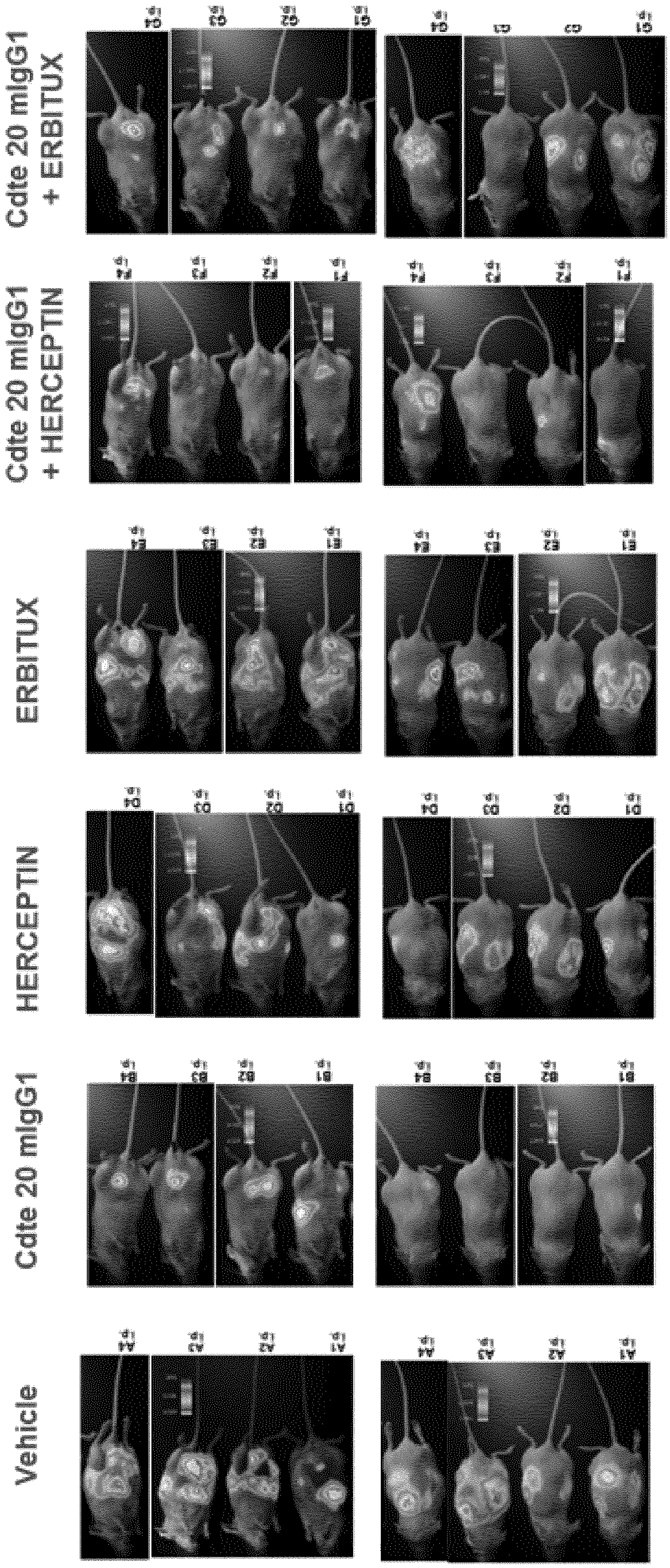

Vehicle
Cdte 20 mIgG1
HERCEPTIN
ERBITUX
Cdte 20 mIgG1 + HERCEPTIN
Cdte 20 mIgG1 + ERBITUX

Figure 10A

Figure 10B

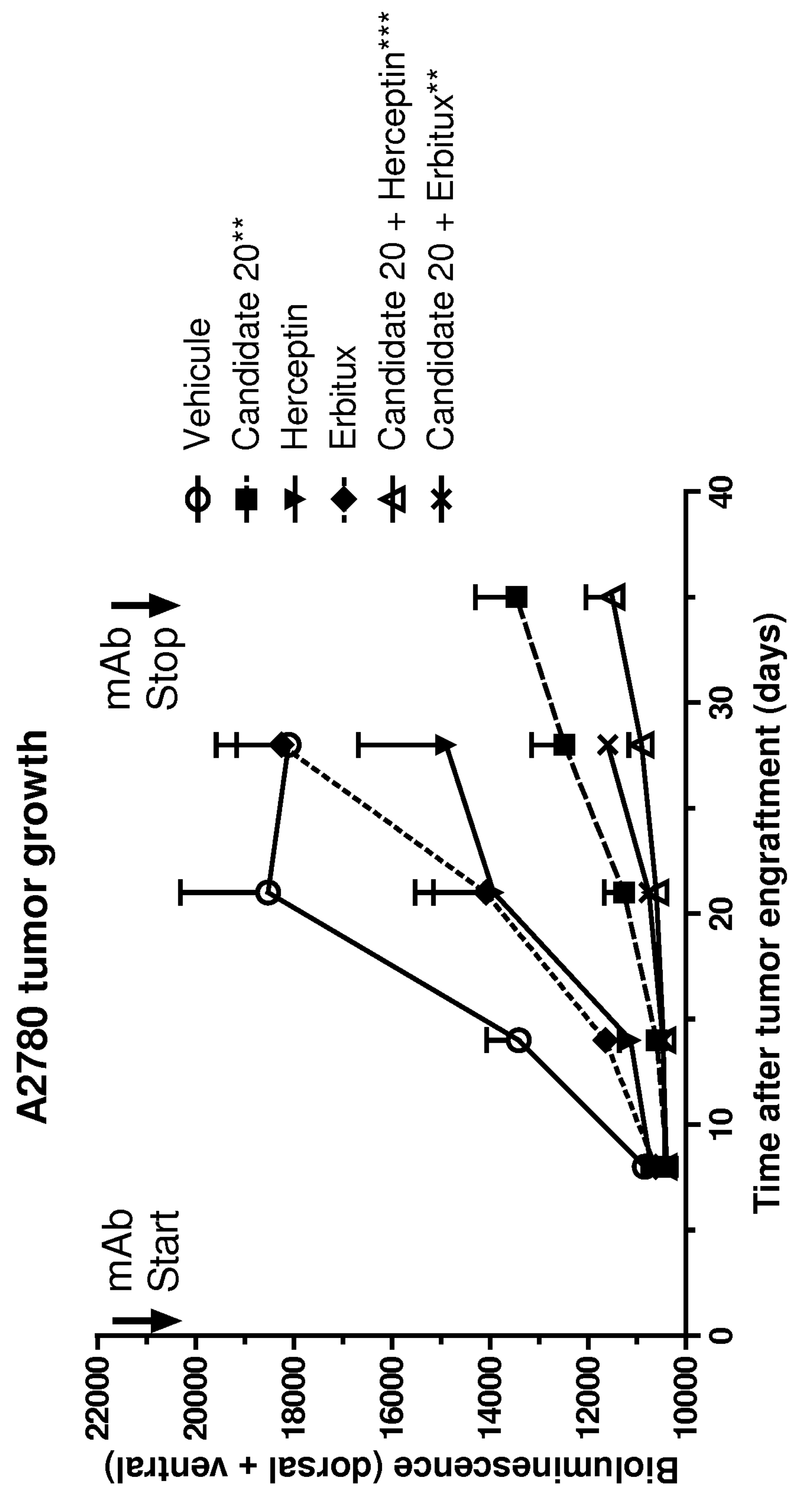
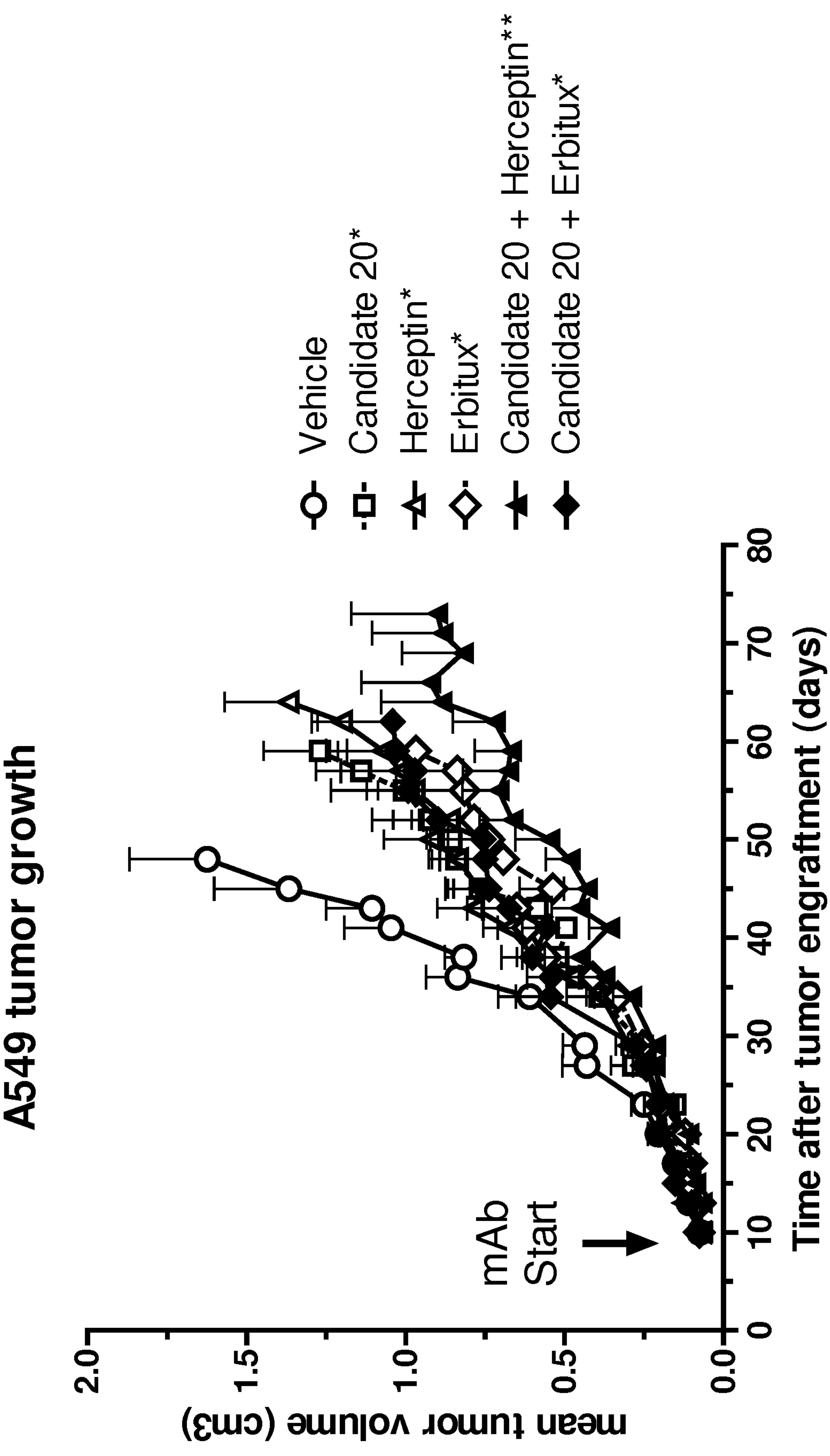

A2780 tumor growth
mAb Start
mAb Stop
Bioluminescence (dorsal + ventral)
22000
20000
18000
16000
14000
12000
10000
0
10
20
30
40
Time after tumor engraftment (days)
Vehicule
Candidate 20**
Herceptin
Erbitux
Candidate 20 + Herceptin***
Candidate 20 + Erbitux**

Figure 11A



A549 tumor growth
mean tumor volume (cm3)
Time after tumor engraftment (days)
mAb Start
0.0
0.5
1.0
1.5
2.0
0
10
20
30
40
50
60
70
80
Vehicle
Candidate 20*
Herceptin*
Erbitux*
Candidate 20 + Herceptin**
Candidate 20 + Erbitux*

Figure 11B

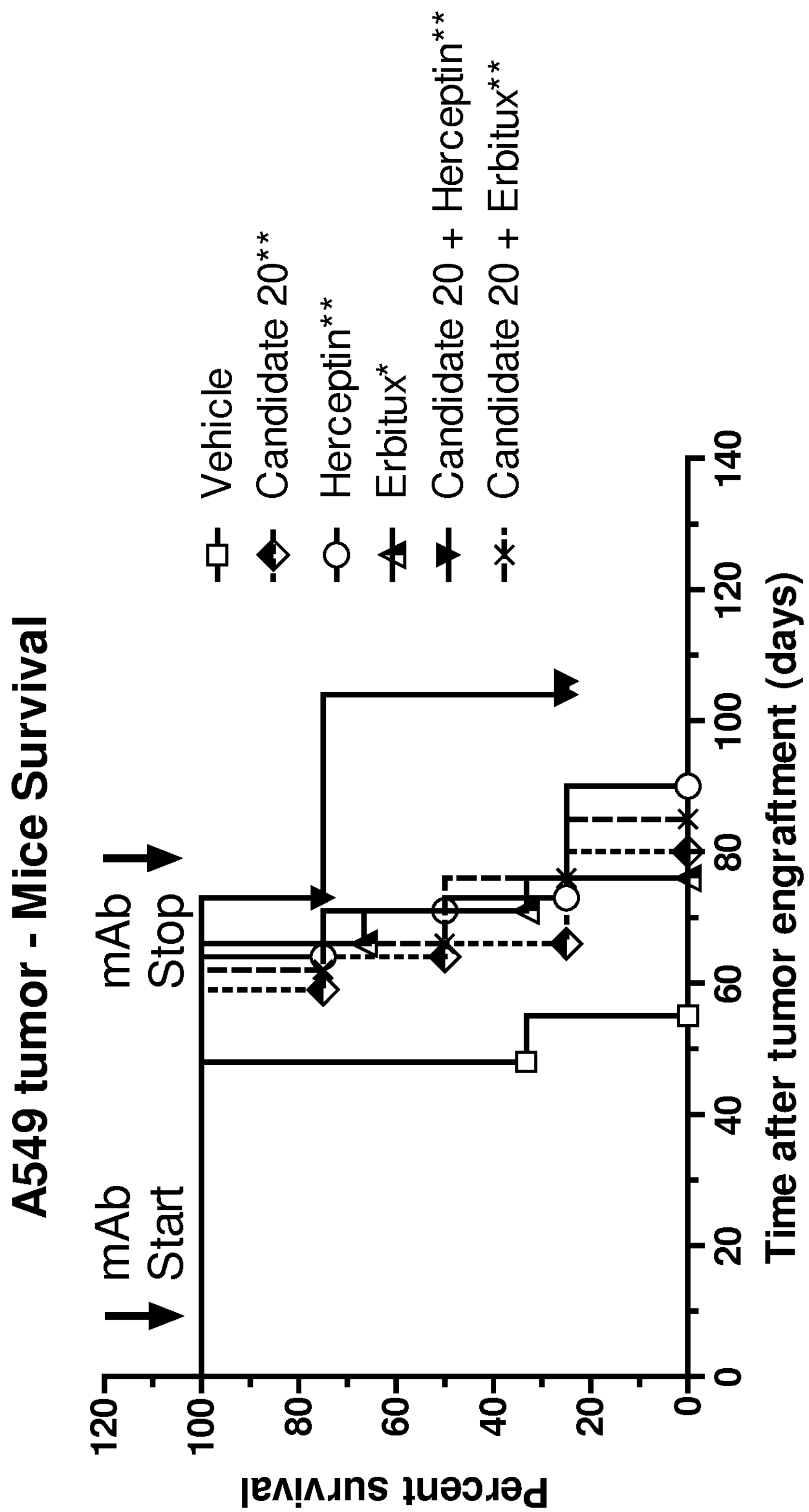

A549 tumor - Mice Survival
mAb Start
mAb Stop
Vehicle
Candidate 20**
Herceptin**
Erbitux*
Candidate 20 + Herceptin**
Candidate 20 + Erbitux**
Percent survival
120
100
80
60
40
20
0
0
20
40
60
80
100
120
140
Time after tumor engraftment (days)

Figure 12A,B

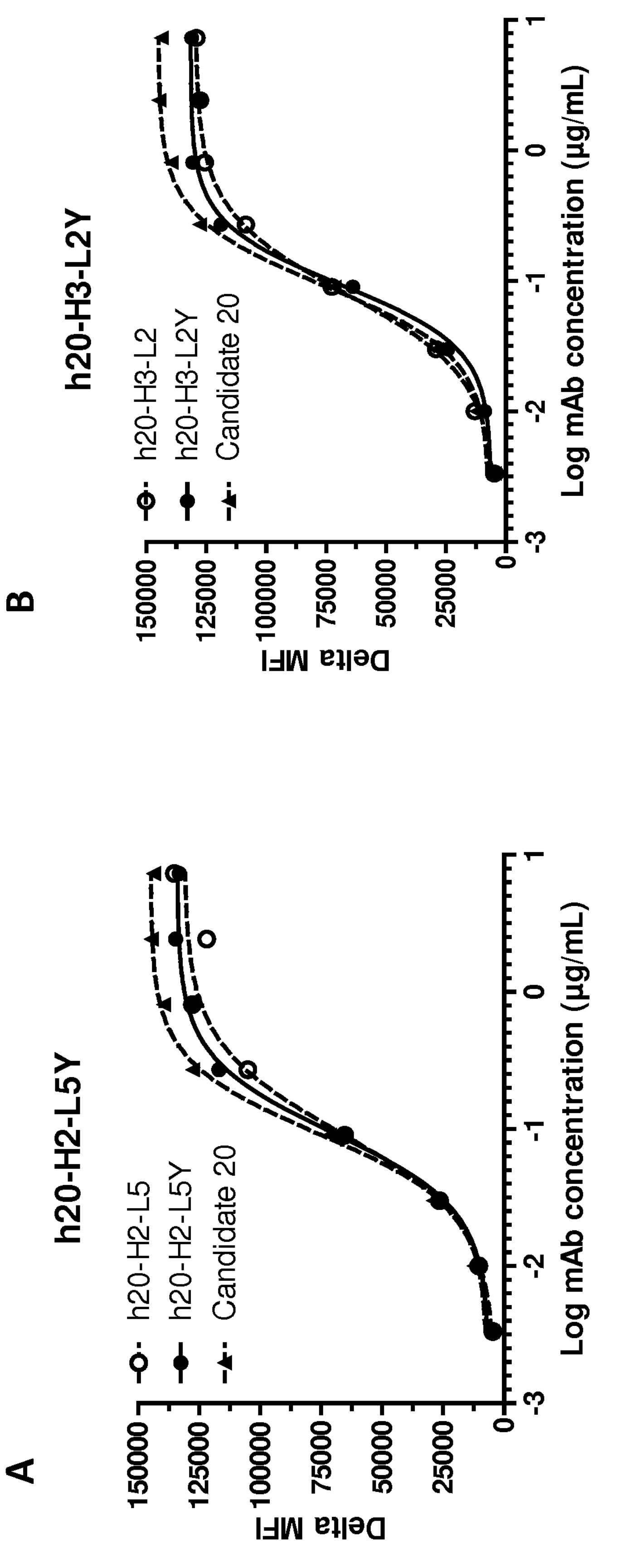

A
h20-H2-L5Y
h20-H2-L5
h20-H2-L5Y
Candidate 20
Delta MFI
150000
125000
100000
75000
50000
25000
0
-3
-2
-1
0
1
Log mAb concentration (µg/mL)
B
h20-H3-L2Y
h20-H3-L2
h20-H3-L2Y
Candidate 20
Delta MFI
150000
125000
100000
75000
50000
25000
0
-3
-2
-1
0
1
Log mAb concentration (µg/mL)

Figure 12C,D

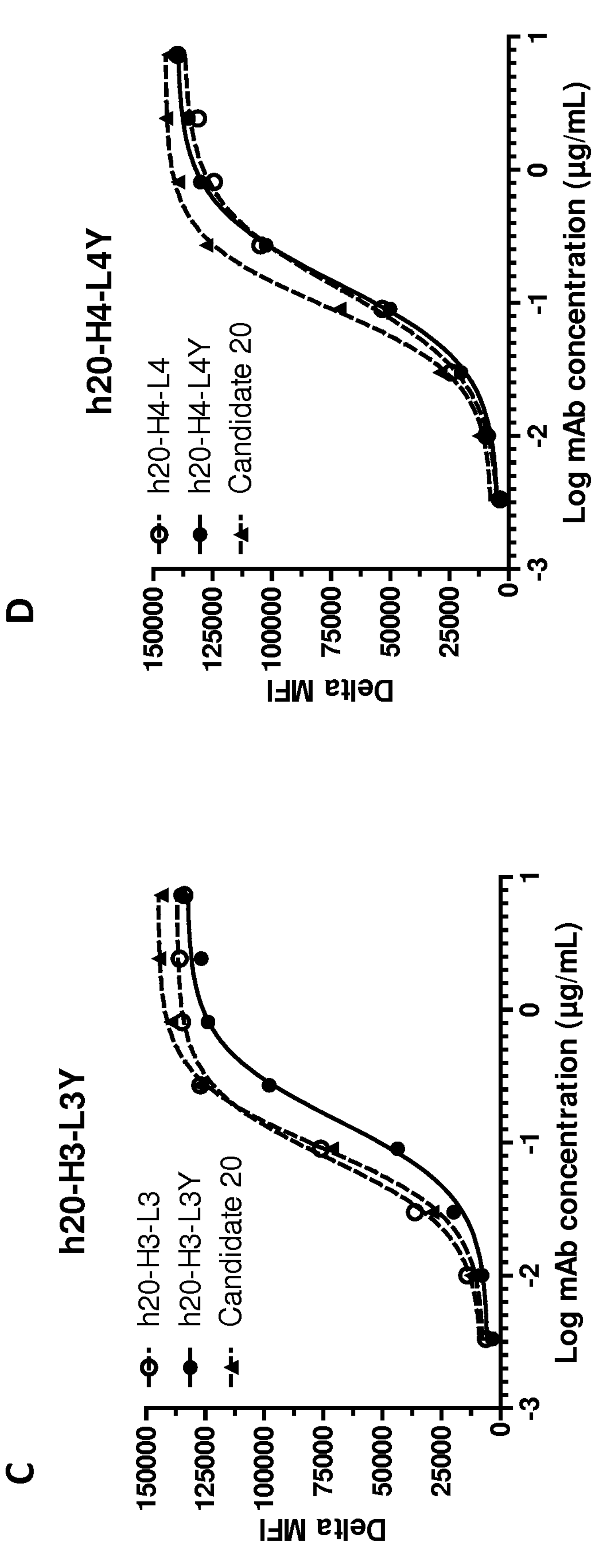

C
h20-H3-L3Y
h20-H3-L3
h20-H3-L3Y
Candidate 20
Delta MFI
150000
125000
100000
75000
50000
25000
0
-3
-2
-1
0
1
Log mAb concentration (µg/mL)
D
h20-H4-L4Y
h20-H4-L4
h20-H4-L4Y
Candidate 20
Delta MFI
150000
125000
100000
75000
50000
25000
0
-3
-2
-1
0
1
Log mAb concentration (µg/mL)

Figure 12E

E

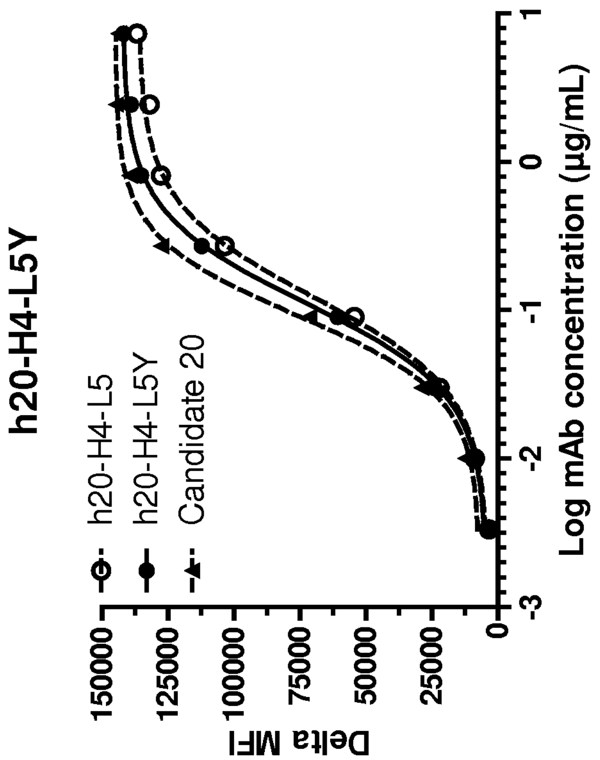

h20-H4-L5Y
h20-H4-L5
h20-H4-L5Y
Candidate 20
Delta MFI
150000
125000
100000
75000
50000
25000
0
-3
-2
-1
0
1
Log mAb concentration (µg/mL)

Figure 13A,B

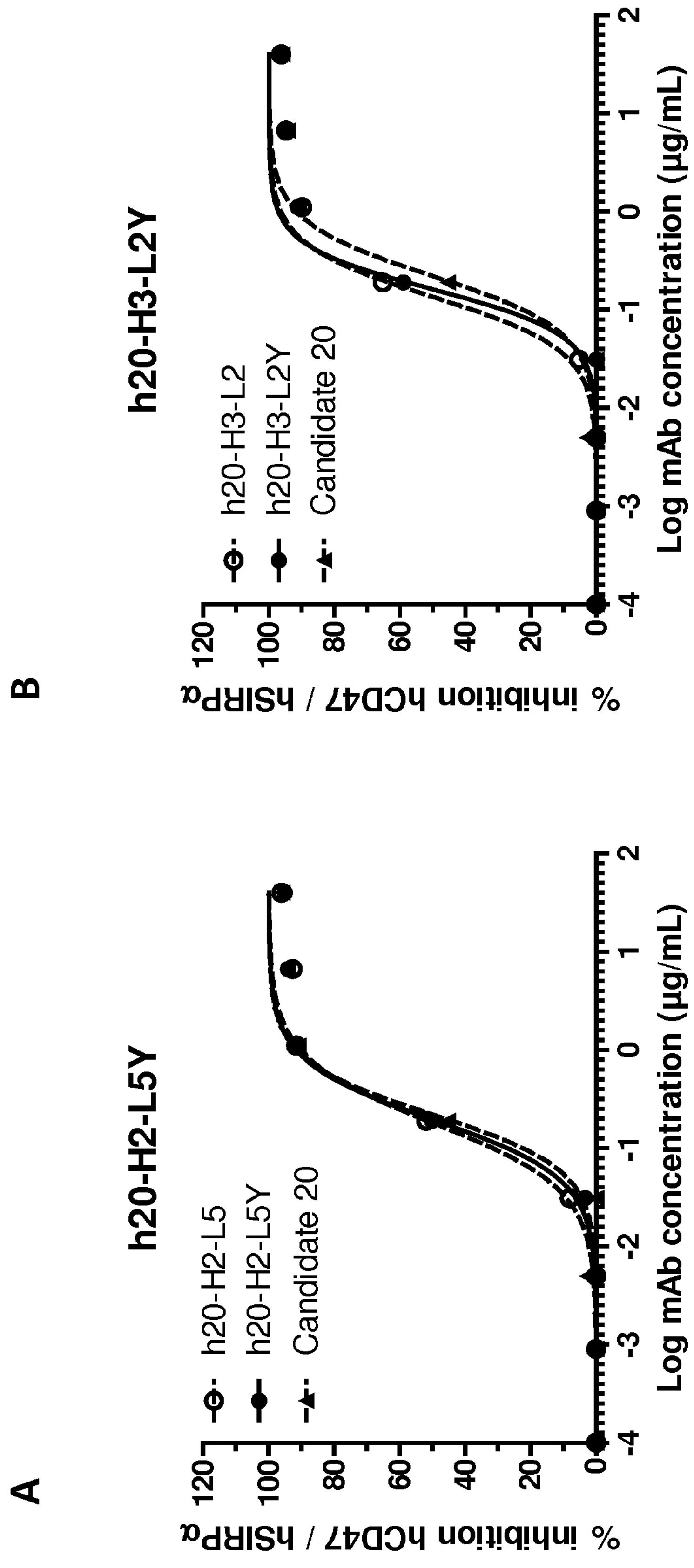

A
h20-H2-L5Y
h20-H2-L5
h20-H2-L5Y
Candidate 20
% inhibition hCD47 / hSIRPα
Log mAb concentration (µg/mL)
B
h20-H3-L2Y
h20-H3-L2
h20-H3-L2Y
Candidate 20
% inhibition hCD47 / hSIRPα
Log mAb concentration (µg/mL)

Figure 13C,D

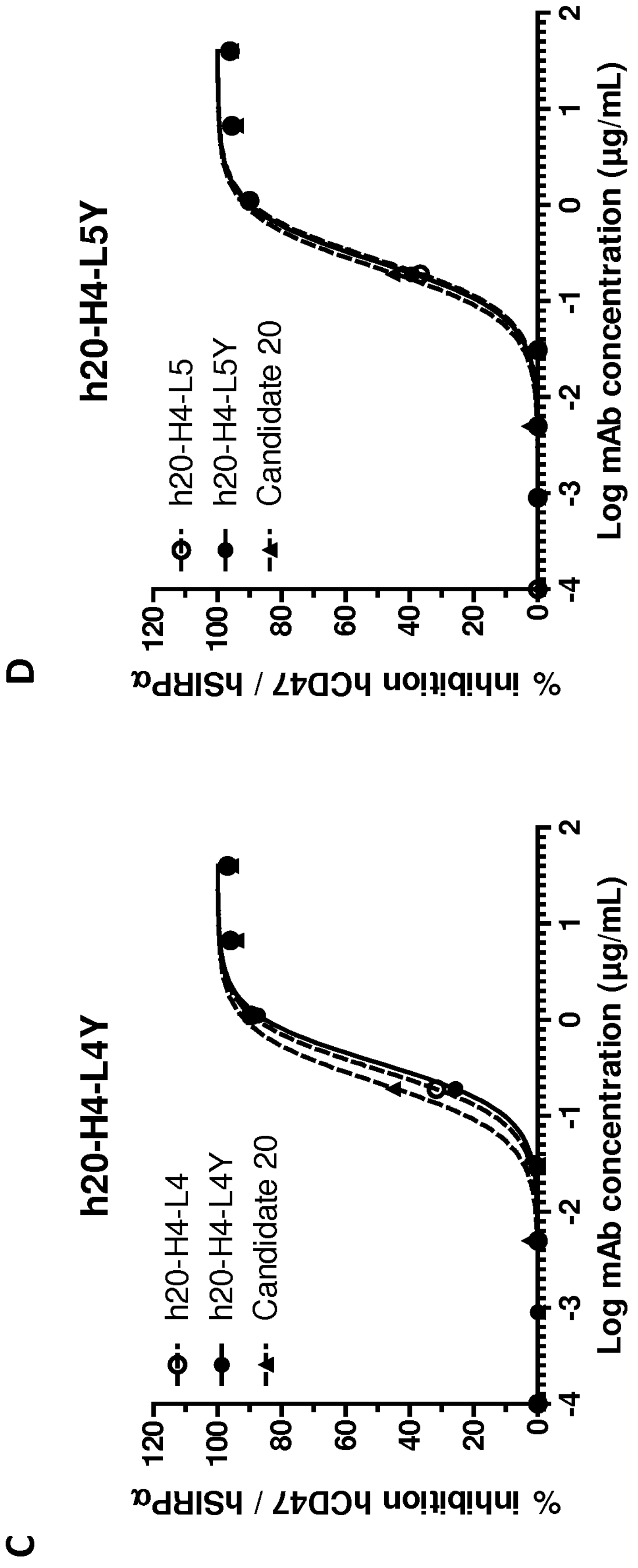

C
h20-H4-L4Y
h20-H4-L4
h20-H4-L4Y
Candidate 20
% inhibition hCD47 / hSIRPα
Log mAb concentration (µg/mL)
D
h20-H4-L5Y
h20-H4-L5
h20-H4-L5Y
Candidate 20
% inhibition hCD47 / hSIRPα
Log mAb concentration (µg/mL)

Figure 13E

E

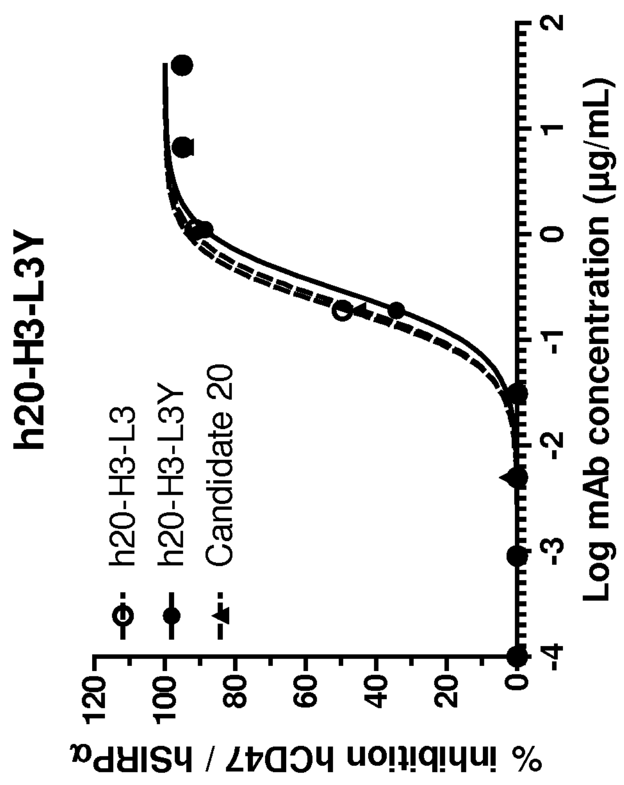

h20-H3-L3Y
h20-H3-L3
h20-H3-L3Y
Candidate 20
% inhibition hCD47 / hSIRPα
120
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2
Log mAb concentration (µg/mL)

Figure 14

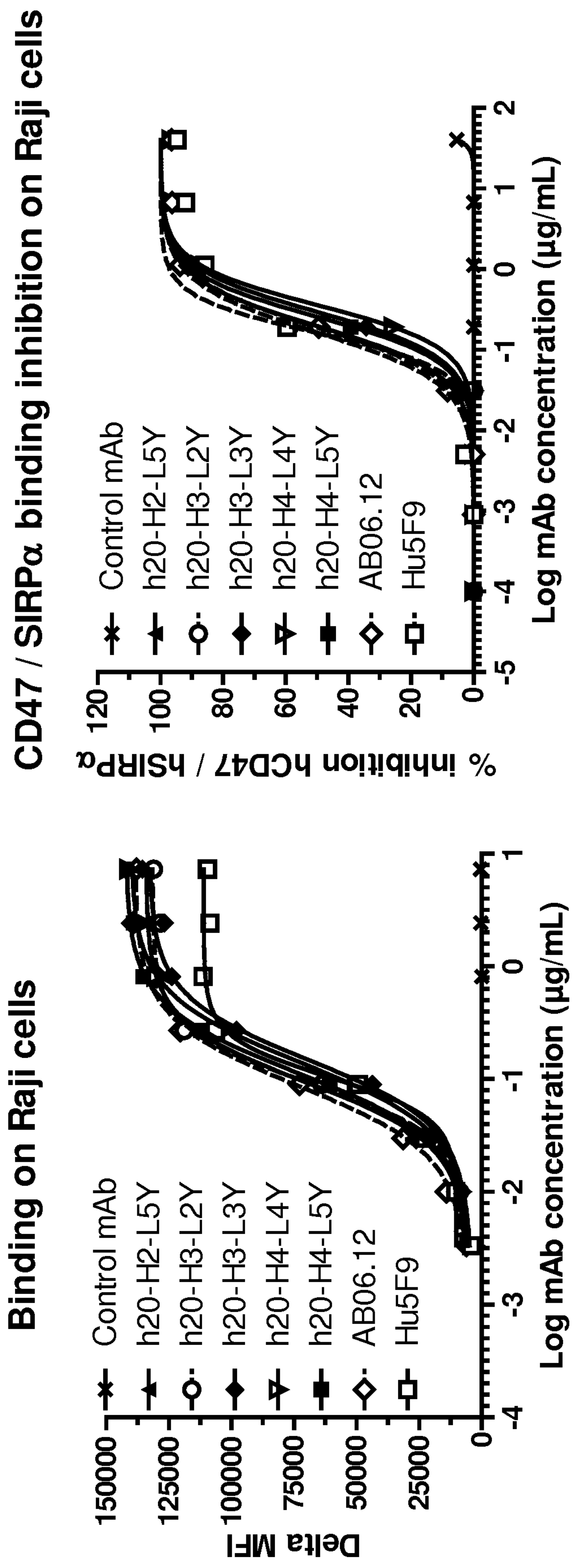

Binding on Raji cells
Delta MFI
150000
125000
100000
75000
50000
25000
0
-4
-3
-2
-1
0
1
Log mAb concentration (µg/mL)
Control mAb
h20-H2-L5Y
h20-H3-L2Y
h20-H3-L3Y
h20-H4-L4Y
h20-H4-L5Y
AB06.12
Hu5F9
CD47 / SIRPα binding inhibition on Raji cells
% inhibition hCD47 / hSIRPα
120
100
80
60
40
20
0
-5
-4
-3
-2
-1
0
1
2
Log mAb concentration (µg/mL)
Control mAb
h20-H2-L5Y
h20-H3-L2Y
h20-H3-L3Y
h20-H4-L4Y
h20-H4-L5Y
AB06.12
Hu5F9

Figure 15

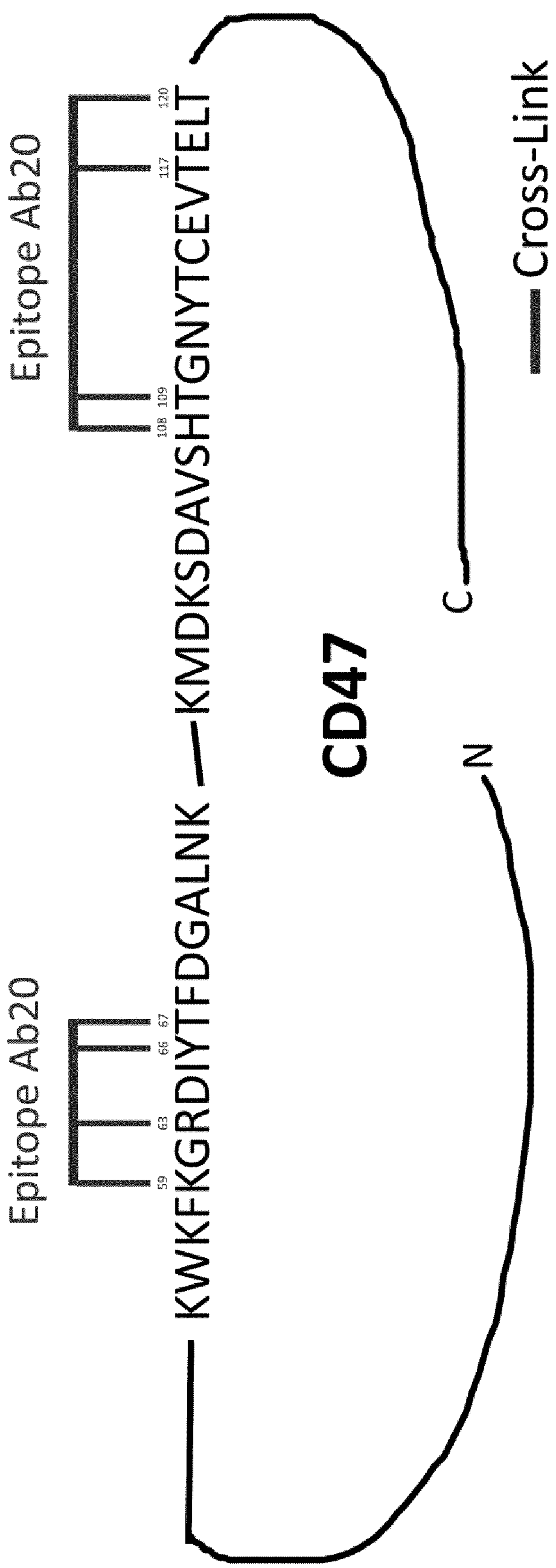

Epitope Ab20
59
63
66
67
KWKFKGRDIYTFDGALNK
Epitope Ab20
108
109
117
120
KMDKSDAVSHTGNYTCEVTELT
CD47
N
C
Cross-Link

Figure 16

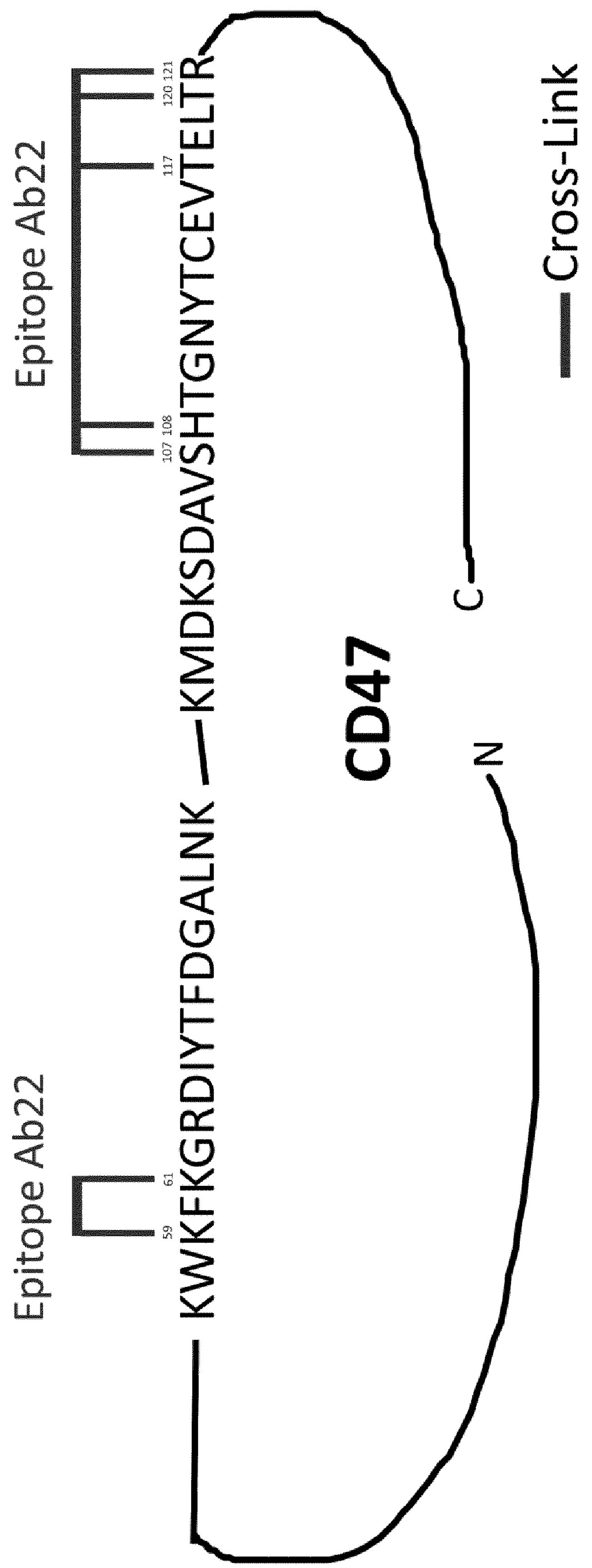

Epitope Ab22
59
61
KWKFKGRDIYTFDGALNK
Epitope Ab22
107 108
117
120 121
KMDKSDAVSHTGNYTCEVTELTR
CD47
N
C
Cross-Link

Figure 17

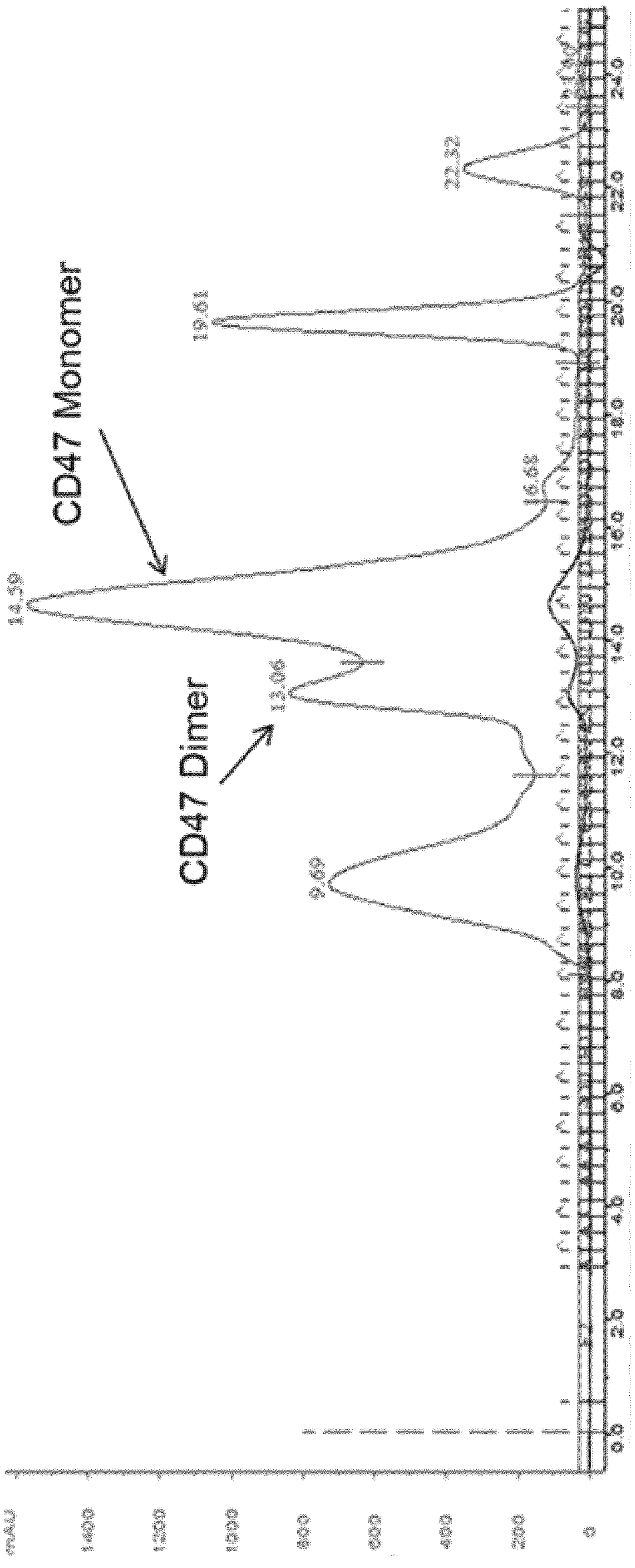

CD47 Monomer
CD47 Dimer
9.69
13.06
14.59
16.68
19.61
22.32
mAU
1400
1200
1000
800
600
400
200
0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
24.0

Figure 18

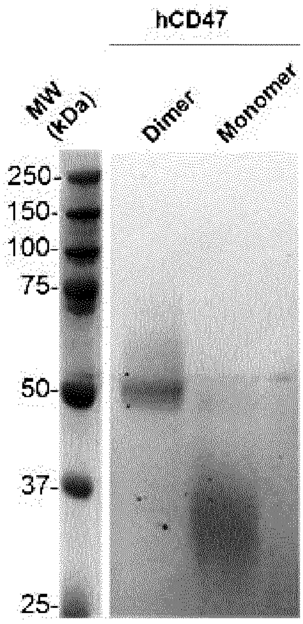


Figure 19

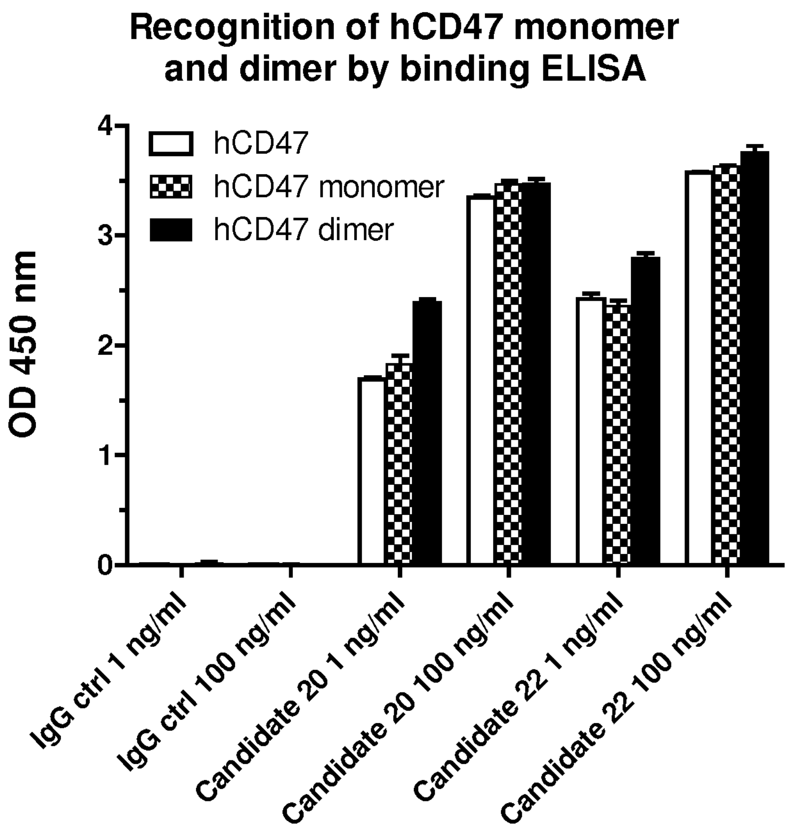


Figure 20

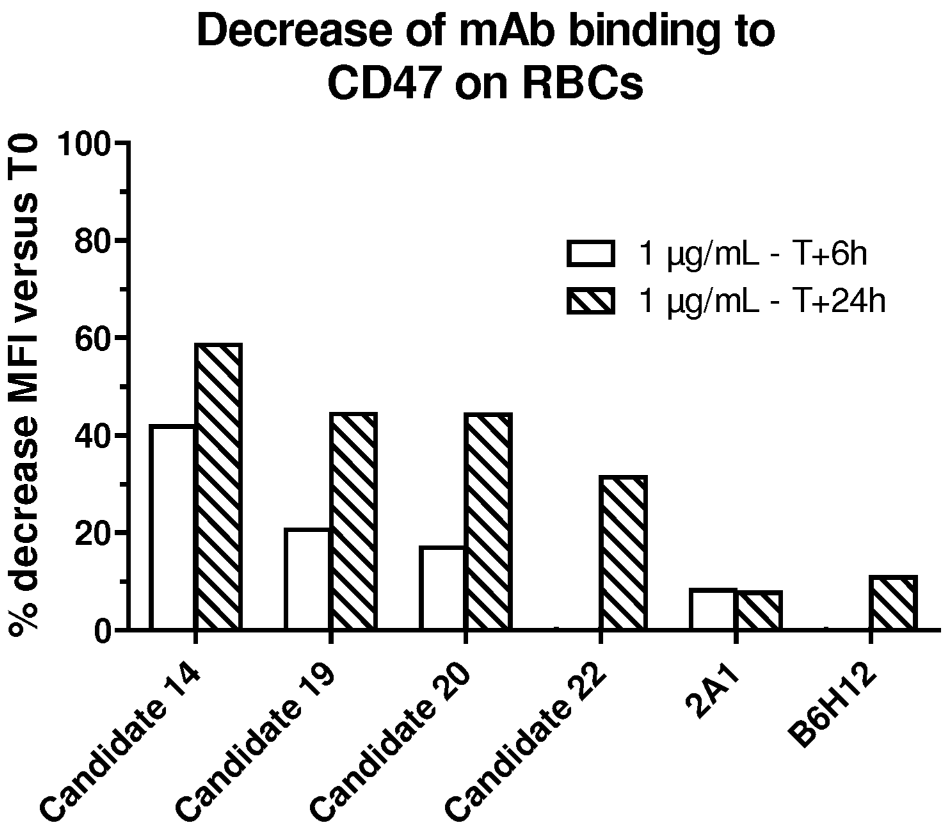
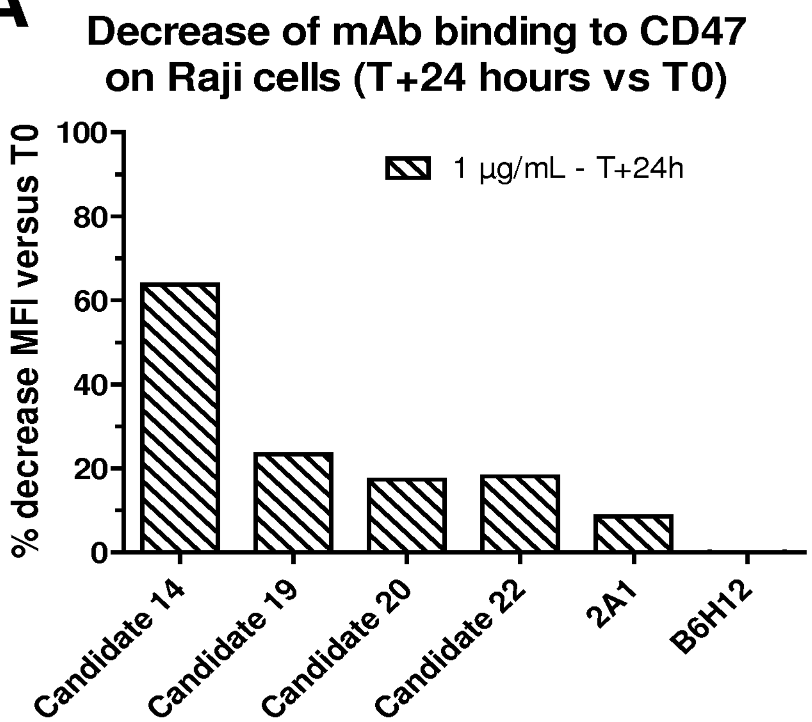

Decrease of mAb binding to CD47 on RBCs
% decrease MFI versus T0
100
80
60
40
20
0
1 µg/mL - T+6h
1 µg/mL - T+24h
Candidate 14
Candidate 19
Candidate 20
Candidate 22
2A1
B6H12

Figure 21

A


Decrease of mAb binding to CD47
on Raji cells (T+24 hours vs T0)
% decrease MFI versus T0
100
80
60
40
20
0
1 µg/mL - T+24h
Candidate 14
Candidate 19
Candidate 20
Candidate 22
2A1
B6H12

B

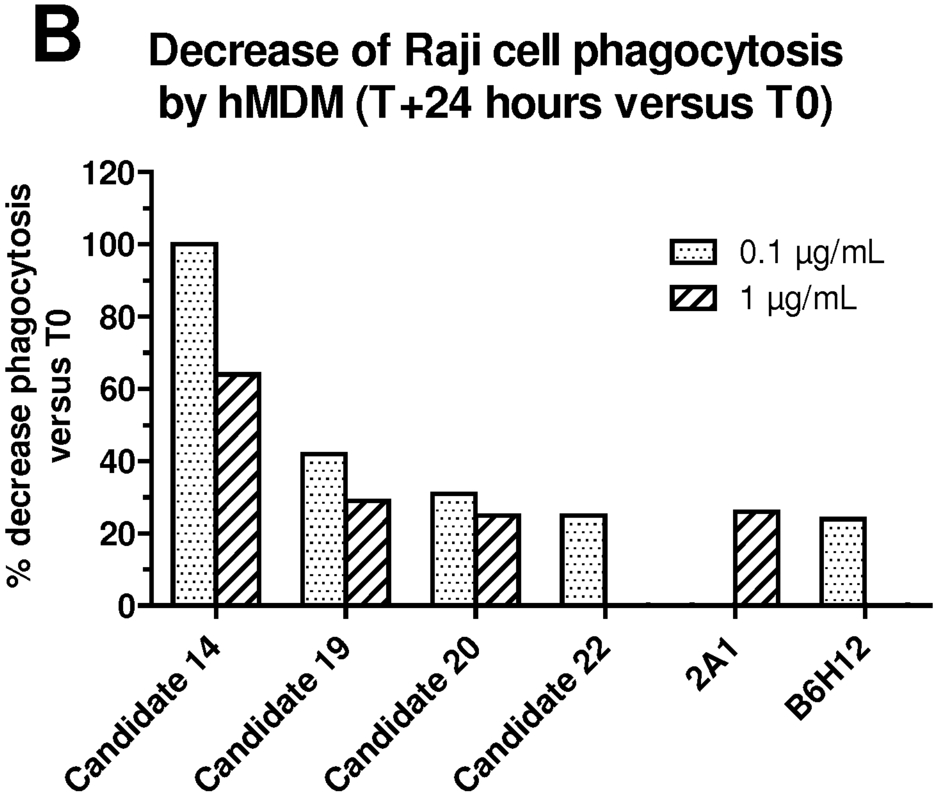
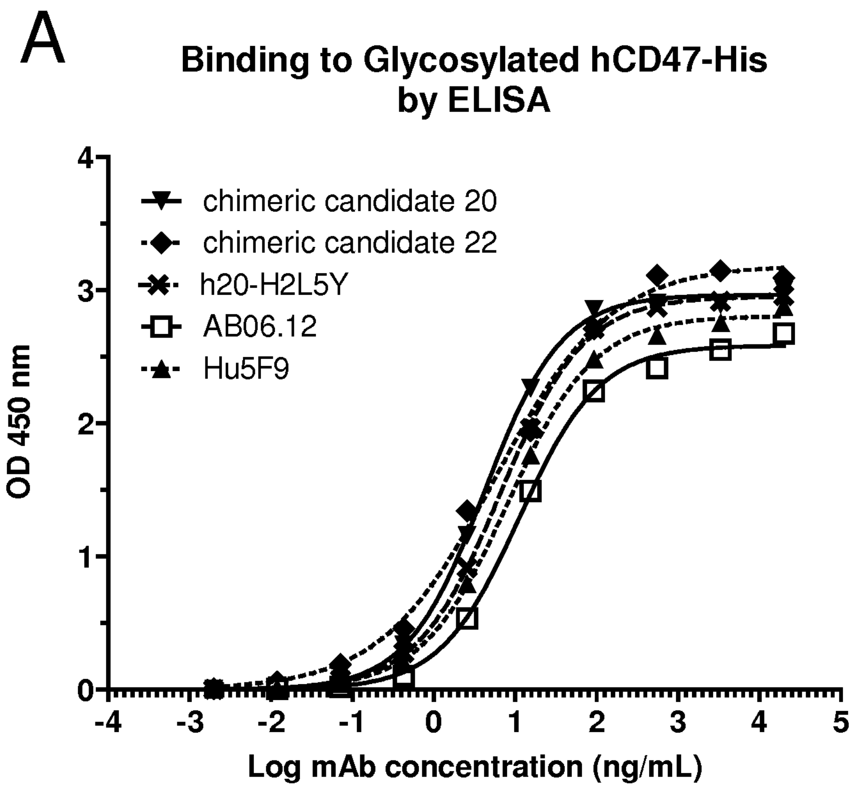

Decrease of Raji cell phagocytosis
by hMDM (T+24 hours versus T0)
% decrease phagocytosis
versus T0
120
100
80
60
40
20
0
0.1 µg/mL
1 µg/mL
Candidate 14
Candidate 19
Candidate 20
Candidate 22
2A1
B6H12

Figure 22


A
Binding to Glycosylated hCD47-His by ELISA
chimeric candidate 20
chimeric candidate 22
h20-H2L5Y
AB06.12
Hu5F9
OD 450 nm
Log mAb concentration (ng/mL)
0
1
2
3
4
-4
-3
-2
-1
5

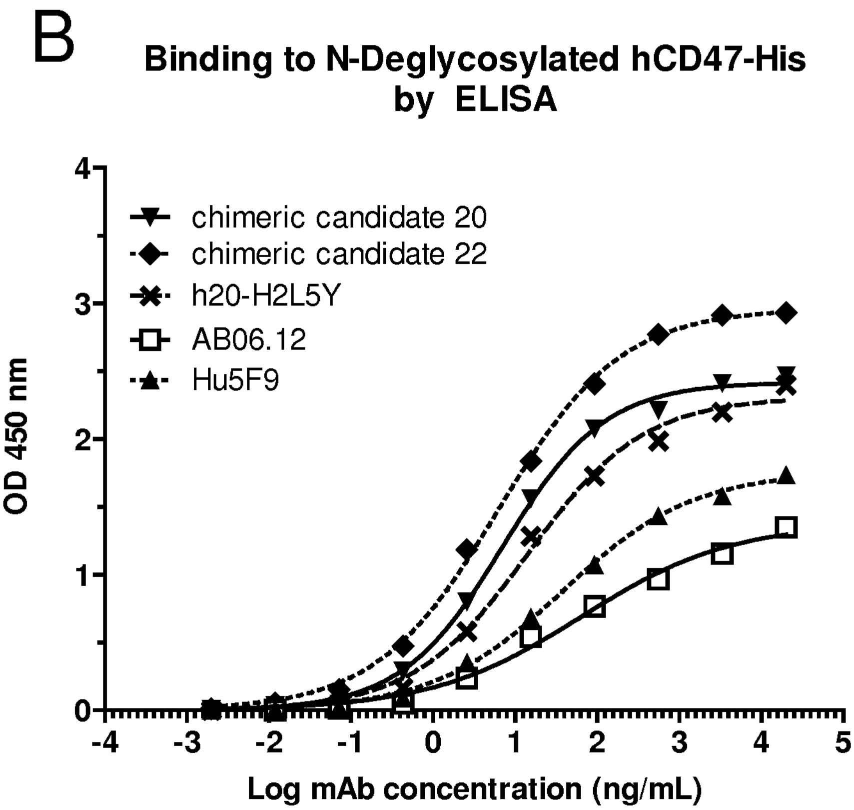

B
Binding to N-Deglycosylated hCD47-His by ELISA
chimeric candidate 20
chimeric candidate 22
h20-H2L5Y
AB06.12
Hu5F9
OD 450 nm
Log mAb concentration (ng/mL)
0
1
2
3
4
-4
-3
-2
-1
5

Figure 23

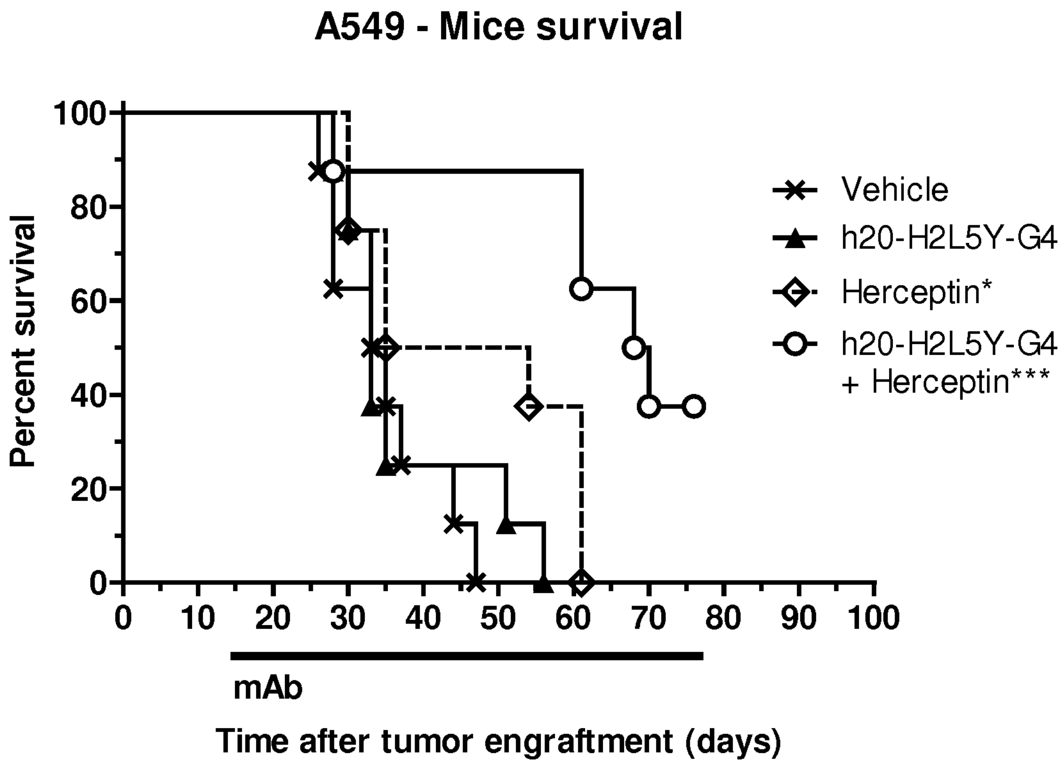
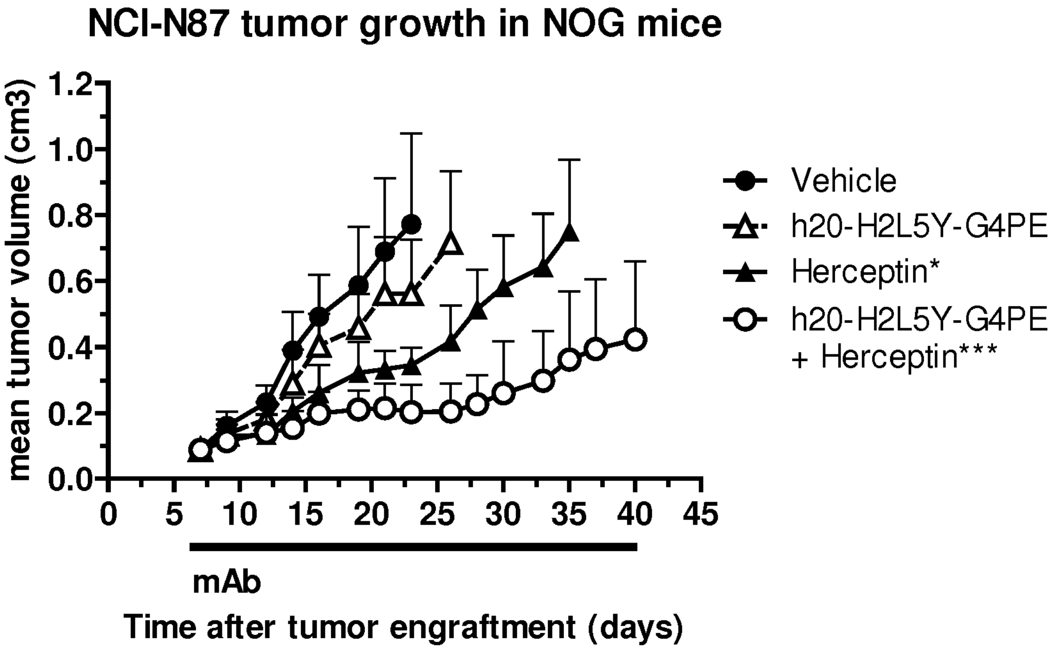

A549 - Mice survival
Percent survival
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
100
mAb
Time after tumor engraftment (days)
Vehicle
h20-H2L5Y-G4
Herceptin*
h20-H2L5Y-G4
+ Herceptin***

Figure 24


NCI-N87 tumor growth in NOG mice
mean tumor volume (cm3)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
35
40
45
mAb
Time after tumor engraftment (days)
Vehicle
h20-H2L5Y-G4PE
Herceptin*
h20-H2L5Y-G4PE
+ Herceptin***

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/050508

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 C07K16/30 A61P35/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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, EMBASE, 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	WO 2014/123580 A1 (INHIBRX LLC [US]) 14 August 2014 (2014-08-14) examples	1-32
X	WO 01/48020 A1 (MEDICAL RES COUNCIL [GB]; BARCLAY NEIL [GB]) 5 July 2001 (2001-07-05) figures examples	1-32

☐ Further documents are listed in the continuation of Box C.

☒ 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
28 March 2017	04/04/2017

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	Covone-van Hees, M

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/050508

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		PH 12015501729 A1	19-10-2015
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		WO 0148020 A1	05-07-2001