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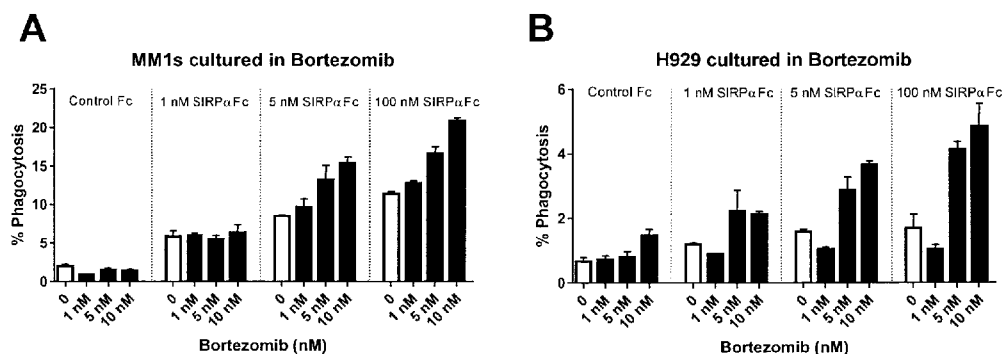
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(54) Title: ENHANCEMENT OF CD47 BLOCKADE THERAPY BY PROTEASOME INHIBITORS

Figure 1



(57) Abstract: CD47+ disease cells such as cancer cells are treated using a combination of CD47 blockade drug and a proteasome inhibitor. The anti-cancer effect of one drug enhances the 5 anti-cancer effect of the other. Specific combinations include SIRP $\alpha$ Fc as CD47 blockade drug, and one of bortezomib, ixazomib and carfilzomib as proteasome inhibitor. These combinations are useful particularly to treat blood cancers including lymphomas, leukemias and myelomas.

WO 2018/081897 A1

**ENHANCEMENT OF CD47 BLOCKADE THERAPY BY PROTEASOME  
INHIBITORS**

[001] This application claims the benefit under 35 USC §119(e) from U.S.  
5 Provisional patent application serial number 62/416,936, filed November 3, 2016, which is incorporated herein by reference in its entirety.

**Field**

[002] The disclosure relates to methods and uses of a drug that blocks the  
CD47/SIRP $\alpha$  interaction. More particularly, the disclosure relates to methods and means  
10 that, in combination, are useful for improving cancer therapy.

**Background**

[003] Cancer cells are targeted for destruction by antibodies that bind to cancer  
cell antigens, and through recruitment and activation of macrophages by way of Fc  
receptor binding to the Fc portion of that antibody. Binding between CD47 on cancer  
15 cells and SIRP $\alpha$  on macrophages transmits a “don’t eat me” signal that enables many  
tumour cells to escape destruction by macrophages. It has been shown that inhibition of  
the CD47/SIRP $\alpha$  interaction (CD47 blockade) will allow macrophages to “see” and  
destroy the target CD47+ cancer cell. The use of SIRP $\alpha$  to treat cancer by CD47 blockade  
is described in WO2010/130053.

[004] In Applicant’s WO2014/094122, a protein drug that inhibits the interaction  
20 between CD47 and SIRP $\alpha$  is described. This CD47 blockade drug is a form of human  
SIRP $\alpha$  that incorporates a particular region of its extracellular domain linked with a  
particularly useful form of an IgG1-based Fc region. In this form, the SIRP $\alpha$ Fc drug  
shows dramatic effects on the viability of cancer cells that present with a CD47+  
25 phenotype. The effect is seen particularly on acute myelogenous leukemia (AML) cells,  
and many other types of cancer. A soluble form of SIRP $\alpha$  having significantly altered  
primary structure and potent CD47 binding affinity is described in WO2013/109752.

[005] Other CD47 blockade drugs have been described, and these include various  
CD47 antibodies (see for instance Stanford’s US8562997, and InhibRx’  
30 WO2014/123580), each comprising different antigen binding sites but having, in common,  
the ability to compete with endogenous SIRP $\alpha$  for binding to CD47, to interact with  
macrophages and, ultimately, to increase CD47+ disease cell depletion. These CD47  
antibodies have activities *in vivo* that are quite different from those intrinsic to drugs that

incorporate SIRP $\alpha$  structure. The latter, for instance, display negligible binding to red blood cells whereas the opposite property seen in CD47 antibodies, and in high affinity SIRP $\alpha$  variants, creates a need for strategies that accommodate a drug “sink” that follows administration.

5 [006] Still other agents are proposed for use in blocking the CD47/SIRP $\alpha$  axis. These include CD47Fc proteins described in Viral Logic’s WO2010/083253, and SIRP $\alpha$  antibodies as described in University Health Network’s WO2013/056352, Eberhard’s US 6913894, and elsewhere.

10 [007] The CD47 blockade approach in anti-cancer drug development shows great promise. There is a need to provide methods and means for improving the effect of these drugs, and in particular for improving the effect of the CD47 blockade drugs that incorporate SIRP $\alpha$ .

### Summary

15 [008] It is now shown that the anti-cancer effect of a CD47 blockade drug is improved when combined with an agent that inhibits proteasome activity. More particularly, significant improvement in cancer cell depletion is seen when CD47+ cancer cells are treated with a CD47 blockade drug, such as a SIRP $\alpha$ -based drug or a CD47 antibody, in combination with a proteasome inhibitor. The two drugs cooperate and/or synergize in their effects on cancer cells, and result in the depletion of more cancer cells  
20 than can be accounted for by their separate, individual effects.

[009] In one aspect, there is provided a method for treating a subject with CD47+ disease cells, comprising administering an effective amount of a drug combination comprising a CD47 blockade drug, such as a CD47-binding form of SIRP $\alpha$ , and a proteasome inhibitor, such as bortezomib, ixazomib and carfilzomib.

25 [0010] In a related aspect, there is provided a use of a CD47 blockade drug, such as a SIRP $\alpha$ -based drug, in combination with a proteasome inhibitor for the treatment of a subject with CD47+ disease cells.

[0011] In one embodiment, the CD47 blockade drug can be administered to a subject already treated with a proteasome inhibitor, or the proteasome inhibitor can be  
30 administered to a subject already treated with a CD47 blockade drug. The treatment should take advantage of the combined effects of the drug within the recipient.

[0012] In another aspect there is provided a combination comprising a CD47 blockade drug and proteasome inhibitor for use in the treatment of CD47+ disease cells.

[0013] There is also provided, in another aspect, a kit comprising a combination of a CD47 blockade drug, such as a soluble SIRP $\alpha$ -based drug, and a proteasome inhibitor, together with instructions teaching their use in the treatment of CD47+ disease cells.

[0014] In a specific embodiment, the combination of the CD47 blockade drug and proteasome inhibitor is for use in the treatment of cancer.

[0015] Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the disclosure are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

[0016] These and other aspects of the disclosure are now described in greater detail with reference to the accompanying drawings, in which:

#### **Brief Reference to the Drawings**

[0017] Figure 1 shows results when the multiple myeloma cell lines MM1s and H929 are cultured in the presence of the proteasome inhibitor bortezomib (at either 1, 5 or 10 nM) for 48 hours. The 0 result represents phagocytosis of cells that were not treated with bortezomib. Cells are then washed; macrophages and SIRP $\alpha$ Fc (at 1, 5 or 100 nM) or Control Fc are added and the mixture is then subjected to the phagocytosis assay described herein. As shown in Figure 1, culturing MM1s (A) and H929 (B) in bortezomib for 48 hours results in increased SIRP $\alpha$ Fc-mediated phagocytosis.

[0018] Figure 2 shows results from an experiment in which bortezomib is replaced by the proteasome inhibitor carfilzomib, which is then investigated as described in Figure 1. The 0 result represents phagocytosis of cells that were not treated with carfilzomib. Culturing of MM1s (A) and H929 (B) in 10 nM carfilzomib resulted in a significant increase in SIRP $\alpha$ Fc-mediated phagocytosis, at all concentrations of SIRP $\alpha$ Fc tested.

[0019] Figure 3 shows the effect of proteasome inhibition on phagocytosis mediated by CD47 blockade, from an experiment supplemental to that represented in Figures 1 and 2. The diffuse large cell lymphoma (DLBCL) cell line SU-DHL-6 and the multiple myeloma (MM) cell line MM1.S were cultured in the presence or absence of the proteasome inhibitors bortezomib (10 nM), carfilzomib (10 nM) or ixazomib (25 nM) for 48 hours. Cells were thereafter washed; macrophages and SIRP $\alpha$ Fc proteins, CD47 monoclonal antibody (CD47 mAb) or Control Fc (at 100 nM) were added, and the mixture

was then subjected to the phagocytosis assay as described *infra*. As shown in Figure 3, culturing SU-DHL-6 and MM1.S cells in bortezomib (10 nM), carfilzomib (10 nM) or ixazomib (25 nM) for 48 hours results in significantly increased SIRP $\alpha$ Fc-mediated phagocytosis or CD47 mAb-mediated phagocytosis.

5 **Detailed Description**

[0020] The present disclosure provides an improved method, use, combination and kits for treating subjects that present with disease cells that have a CD47<sup>+</sup> phenotype. In particular, it is demonstrated herein that the combination of a CD47 blockade drug and a proteasome inhibitor exhibits an effect that is superior to the effects of either agent alone or of both agents in addition. This statistically significant effect, or benefit, results particularly when the CD47 blockade drug is a soluble SIRP $\alpha$ -based agent. The effect is also seen when the CD47 blockade drug is a CD47-binding antibody. The effect is pronounced when the CD47<sup>+</sup> disease cells are CD47<sup>+</sup> cancer cells and tumours.

10 [0021] In one aspect, there is provided a method for treating a subject with CD47<sup>+</sup> disease cells, comprising administering an effective amount of a drug combination comprising a CD47 blockade drug and a proteasome inhibitor.

[0022] In a related aspect, there is provided a use of a CD47 blockade drug in combination with a proteasome inhibitor for the treatment of a subject with CD47<sup>+</sup> disease cells.

20 [0023] In another aspect, there is provided a combination comprising a CD47 blockade drug and proteasome inhibitor for use in the treatment of a CD47<sup>+</sup> disease.

[0024] In a further aspect, there is provided a kit comprising a combination comprising a CD47 blockade drug and proteasome inhibitor together with instructions for the use in the treatment of CD47<sup>+</sup> disease cells.

25 [0025] There is also provided, in another aspect, a kit comprising a combination of a CD47 blockade drug and a proteasome inhibitor, together with instructions teaching their use in the treatment of CD47<sup>+</sup> disease cells.

[0026] The term CD47<sup>+</sup> disease cells means cells having the phenotype CD47<sup>+</sup> and are associated with a disease. Cells that are CD47<sup>+</sup> can be identified using the methods disclosed herein. In one embodiment, the CD47<sup>+</sup> disease cells are cancer cells.

30 [0027] As used herein, a CD47 blockade drug can be any drug or agent that interferes with and dampens or blocks signal transmission that results when CD47 interacts with macrophage-presented SIRP $\alpha$ . The CD47 blockade drug is an agent that inhibits CD47

interaction with SIRP $\alpha$ . The CD47 blockade drug is preferably an agent that binds CD47 and blocks its interaction with SIRP $\alpha$ . The CD47 blockade drug can be an antibody or antibody-based antagonist of the CD47/SIRP $\alpha$  signaling axis, such as an antibody that binds CD47 and blocks interaction of CD47 with SIRP $\alpha$ . Desirably, but not essentially, the CD47 blockade drug comprises a constant region, i.e., an Fc region, that can be bound by macrophages that are activated to destroy cells to which the CD47 blockade drug is bound, such as cancer cells. The CD47 blockade drug Fc region preferably has effector function, and is derived from either IgG1 or IgG4 including IgG4(S228P). In the alternative, the Fc region can be one that is altered by amino acid substitution to reduce effector function to an inactive state.

**[0028]** CD47-binding forms of human SIRP $\alpha$  are the preferred CD47 blockade drugs for use in the combination herein disclosed. These drugs are based on the extracellular region of human SIRP $\alpha$ . They comprise at least a part of the extracellular region sufficient to confer effective CD47 binding affinity and specificity. So-called “soluble” forms of SIRP $\alpha$ , lacking the membrane anchoring component of SIRP $\alpha$ , are useful and are described in the literature and include those referenced in Novartis’ WO 2010/070047, Stanford’s WO2013/109752, Merck’s WO2016/024021 and Trillium’s WO2014/094122.

**[0029]** In a preferred embodiment, the soluble CD47-binding form of SIRP $\alpha$  is an Fc fusion. More particularly, the drug suitably comprises the human SIRP $\alpha$  protein, in a form fused directly, or indirectly, with an antibody constant region, or Fc (fragment crystallisable) Unless otherwise stated, the term “human SIRP $\alpha$ ” as used herein refers to a wild type, endogenous, mature form of human SIRP $\alpha$ . In humans, the SIRP $\alpha$  protein is found in two major forms. One form, the variant 1 or V1 form, has the amino acid sequence set out as NCBI RefSeq NP\_542970.1 (residues 27-504 constitute the mature form). Another form, the variant 2 or V2 form, differs by 13 amino acids and has the amino acid sequence set out in GenBank as CAA71403.1 (residues 30-504 constitute the mature form). These two forms of SIRP $\alpha$  constitute about 80% of the forms of SIRP $\alpha$  present in humans, and both are embraced herein by the term “human SIRP $\alpha$ ”. Also embraced by the term “human SIRP $\alpha$ ” are the minor forms thereof that are endogenous to humans and have the same property of triggering signal transduction through CD47 upon binding thereto. The present disclosure is directed in some embodiments to the drug

combinations that include a CD47 blockade drug that comprises the V region of the V2 form of human SIRP $\alpha$ .

**[0030]** In the present drug combination, useful SIRP $\alpha$ Fc fusion proteins comprise at least one, such as only one, of the three so-called immunoglobulin (Ig) domains that lie within the extracellular region of human SIRP $\alpha$ . More particularly, the present SIRP $\alpha$ Fc proteins incorporate at least residues 32-137 of human SIRP $\alpha$  (a 106-mer), which constitute and define the IgV domain of the V2 form of human SIRP $\alpha$ , according to current nomenclature. This SIRP $\alpha$  sequence, shown below, is referenced herein as SEQ ID No. 1.

5  
10 EELQVIQPKSVSVAAGESAILHCTVTSVIPVGPVWFRGAGPARELIYNQKEGHFP  
RVTTVSESTKRENMDFSISISNITPADAGTYVCVKFRKGGSPDTEFKSGA (SEQ ID  
No.1)

**[0031]** In a preferred embodiment, the SIRP $\alpha$ Fc fusion proteins incorporate the IgV domain as defined by SEQ ID No. 1, and additional, flanking residues that can be contiguous within the SIRP $\alpha$  sequence. This preferred form of the IgV domain, represented by residues 31-148 of the V2 form of human SIRP $\alpha$ , is a 118-mer having SEQ ID No. 2 shown below:

15  
20 EEELQVIQPKSVSVAAGESAILHCTVTSVIPVGPVWFRGAGPARELIYNQKEGHF  
PRVTTVSESTKRENMDFSISISNITPADAGTYVCVKFRKGGSPDTEFKSGAGTELSVR  
AKPS (SEQ ID No. 2)

**[0032]** Desirable SIRP $\alpha$  fusion proteins incorporate an Fc region that preferably also has effector function. Fc refers to “fragment crystallisable” and represents the constant region of an antibody comprised principally of the heavy chain constant region and components within the hinge region. An Fc component “having effector function” is an Fc component having at least some natural or engineered function, such as at least some contribution to antibody-dependent cellular cytotoxicity or some ability to fix complement. Also, the Fc will at least bind to Fc receptors. These properties can be revealed using assays established for this purpose. Functional assays include the standard chromium release assay that detects target cell lysis. By this definition, an Fc region that is wild type IgG1 or IgG4 has effector function, whereas the Fc region of a human IgG4 mutated to alter effector function, such as by incorporation of an alteration series that includes Pro233, Val234, Ala235 and deletion of Gly236 (EU), is considered not to have effector function. In a preferred embodiment, the Fc is based on human antibodies of the

IgG1 isotype. The Fc region of these antibodies will be readily identifiable to those skilled in the art. In embodiments, the Fc region includes the lower hinge-CH2-CH3 domains.

**[0033]** In a specific embodiment, the Fc region is based on the amino acid sequence of a human IgG1 set out as P01857 in UniProtKB/Swiss-Prot, residues 104-330, and has the amino acid sequence shown below and referenced herein as SEQ ID No. 3:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN  
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL  
 PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN  
 10 GQPENNYKTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQ  
 KSLSLSPGK\* (SEQ ID No. 3)

**[0034]** Thus, in embodiments, the Fc region has either a wild type or consensus sequence of an IgG1 constant region. In alternative embodiments, the Fc region incorporated in the fusion protein is derived from any IgG1 antibody having a typical effector-active constant region. The sequences of such Fc regions can correspond, for example, with the Fc regions of any of the following IgG1 sequences (all referenced from GenBank), for example: BAG65283 (residues 242-473), , BAC04226.1 (residues 247-478), BAC05014.1 (residues 240-471), CAC20454.1 (residues 99-320), BAC05016.1 (residues 238-469), BAC85350.1 (residues 243-474), BAC85529.1 (residues 244-475),  
 20 and BAC85429.1 (residues (238-469).

**[0035]** In the alternative, the Fc region can be a wild type or consensus sequence of an IgG2 or IgG3 sequence, examples thereof being shown below:

a human IgG2, for example:

APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN  
 25 AKTKPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKG  
 QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTTPP  
 MLDSGDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK  
 (SEQ ID No. 4), as comprised in P01859 of the UniProtKB/Swiss-Prot database;

a human IgG3, for example:

30 APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVH  
 NAKTKPREEQYNSTFRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTK  
 GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPP

MLSDSGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK (SEQ ID No. 5), as comprised in P01860 of the UniProtKB/Swiss-Prot database;

**[0036]** In other embodiments, the Fc region has a sequence of a wild type human IgG4 constant region. In alternative embodiments, the Fc region incorporated in the fusion protein is derived from any IgG4 antibody having a constant region with effector activity that is present but, naturally, is significantly less potent than the IgG1 Fc region. The sequences of such Fc regions can correspond, for example, with the Fc regions of any of the following IgG4 sequences: P01861 (residues 99-327) from UniProtKB/Swiss-Prot and CAC20457.1 (residues 99-327) from GenBank.

**[0037]** In a specific embodiment, the Fc region is based on the amino acid sequence of a human IgG4 set out as P01861 in UniProtKB/Swiss-Prot, residues 99-327, and has the amino acid sequence shown below and referenced herein as SEQ ID No. 6:

ESKYGPPCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQF  
 NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESN  
 GQPENNYKTTTPVLDSGSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ  
 KSLSLSLGK (SEQ ID No. 6)

**[0038]** In embodiments, the Fc region incorporates one or more alterations, usually not more than about 10, e.g., up to 5 such alterations, including amino acid substitutions that affect certain Fc properties. In one specific and preferred embodiment, the Fc region incorporates an alteration at position 228 (EU numbering), in which the serine at this position is substituted by a proline (S228P), thereby to stabilize the disulfide linkage within the Fc dimer. Other alterations within the Fc region can include substitutions that alter glycosylation, such as substitution of Asn297 by glycine or alanine; half-life enhancing alterations such as T252L, T253S, and T256F as taught in US62777375, and many others. Particularly useful are those alterations that enhance Fc properties while remaining silent with respect to conformation, e.g., retaining Fc receptor binding.

**[0039]** In a specific embodiment, and in the case where the Fc component is an IgG4 Fc, the Fc incorporates at least the S228P mutation, and has the amino acid sequence set out below and referenced herein as SEQ ID No. 7:

ESKYGPPCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQF  
 NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESN

GQPENNYKTTTPVLDSGDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ  
KSLSLSLGK (SEQ ID No. 7)

[0040] The CD47 blockade drug used in the combination is thus preferably a  
SIRP $\alpha$  fusion protein useful to inhibit the binding of human SIRP $\alpha$  with human CD47,  
5 thereby to inhibit or reduce transmission of the signal mediated via SIRP $\alpha$ -bound CD47.  
In embodiments, the fusion protein comprises a human SIRP $\alpha$  component and, fused  
therewith, an Fc component, wherein the SIRP $\alpha$  component comprises or consists of a  
single IgV domain of human SIRP $\alpha$  V2 and the Fc component is the constant region of a  
human IgG having effector function.

10 [0041] In one embodiment, the fusion protein comprises a SIRP $\alpha$  component  
consisting at least of residues 32-137 of the V2 form of wild type human SIRP $\alpha$ , i.e., SEQ  
ID No. 1. In a preferred embodiment, the SIRP $\alpha$  component consists of residues 31-148  
of the V2 form of human SIRP $\alpha$ , i.e., SEQ ID No. 2. In another embodiment, the Fc  
component is the Fc component of the human IgG1 designated P01857, and in a specific  
15 embodiment has the amino acid sequence that incorporates the lower hinge-CH2-CH3  
region thereof i.e., SEQ ID No. 3.

[0042] In a preferred embodiment, therefore, the SIRP $\alpha$ Fc fusion protein is  
provided and used in a secreted dimeric fusion form, wherein the fusion protein  
incorporates a SIRP $\alpha$  component having SEQ ID No. 1 and preferably SEQ ID No. 2 and,  
20 fused therewith, an Fc region having effector function and having SEQ ID No.3. When  
the SIRP $\alpha$  component is SEQ ID No. 1, this fusion protein comprises SEQ ID No. 8,  
shown below:

EEELQVIQPKSVSVAAGESAILHCTVTSLIPVGPIQWFRGAGPARELIYNQKEGHF  
PRVTTVSESTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSGAGTELSVR  
25 AKPSDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE  
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE  
WESNGQPENNYKTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN  
HYTQKSLSLSPGK\* (SEQ ID No. 8)

30 [0043] When the SIRP $\alpha$  component is SEQ ID No. 2, this fusion protein comprises  
SEQ ID No. 9, a preferred CD47 blockade drug species, shown below:

EEELQVIQPKSVSVAAGESAILHCTVTSLIPVGPIQWFRGAGPARELIYNQKEGHF  
PRVTTVSESTKRENMDFSISISNITPADAGTYYCVKFRKGSPDTEFKSGAGTELSVR

AKPSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE  
 VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
 NKALPAIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE  
 WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHN  
 5 HYTQKSLSLSPGK (SEQ ID No. 9)

[0044] In alternative embodiments, the Fc component of the fusion protein is based on an IgG4, and preferably an IgG4 that incorporates the S228P mutation. In the case where the fusion protein incorporates the preferred SIRP $\alpha$  IgV domain of SEQ ID No. 2, the resulting IgG4-based SIRP $\alpha$ -Fc protein, another preferred CD47 blockade drug species, has SEQ ID No. 10 shown below:

EEELQVIQPKSVSVAAGESAILHCTVTSVIPVGPVWFRGAGPARELIYNQKEGHF  
 PRVTTVSESTKRENMDFSISISNITPADAGTYVCVKFRKGSPDTEFKSGAGTELSVR  
 AKPSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDP  
 EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKV  
 15 NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE  
 WESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFCSCVMHEALHN  
 HYTQKSLSLSLGK (SEQ ID No. 10)

[0045] In preferred embodiment, the fusion protein comprises, as the SIRP $\alpha$  IgV domain of the fusion protein, a sequence that is SEQ ID No. 2. The preferred SIRP $\alpha$ -Fc is SEQ ID No. 9.

[0046] The SIRP $\alpha$  sequence incorporated within the CD47 blockade drug can be varied, as described in the literature. That is, useful substitutions within SIRP $\alpha$  include one or more of the following: L4V/I, V6I/L, A21V, V27I/L, 131T/S/F, E47V/L, K53R, E54Q, H56P/R, S66T/G, K68R, V92I, F94V/L, V63I, and/or F103V. In embodiments, 25 these variants can incorporate a set of amino acid substitutions, such as V6I + V27I + 131 F + E47V + K53R + E54Q + H56P + S66T + V92I. CD47-binding SIRP $\alpha$  variants of this type can be used either per se or as Fc fusion proteins, such as G4 Fc fusions and other low effector activity Fc regions including mutated G4.

[0047] In a embodiment, the CD47 blockade drug is a variant of human SIRP $\alpha$  30 having higher binding affinity for human CD47 than wild type SIRP $\alpha$ . In a specific embodiment, the variant SIRP $\alpha$  has the sequence shown in SEQ ID No. 11:

EEELQIIQPKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARVLIYNQRQGPFP  
 RVTTVSETTKRENMDFSISISNITPADAGTYCYCIKFRKGGSPDTEFKSGAGTELSVRA  
 KP (SEQ ID No. 11)

[0048] This SIRP $\alpha$  variant comprises the following amino acid substitutions  
 5 relative to wild type SIRP $\alpha$ :

V<sup>6</sup>I + V<sup>27</sup>I + I<sup>31</sup>F + E<sup>47</sup>V + K<sup>53</sup>R + E<sup>54</sup>Q + H<sup>56</sup>P + S<sup>66</sup>T + V<sup>92</sup>I. In a specific embodiment,  
 this variant SIRP $\alpha$  sequence can be fused with a mutated IgG4 Fc region including a  
 Ser<sup>228</sup>Pro (EU) having virtually no effector function, to yield a CD47 blockade drug  
 having the sequence:

10 EEELQIIQPKSVSVAAGESAILHCTITSLFPVGPIQWFRGAGPARVLIYNQRQGPFP  
 RVTTVSETTKRENMDFSISISNITPADAGTYCYCIKFRKGGSPDTEFKSGAGTELSVRA  
 KPSESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEV  
 QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN  
 KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEV  
 15 ESNQGPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH  
 YTQKLSLSLGLK\* (SEQ ID No. 12)

[0049] Still other types of CD47 blockade drugs can be used in the present method  
 and combination, instead of or in addition to the SIRP $\alpha$ -based drugs. These other drugs  
 include particularly anti-CD47 antibodies, which bind to CD47 and antagonize the  
 20 interaction with SIRP $\alpha$ . By blocking that interaction, and because of the Fc region of the  
 antibody, the effect of the CD47 antibodies can be similar to the effect of the SIRP $\alpha$ -based  
 Fc fusion drugs. Examples of CD47 antibodies are described in the literature such as  
 Chugai's US2008/0107654; Stanford's WO2009/091601; InhibRx WO2013/119714,  
 Celgene's WO2016/109415; and Janssen's WO2016/081423. Because these antibodies  
 25 bind red blood cells, a dosing regimen that takes this into account has been developed and  
 is described in WO2014/149477. The properties of a useful anti-CD47 antibody include  
 simply the ability to bind to CD47 in a way that ultimately inhibits signaling by SIRP $\alpha$ ,  
 i.e., as an antagonist.

[0050] In one embodiment, the CD47 blockade drug is an anti-CD47 antibody that  
 30 is a chimeric, humanized, human or otherwise recombinant, monoclonal or polyclonal  
 antibody based on the sequence of antibody B6H12 known from the literature and  
 including the sequences:

[0051] Amino acid sequence of B6H12 heavy chain variable region:  
EVQLVESGGDLVKPGGSLKLSAASGFTFSGYGMSWVRQTPDKRLEWVATITSG  
GTYYTYPDSVKGRFTISRDNKNTLYLQIDSLKSEDTAIYFCARSLAGNAMDYWG  
QGTSVTVSS (SEQ ID No. 13)

5 [0052] Amino acid sequence of B6H12 light chain variable region  
DIVMTQSPATLSVTPGDRVSLSCRASQTISDYLHWYQQKSHESPRLLIKFASQSIG  
IPSRFSGSGSGSDFTLINSVPEPDVGVYYCQNGHGFPRTFGGGTKLEIK (SEQ ID  
No. 14)

[0053] A full sequence for this antibody and the CDR sequences therein, are  
10 available from Figure 1 in US21030142786, the entire contents of which are incorporated  
herein by reference.

[0054] Other CD47 blockade drugs include CD47Fc proteins, as taught by Viral  
Logic in WO2010/083253 and by Stanford in US8377448), as well as SIRP $\alpha$  antibodies,  
as described in UHN's WO2013/056352, Stanford's WO2016/022971, Eberhard's US  
15 6913894, and elsewhere.

[0055] In a SIRP $\alpha$ Fc fusion protein, the SIRP $\alpha$  component and the Fc component  
are fused, either directly or indirectly, to provide a single chain polypeptide that is  
ultimately produced as a dimer in which the single chain polypeptides are coupled through  
intrachain disulfide bonds formed within the Fc region. The nature of the fusing region is  
20 not critical. The fusion may be direct between the two components, with the SIRP  
component constituting the N-terminal end of the fusion and the Fc component  
constituting the C-terminal end. Alternatively, the fusion may be indirect, through a linker  
comprised of one or more amino acids, desirably genetically encoded amino acids, such as  
two, three, four, five, six, seven, eight, nine or ten amino acids, or any number of amino  
25 acids between 5 and 100 amino acids, such as between 5 and 50, 5 and 30 or 5 and 20  
amino acids. A linker may comprise a peptide that is encoded by DNA constituting a  
restriction site, such as a BamHI, ClaI, EcoRI, HindIII, PstI, SalI and XhoI site and the  
like.

[0056] The linker amino acids typically and desirably have some flexibility to  
30 allow the Fc and the SIRP components to adopt their active conformations. Residues that  
allow for such flexibility typically are Gly, Asn and Ser, so that virtually any combination  
of these residues (and particularly Gly and Ser) within a linker is likely to provide the  
desired linking effect. In one example, such a linker is based on the so-called G4S

sequence (Gly-Gly-Gly-Gly-Ser) (SEQ ID No. 15) which may repeat as (G4S)<sub>n</sub> where n is 1, 2, 3 or more, or is based on (Gly)<sub>n</sub>, (Ser)<sub>n</sub>, (Ser-Gly)<sub>n</sub> or (Gly-Ser)<sub>n</sub> and the like. In another embodiment, the linker is GTELSVRAKPS (SEQ ID No. 16). This sequence constitutes SIRP $\alpha$  sequence that C-terminally flanks the IgV domain (it being understood that this flanking sequence could be considered either a linker or a different form of the IgV domain when coupled with the IgV minimal sequence described above). It is necessary only that the fusing region or linker permits the components to adopt their active conformations, and this can be achieved by any form of linker useful in the art.

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[0057] As noted, the CD47 blockade drug such as a SIRP $\alpha$ Fc fusion is useful to inhibit interaction between SIRP $\alpha$  and CD47, thereby to block signalling across this axis. Stimulation of SIRP $\alpha$  on macrophages by CD47 is known to inhibit macrophage-mediated phagocytosis by deactivating myosin-II and the contractile cytoskeletal activity involved in pulling a target into a macrophage. Activation of this cascade is therefore important for the survival of CD47+ disease cells, and blocking this pathway enables macrophages to eradicate or at least reduce the active CD47+ disease cell population.

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[0058] The term "CD47+" is used with reference to the phenotype of cells targeted for binding by the present CD47 blockade drug. Cells that are CD47+ can be identified by flow cytometry using CD47 antibody as the affinity ligand. CD47 antibodies that are labeled appropriately are available commercially for this use (for example, the antibody product of clone B6H12 is available from Santa Cruz Biotechnology). The cells examined for CD47 phenotype can include standard tumour biopsy samples including particularly blood samples taken from the subject suspected of harbouring endogenous CD47+ cancer cells. CD47 disease cells of particular interest as targets for therapy with the present drug combinations are those that "over-express" CD47. These CD47+ cells typically are disease cells, and present CD47 at a density on their surface that exceeds the normal CD47 density for a cell of a given type. CD47 overexpression will vary across different cell types, but is meant herein to refer to any CD47 level that is determined, for instance by flow cytometry or by immunostaining or by gene expression analysis or the like, to be greater than the level measurable on a healthy counterpart cell having a CD47 phenotype that is normal for that cell type.

[0059] The present drug combination comprises both a CD47 blockade drug that preferably comprises a soluble form of a SIRP $\alpha$ , as just described, and an inhibitor of a proteasome. In a preferred embodiment, the proteasome inhibitor is bortezomib, or

carfilzomib, or ixazomib, or an analog thereof including certain fluorinated analogs, as described herein.

5 [0060] The multi-catalytic proteasome is the ubiquitous proteinase found in cells throughout the plant and animal kingdoms that is responsible for the ubiquitin-dependent degradation of intracellular proteins. Thousands of copies are found in all cells, in both the cytoplasm and the nucleus, which constitute up to 3% of all cellular protein content. Proteasomes serve multiple intracellular functions, including the degradation of damaged proteins and the modulation of many regulatory proteins that affect inflammatory processes, viral shedding, the cell cycle, growth, and differentiation.

10 [0061] The ubiquitin-proteasome pathway (UPP), also known as the ubiquitin-proteasome system (UPS), regulates the degradation of intracellular proteins with specificity as to target, time and space. The pathway plays a central role in recognizing and degrading misfolded and abnormal proteins in most mammalian cells. In this pathway, the 26S proteasome is the main proteolytic component, which is found in all eukaryotic cells and is made up of the cylinder-shaped multi-catalytic proteinase complex (MPC) 20S proteasome and two regulatory particles (RP) 19S proteasomes. The 19S proteasome located at each end of the 20S proteasome is made up of 18 subunits, and controls the recognition, unfolding, and translocation of protein substrates into the lumen of the 20S proteasome. The 20S proteasome is composed of 28 protein subunits arranged in four stack rings, with each ring made up of seven  $\alpha$ - and  $\beta$ -type subunits, following an  $\alpha$ 1-7 $\beta$ 1-7 stoichiometry. The two outer chambers are formed by  $\alpha$  subunits, while the central chamber, containing the proteolytic active sites, is made up of  $\beta$  subunits. Three of the 14  $\beta$  subunits are responsible for the post-glutamyl peptide hydrolysis activity (PGPH, attributed to  $\beta$ 1), trypsin-like activity (T-L,  $\beta$ 2), and chymotrypsin-like activity (CT-L,  $\beta$ 5), respectively, and all these three active subunits hydrolyze the amide bond of protein substrates with the hydrophilic  $\gamma$ -hydroxyl group of the N-terminal threonine ( $O\gamma$ -Thr1).

25 [0062] Proteasome inhibitors include those agents that inhibit at least one of the activities of a proteasome subunit or a proteasome complex, such as inhibition of an enzymatic activity. Other proteasome inhibitors include those agents that inhibit formation or interaction of active proteasome complexes.

30 [0063] Useful in combination with a CD47 blockade drug is the first-in-class proteasome inhibitor, bortezomib, a potent, selective, and reversible proteasome inhibitor which targets the 26S proteasome complex and inhibits its function. The successful

development of bortezomib (Velcade®) for treatment of relapsed/refractory multiple myeloma (MM) and mantle cell lymphoma, has shown proteasome inhibition to be a useful anti-cancer strategy. Bortezomib primarily inhibits chymotryptic activity, without altering tryptic or caspase-like, proteasome activity. It has pleiotropic effects on multiple myeloma biology by targeting a) cell-cycle regulatory proteins; b) the unfolded protein response (UPR) pathway via modulating the transcriptional activity of plasma cell differentiation factor X-box binding protein-1 (XBP-1); c) p53-mediated apoptosis/MDM2; d) DNA repair mechanisms; and e) classical stress-response pathways via both intrinsic (caspase-9 mediated) and extrinsic (caspase-3 mediated) cell death cascades. Specifically, bortezomib activates c-Jun N-terminal kinase (JNK), which triggers mitochondrial apoptotic signalling: release of cytochrome-c (cyto-c) and second mitochondrial activator of caspases (Smac) from mitochondria to cytosol, followed by activation of caspase-9 and caspase-3.

[0064] Another proteasome inhibitor useful in the present combination is a structural analogue of the microbial natural product epoxomicin, now known as carfilzomib (also called PR-171). Carfilzomib selectively inhibits the CTL activity of the 20S proteasome with minimal cross reactivity to other proteasome classes.

[0065] Clinical studies have demonstrated that consecutive daily dosing schedules with carfilzomib are both well-tolerated and promote antitumor activity in hematologic malignancies, including patients previously treated with bortezomib.

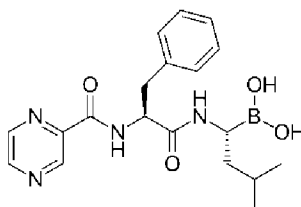
[0066] Thus, in the present method, a CD47 blockade drug is used in combination with a proteasome inhibitor, especially bortezomib, ixazomib and carfilzomib. The proteasome inhibitors useful in the present method also include a number and variety of clinically advanced or marketed compounds such as bortezomib sold as Velcade® (PS-341), carfilzomib sold as Kyprolis® (PR 171), ixazomib (MLN-9708/2238), delanzomib (CEP-18770), oprozomib (ONX-0912, PR-047) and marizomib (NPI-0052, salinosporamide A).

[0067] Proteasome inhibitors useful in the present method, use and combination thus include, as a class, a variety of boron-containing peptide-based structures, i.e., the peptidic boronic acids that include bortezomib, ixazomib, and delanzomib, and numerous analogs.

[0068] Proteasome inhibitors useful in the present method, use and combination also include, as a class, a variety of peptide epoxyketones that include carfilzomib, and oprozomib, and numerous analogs.

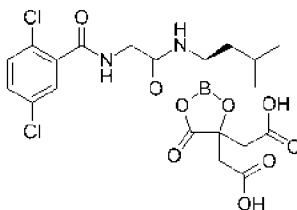
[0069] Still other proteasome inhibitors useful in the present method, use and combination include lactacystin, disulfiram, expoxomicin, G132,  $\beta$ -hydroxy  $\beta$ -methylbutyrate, epigallocatechin-3-gallate, MLN9708, and CD P-18770.

[0070] In a specific embodiment of the present method, use and combination, the CD47 blockade drug is used in combination with bortezomib, having the structure:



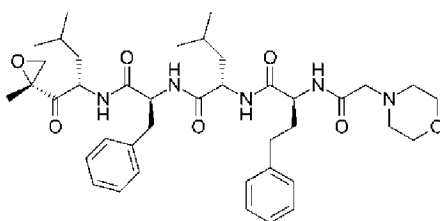
[0071] As noted, bortezomib is marketed under the trademark Velcade® and is provided as a lyophilized powder for intravenous injection. It is a reversible inhibitor with a  $\beta 5 > \beta 1$  inhibition profile. Established dosing is 1.3mg/m<sup>2</sup> with 2 intravenous administrations on days 1, 4, 8 and 11 of a 21 day cycle. It can be used in combination with doxorubicin and dexamethasone, or in combination with thalidomide, melphalan, prednisone, cyclophosphamide and other agents such as etoposide. It can be used in this same manner for purposes of the present disclosure, although cooperation/interaction with the CD47 blockade drug should permit the use of a reduced bortezomib dose or dosing frequency. It is used particularly for the treatment of multiple myeloma, and can be used for this purpose when combined with CD47 blockade drug for treating this type of blood cancer.

[0072] Another boron-containing compound useful the present combination is ixazomib, an orally-available proteasome inhibitor sold as Ninlaro® and used currently in combination with lenalidomide and dexamethasone for the treatment of multiple myeloma. It inhibits proteasome subunit beta type-5. It has the following structure (and is the R-enantiomer):





[0076] Instead of bortezomib or in addition thereto, the drug combination can include the epoxyketone-based proteasome inhibitor known as carfilzomib having the structure of Formula III shown below:

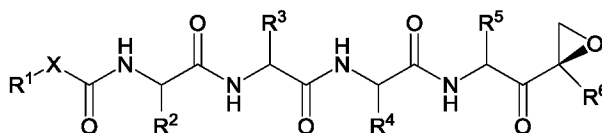


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III

[0077] Carfilzomib interferes with the chymotrypsin-like activity of the 20S proteasome that degrades unwanted cellular proteins, causing a build-up of polyubiquitinated proteins, which may lead to apoptosis, cycle arrest, and tumor growth inhibition. This tetrapeptide epoxyketone (also an epoxomicin analog) is marketed as Kyprolis® for the treatment of multiple myeloma. In this marketed form, i.e., a form also useful in the present combination, the active ingredient is formulated as monotherapy for a 10-minute infusion and is started at 20 mg/m<sup>2</sup> during the first cycle on days 1 and 2. If this dose is tolerated, the dose is increased to 27 mg/m<sup>2</sup> for the remaining cycles.

15 [0078] Potent analogs of carfilzomib have more recently been described in WO2014/026282. These fluorinated analogs have the general structure of Formula IV shown below.



I

IV

20 wherein:

R<sup>1</sup> is selected from morpholinyl, 1,4-oxazepanyl, thiomorpholinyl, 1,4-thiazepanyl, 1,4-thiazepanyl-1-oxide, 1,4-thiazepanyl-1,1-dioxide, 1,4-thiazinanyl-1-oxide, 1,4-thiazinanyl-1,1-dioxide, aziridinyl, azetidiny, pyrrolidinyl, piperazinyl and 1,4-diazepanyl;

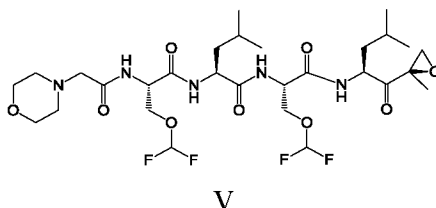
25 X is C<sub>1-4</sub>alkylene;

R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup> and R<sup>5</sup> are each independently selected from the group consisting of C<sub>1-6</sub>alkyl, C<sub>1-4</sub>alkylene-phenyl, C<sub>1-4</sub>alkylene-O-CH<sub>2</sub>F, C<sub>1-4</sub>alkylene-O-CHF<sub>2</sub> and

$C_{1-4}$ alkylene-O-CF<sub>3</sub>, wherein at least one of R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup> and R<sup>5</sup> is  $C_{1-4}$ alkylene-O-CH<sub>2</sub>F,  $C_{1-4}$ alkylene-O-CHF<sub>2</sub> or  $C_{1-4}$ alkylene-O-CF<sub>3</sub>; and

R<sup>6</sup> is  $C_{1-6}$ alkyl.

[0079] In embodiments of the present disclosure, the drug combination comprises  
5 a species of fluorinated carfilzomib analogs of formula V:



[0080] Still other CD47 blockade drug combinations can include such proteasome inhibitors as the natural product lactacystin, disulfiram, epigallocatechin-3-gallate,  
10 epoxomicin, G132, and  $\beta$ -hydroxy  $\beta$ -methylbutyrate (a proteasome inhibitor in human skeletal muscle). Also, the CD47 blockade drug can be used in combination with a proteasome inhibitor that is an aldehyde (IPSI-001), or a compound that targets ubiquitin E3 ligase such as a cis-imidazoline (nutline-3 and RO5045337 and RO5503781) and a Smac peptide mimetic (LCL161), or an IAP anti-sense termed AEG 35156. The  
15 proteasome inhibitor can also be a compound that targets 19S proteasome particularly, such as the quinoline-based ubistatins, and a bis-nitrobenzylidene-piperidinone. Still other compounds useful as proteasome inhibitors include P5091, P22077 as well as WP-1130 which all target DUBs (deubiquitinases).

[0081] Each drug included in the combination can be formulated separately for use  
20 in combination. The drugs are said to be used “in combination” when, in a recipient of both drugs, the effect of one drug enhances or at least influences the effect of the other drug.

[0082] The two drugs in the combination cooperate to provide an effect on target CD47+ cells that is greater than the effect of either drug alone. This benefit manifests as a  
25 statistically significant improvement in a given parameter of target cell fitness or vitality. For instance, a benefit in CD47+ cancer cells when a given combination of CD47 blockade drug and proteasome inhibitor is used could be a statistically significant decrease in the number of living cancer cells (hence a depletion), relative to non-treatment, or an increase in the number or size of cancer cells or tumours, or an improvement in the  
30 endogenous location or distribution of any particular tumour type. In embodiments, the improvement resulting from treatment with the drug combination can manifest as an effect

that is at least additive and desirably synergistic, relative to results obtained when only a single agent is used.

**[0083]** In use, each drug in the combination can be formulated as it would be for monotherapy, in terms of dosage size and form and regimen. In this regard, the synergy  
5 resulting from their combined use may permit the use of somewhat reduced dosage sizes or frequencies, as would be revealed in an appropriately controlled clinical trial.

**[0084]** The mechanism by which a proteasome inhibitor contributes to the activity of a CD47 blockade drug, in the present combination, is not known. The proteasome inhibitors likely have a direct activity on some tumour cells, and preliminary data suggest  
10 that treatment of tumor cells with proteasome inhibitors results in upregulation of pro-phagocytic (“eat-me”) signals such as galectin-3 and galectin-9 on the surface of tumor cells.

**[0085]** In this approach, each drug is provided in a dosage form comprising a pharmaceutically acceptable carrier, and in a therapeutically effective amount. As used  
15 herein, “pharmaceutically acceptable carrier” means any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible and useful in the art of protein/antibody formulation. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise  
20 minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the pharmacological agent. Each CD47 blockade drug that is a protein such as SIRP $\alpha$ Fc fusion protein and CD47 antibody is formulated using practises standard in the art of therapeutic protein drug formulation. Solutions that are suitable for intravenous administration, such as by injection or infusion, are particularly useful. The inhibitor will of course be formulated as permitted by the regulatory agencies that have approved its use  
25 in humans.

**[0086]** Sterile solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients noted above, as required, followed by sterilization microfiltration. Generally, dispersions

are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

5 [0087] As used herein, “effective amount” refers to an amount effective, at dosages and for a particular period of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of each drug in the combination may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the drug to elicit a desired response in the recipient. A therapeutically effective amount is also one in which any toxic or detrimental effects of the pharmacological agent are outweighed by the therapeutically beneficial effects. The proteasome inhibitor will of course be formulated in amounts that are suitable for patient dosing, as permitted by the regulatory agencies that have approved its use in humans. The CD47 blockade drug can also be administered in amounts that are effective according to clinical trial results. The SIRP $\alpha$ Fc having SEQ ID No. 9 can be delivered as a 3mg dose by intratumoural injection. Some additional guidance can be gleaned from the experimental drug concentrations used with cell-based assays described in the examples herein.

20 [0088] The SIRP $\alpha$ Fc fusion protein can be administered to the subject through any of the routes established for protein delivery, in particular intravenous, intradermal and subcutaneous injection or infusion, or by oral or nasal administration.

[0089] The drugs in the present combination can be administered sequentially or, essentially at the same time. In embodiments, the proteasome inhibitor can be given before administration of SIRP $\alpha$ Fc. In the alternative, the proteasome inhibitor can be given after or during administration of SIRP $\alpha$ Fc, or any other CD47 blockade drug alternative. Thus, in embodiments, the subject undergoing therapy is a subject already treated with one of the combination drugs, such as a proteasome inhibitor, that is then treated with the other of the combination drugs, such as a CD47 blockade drug.

30 [0090] Dosing regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus of each drug may be administered, or several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the medical situation. It is especially

advantageous to formulate parenteral compositions in unit dosage form for ease of administration and uniformity of dosage. "Unit dosage form" as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[0091] The drugs can be formulated in combination, so that the combination can be introduced to the recipient in one administration, e.g., one injection or one infusion. Alternatively, and for marketing, the drugs can be combined as separate units that are provided together in a single package, and with instructions for the use thereof according to the present method. In another embodiment, an article of manufacture containing the SIRP $\alpha$ Fc drug and proteasome inhibitor combination in an amount useful for the treatment of the disorders described herein is provided. The article of manufacture comprises one or both drugs of the present combination, as well as 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 vial having a stopper pierceable by a hypodermic injection needle). The label on or associated with the container indicates that the composition is to be used so that a recipient receives both the CD47 blockade drug, e.g., a SIRP-based protein, and the proteasome inhibitor in accordance with the present disclosure, thereby to elicit a synergistic effect on the CD47+ disease cells. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other matters desirable from a commercial and use standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[0092] For administration the dose for the CD47 blockade drug will be within the range from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example SIRP $\alpha$ Fc dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 0.1 - 100 mg/kg.

[0093] The SIRP $\alpha$ Fc protein displays negligible binding to red blood cells. There is accordingly no need to account for an RBC "sink" when dosing with the drug

combination. Relative to other CD47 blockade drugs that are bound by RBCs, it is estimated that the present SIRP $\alpha$ Fc fusion can be effective at doses that are less than half the doses required for drugs that become RBC-bound, such as CD47 antibodies. Moreover, the SIRP $\alpha$ -Fc fusion protein is a dedicated antagonist of the SIRP $\alpha$ -mediated  
5 signal, as it displays negligible CD47 agonism when binding thereto. There is accordingly no need, when establishing medically useful unit dosing regimens, to account for any stimulation induced by the drug.

**[0094]** The drug combination is useful to treat a variety of CD47+ disease cells. These include particularly CD47+ cancer cells, including liquid and solid tumours. Solid  
10 tumours can be treated with the present drug combination, to reduce the size, number, distribution or growth rate thereof and to control growth of cancer stem cells. Such solid tumours include CD47+ tumours in bladder, brain, breast, lung, colon, ovary, prostate, liver and other tissues as well. In one embodiment, the drug combination can be used to inhibit the growth or proliferation of hematological cancers. As used herein,  
15 “hematological cancer” refers to a cancer of the blood, and includes leukemia, lymphoma and myeloma among others. “Leukemia” refers to a cancer of the blood, in which too many white blood cells that are ineffective in fighting infection are made, thus crowding out the other parts that make up the blood, such as platelets and red blood cells. It is understood that cases of leukemia are classified as acute or chronic. Certain forms of  
20 leukemia may be, by way of example, acute lymphocytic leukemia (ALL); acute myeloid leukemia (AML); chronic lymphocytic leukemia (CLL); chronic myelogenous leukemia (CML); myeloproliferative disorder/neoplasm (MPDS); and myelodysplastic syndrome. “Lymphoma” may refer to a Hodgkin’s lymphoma, both indolent and aggressive non-Hodgkin’s lymphoma, cutaneous T cell lymphoma (CTCL), Burkitt’s lymphoma, Mantle  
25 cell lymphoma (MCL) and follicular lymphoma (small cell and large cell), among others. Myelomas include multiple myeloma (MM), giant cell myeloma, heavy-chain myeloma, and light chain myeloma and Bence-Jones myeloma.

**[0095]** In some embodiments, the hematological cancer treated with the drug combination is a CD47+ leukemia, preferably selected from acute lymphocytic leukemia,  
30 acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, and myelodysplastic syndrome, preferably, human acute myeloid leukemia.

**[0096]** In other embodiments, the hematological cancer treated with the drug combination is a CD47+ lymphoma or myeloma selected from Hodgkin’s lymphoma, both

indolent and aggressive non-Hodgkin's lymphoma, diffuse large cell lymphoma (DLBCL), mantle cell lymphoma, T cell lymphoma including mycosis fungoides, Sezary's syndrome, Burkitt's lymphoma, follicular lymphoma (small cell and large cell), multiple myeloma (MM), giant cell myeloma, heavy-chain myeloma, and light chain or Bence-Jones myeloma as well as leiomyosarcoma.

5 [0097] In a specific embodiment, the cancer treated with the present combination is multiple myeloma. In another specific embodiment, the targeted cancer is mantle cell lymphoma. In another specific embodiment, the CD47 blockade drug is SIRP $\alpha$ Fc. In a further specific embodiment the proteasome inhibitor is bortezomib or carfilzomib or  
10 ixazomib.

[0098] In still other embodiments, the proteasome inhibitor is bortezomib in combination with SIRP $\alpha$ Fc, such as SEQ ID No. 9 or SEQ ID No. 10, such as for the treatment of mantle cell lymphoma, multiple myeloma, or diffuse large cell lymphoma.

[0099] Thus, in embodiments, there is provided the use of a CD47 blockade drug  
15 in combination with a proteasome inhibitor for the treatment of a particular CD47+ cancer, wherein:

- i) the CD47 blockade drug is SIRP $\alpha$ Fc of SEQ ID No. 9 and the proteasome inhibitor is bortezomib, such as for the treatment of a cancer that is mantle cell lymphoma or multiple myeloma;
- 20 ii) the CD47 blockade drug is SIRP $\alpha$ Fc of SEQ ID No. 10 and the proteasome inhibitor is bortezomib, such as for the treatment of a cancer that is mantle cell lymphoma or multiple myeloma;
- iii) the CD47 blockade drug is SIRP $\alpha$ Fc of SEQ ID No. 9 and the proteasome inhibitor is carfilzomib, such as for multiple myeloma treatment;
- 25 iv) the CD47 blockade drug is SIRP $\alpha$ Fc of SEQ ID No. 10 and the proteasome inhibitor is carfilzomib, such as for multiple myeloma treatment;
- v) the CD47 blockade drug is SIRP $\alpha$ Fc of SEQ ID No. 9 and the proteasome inhibitor is ixazomib; such as for multiple myeloma treatment; and
- 30 vi) the CD47 blockade drug is SIRP $\alpha$ Fc of SEQ ID No. 10 and the proteasome inhibitor is ixazomib, such as for multiple myeloma treatment.

[00100] It will be appreciated that other CD47 blockade drugs can be used in combination with other proteasome inhibitors, as discussed supra. Desirable combinations will show a statistically significant improvement in cancer cell response. This can be

demonstrated as a statistically significant improvement in proteasome inhibitor activity caused by combination with a CD47 blockade drug, or vice versa, where statistical significance is shown as noted in the examples that follow and desirably, provides a p value  $>0.05$  and more desirably  $>0.01$  such as  $>0.001$ .

5 [00101] The combination therapy, comprising CD47 blockade and proteasome inhibition can also be exploited together with any other agent or modality useful in the treatment of the targeted indication, such as surgery as in adjuvant therapy, or with additional chemotherapy as in neoadjuvant therapy.

[00102] The following non-limiting examples are illustrative of the present  
10 disclosure:

### **Examples**

[00103] To generate the results represented in Figures 1 and 2, heparinized whole blood was obtained from normal healthy human donors (Biological Specialty Corporation) and informed consent was obtained from all donors. Peripheral blood mononuclear cells  
15 (PBMCs) were isolated over Ficoll-Paque Plus density gradient (GE Healthcare) and CD14<sup>+</sup> monocytes were isolated from PBMCs by positive selection using CD14 antibody-coated MicroBead separation (Miltenyi Biotec). Monocytes were differentiated into macrophages by culturing for seven days in X-Vivo-15 media (Lonza) supplemented with M-CSF (PeproTech). 24 hours prior to the phagocytosis assay, macrophages were primed  
20 with IFN- $\gamma$  (PeproTech). 48 hours prior to the phagocytosis assay, bortezomib (1, 5 or 10 nM) or carfilzomib (0.5, 2 10 nM) were added to tumor cells. On the day of the phagocytosis assay, macrophages were co-cultured with violet proliferation dye 450 (VPD450)-labeled human multiple myeloma cell lines (MM1s or H929) in the presence of 1, 5 or 100 nM human SIRP $\alpha$ Fc (V region of human SIRP $\alpha$  variant 2 fused with IgG1 Fc),  
25 100 nM control Fc [human IgG1 Fc region (hinge-CH2-CH3)] for two hours. Phagocytosis was assessed as % VPD450<sup>+</sup> cells of live, single CD14<sup>+</sup>CD11b<sup>+</sup> macrophages by flow cytometry. Results shown in Figures 1 and 2 are representative of two independent experiments.

[00104] To generate the results represented in Figure 3, macrophages were prepared  
30 from human peripheral blood mononuclear cells (PBMCs) obtained from healthy donors (BioreclamationIVT); informed consent was obtained from all donors. CD14<sup>+</sup> monocytes were isolated by positive selection using the EasySep<sup>®</sup> human monocyte isolation kit (Stemcell Technologies). Monocytes were differentiated into macrophages by culturing

the cells in X-VIVO 15 media (Lonza) supplemented with human m-CSF (PeproTech) for 10 days. Macrophages were primed with human IFN $\gamma$  (PeproTech) one day prior to the phagocytosis assay. 48 hours prior to the phagocytosis assay, bortezomib (10 nM), carfilzomib (10 nM) or ixazomib (25 nM) were added to tumor cells. On the day of the phagocytosis assay, tumor cells (MM1.S or SU-DHL-6) were labeled with Violet Proliferation Dye 450 (BD Biosciences) and cultured with IFN $\gamma$ -primed macrophages. Macrophages and tumor cells were co-cultured for 2 hours in the presence of 100 nM SIRP $\alpha$ Fc (V region of human SIRP $\alpha$  variant 2 fused with IgG1 Fc), SIRP $\alpha$ Fc (V region of human SIRP $\alpha$  variant 2 fused with IgG4 Fc), vSIRP $\alpha$ Fc (high affinity CV1 variant of V region of human SIRP $\alpha$  fused with mutated IgG4) [SEQ ID No. 12], CD47 monoclonal antibody B6H12 [SEQ ID Nos. 13 and 14] or Control Fc (wild type human IgG4 with stabilized hinge). Cells were subsequently stained with a viability dye, APC-conjugated anti-human CD14 (61D3, eBioscience), and PE-conjugated anti-human CD11b (ICRF44, eBioscience). Macrophages were identified as live, single, CD14+CD11b+ cells. Doublets were excluded by SSC-W and SSC-H discrimination. Percent phagocytosis was assessed as the percent of macrophages that were VPD450+. Unpaired t-tests comparing the percentage of phagocytosis of untreated vs proteasome inhibitor treated tumor cells were performed (\* P  $\leq$  0.05, \*\* P  $\leq$  0.01, \*\*\* P  $\leq$  0.001).

**[00105]** Results in Figure 3 show that treatment of tumor cells (SU-DHL-6 or MM1.S) with proteasome inhibitors leads to a significant increase in phagocytosis as compared to CD47 blockade alone. The CD47 blockade was achieved by treatment with SIRP $\alpha$ Fc (V region of human SIRP $\alpha$  variant 2 fused with IgG1 Fc), SIRP $\alpha$ Fc (V region of human SIRP $\alpha$  variant 2 fused with IgG4 Fc), vSIRP $\alpha$ Fc (high affinity CV1 variant of V region of human SIRP $\alpha$  fused with mutated IgG4) or CD47 monoclonal antibody (CD47 mAb). In embodiments, the improvement in CD47 blockade drug activity is seen particularly when the CD47 blockade drug is a G1 version of SIRP $\alpha$ Fc or a G4 version of SIRP $\alpha$ Fc, and the proteasome inhibitor is a peptidic boronate such as bortezomib and ixazomib or a peptidic epoxyketone such as carfilzomib.

**[00106]** While the present disclosure has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the disclosure is not limited to the disclosed examples. To the contrary, the disclosure is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

[00107] All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

## WE CLAIM:

1. A use of a combination of a CD47 blockade drug and a proteasome inhibitor for treating a subject with CD47+ disease cells.  
5
2. The use according to claim 1, wherein the proteasome inhibitor is selected from an epoxyketone and a boronate.
3. The use according to claim 2, wherein the proteasome inhibitor is a boronate.  
10
4. The use according to claim 3, wherein the inhibitor is bortezomib.
5. The use according to claim 3, wherein the inhibitor is ixazomib.
- 15 6. The use according to claim 2, wherein the proteasome inhibitor is an epoxyketone.
7. The use according to claim 5, wherein the proteasome inhibitor is carfilzomib.
8. The use according to claim 5, wherein the inhibitor is a fluorinated carfilzomib  
20 analog.
9. The use according to any one of claims 1-8, wherein the CD47 blockade drug comprises a CD47-binding form of human SIRP $\alpha$ .
- 25 10. The use according to claim 9, wherein the CD47-binding form of human SIRP $\alpha$  is a CD47-binding fragment of human SIRP $\alpha$ .
11. The use according to claim 10, wherein the CD47 binding fragment of human SIRP $\alpha$  comprises the V region of human SIRP $\alpha$ .  
30
12. The use according to any one of claims 1-11, wherein the CD47 blockade drug is an Fc fusion protein comprising the V region of human SIRP $\alpha$  variant 2.

13. The use according to claim 10, wherein the Fc fusion protein comprises SEQ ID No. 9.
14. The use according to claim 10, wherein the Fc fusion protein comprises SEQ ID No. 10.
15. The use according to claim 9, wherein the CD47 blockade drug comprises soluble SIRP $\alpha$  having one or more amino acid substitutions selected from L<sup>4</sup>V/I, V<sup>6</sup>I/L, A<sup>21</sup>V, V<sup>27</sup>I/L, <sup>131</sup>T/S/F, E<sup>47</sup>V/L, K<sup>53</sup>R, E<sup>54</sup>Q, H<sup>56</sup>P/R, S<sup>66</sup>T/G, K<sup>68</sup>R, V<sup>92</sup>I, F<sup>94</sup>V/L, V<sup>63</sup>I, and F<sup>103</sup>V.
16. The use according to any one of claims 1-8, wherein the CD47 blockade drug is an anti-CD47 antibody that inhibits binding of human SIRP $\alpha$  to human CD47.
17. The use according to any one of claims 1-16, wherein the CD47+ cells are cancer cells.
18. The use according to claim 17 wherein the cancer cells are blood cancer cells or solid tumour cells.
19. The use according to claim 17, wherein the cancer cells are cells of a cancer type selected from acute lymphocytic leukemia (ALL); acute myeloid leukemia (AML); chronic lymphocytic leukemia (CLL); chronic myelogenous leukemia (CML); myeloproliferative disorder/neoplasm (MPDS); and myelodysplastic syndrome.
20. The use according to claim 19, wherein the cancer is selected from Hodgkin's lymphoma, mantle cell lymphoma, T cell lymphoma, Sezary's syndrome, mycosis fungoides, both indolent and aggressive non-Hodgkin's lymphoma, Burkitt's lymphoma, and follicular lymphoma (small cell and large cell).
21. The use according to claim 20, wherein the cancer is selected from multiple myeloma (MM), giant cell myeloma, heavy-chain myeloma, and light chain or Bence-Jones myeloma.

22. The use according to claim 19, wherein the cancer is multiple myeloma (MM).
23. The use according to any one of claims 1-21, wherein the CD47 blockade drug is  
5 for use in a recipient of a proteasome inhibitor.
24. The use according to any one of claims 1-21, wherein the proteasome inhibitor is  
for use in a recipient of a CD47 blockade drug.
- 10 25. A combination comprising an effective amount of a CD47 blockade drug and an  
effective amount of a proteasome inhibitor.
26. The combination according to claim 25, wherein the proteasome inhibitor is  
selected from an epoxyketone and a boronate.
- 15 27. The combination according to claim 25, wherein the proteasome inhibitor is a  
boronate.
28. The combination according to claim 27, wherein the inhibitor is bortezomib.
- 20 29. The combination according to claim 27, wherein the inhibitor is ixazomib.
30. The combination according to claim 26, wherein the proteasome inhibitor is an  
epoxyketone.
- 25 31. The combination according to claim 29, wherein the proteasome inhibitor is  
carfilzomib.
32. The combination according to claim 29, wherein the inhibitor is a fluorinated  
30 carfilzomib analog.
33. The combination according to any one of claims 26-32, wherein the CD47  
blockade drug comprises a CD47-binding form of human SIRP $\alpha$ .

34. The combination according to claim 33, wherein the CD47-binding form of human SIRP $\alpha$  is a CD47-binding fragment of human SIRP $\alpha$ .

5 35. The combination according to claim 34, wherein the CD47 binding fragment of human SIRP $\alpha$  comprises the V region of human SIRP $\alpha$ .

36. The combination according to any one of claims 26-35, wherein the CD47 blockade drug is an Fc fusion protein comprising the V region of human SIRP $\alpha$  variant 2.

10

37. The combination according to claim 34, wherein the Fc fusion protein comprises SEQ ID No. 9.

15 38. The combination according to claim 34, wherein the Fc fusion protein comprises SEQ ID No. 10.

39. The combination according to claim 33, wherein the CD47 blockade drug comprises soluble SIRP $\alpha$  having one or more amino acid substitutions selected from L<sup>4</sup>V/I, V<sup>6</sup>I/L, A<sup>21</sup>V, V<sup>27</sup>I/L, <sup>131</sup>T/S/F, E<sup>47</sup>V/L, K<sup>53</sup>R, E<sup>54</sup>Q, H<sup>56</sup>P/R, S<sup>66</sup>T/G, K<sup>68</sup>R, V<sup>92</sup>I,  
20 F<sup>94</sup>V/L, V<sup>63</sup>I, and F<sup>103</sup>V.

40. The combination according to any one of claims 26-32, wherein the CD47 blockade drug is an anti-CD47 antibody that inhibits binding of human SIRP $\alpha$  to human CD47.

25

41. A kit comprising a combination of any one of claims 25 to 40 together with instructions for the use thereof to treat CD47+ disease cells.

30 42. The kit according to claim 41, wherein the CD47+ disease cells are CD47+ cancer cells.

43. The kit according to claim 42 wherein the CD47+ cancer cells are blood cancer cells.

44. The kit according to claim 43, wherein the cancer cells are cells of a cancer type selected from acute lymphocytic leukemia (ALL); acute myeloid leukemia (AML); chronic lymphocytic leukemia (CLL); chronic myelogenous leukemia (CML);  
5 myeloproliferative disorder/neoplasm (MPDS); myelodysplastic syndrome; giant cell myeloma, heavy-chain myeloma, light chain myeloma and Bence-Jones myeloma.

45. The kit according to claim 43, wherein the cancer is a lymphoma selected from a T cell lymphoma, Hodgkin's lymphoma, Sezary syndrome, mycosis fungoides, non-  
10 Hodgkin's lymphoma, indolent non-Hodgkin's lymphoma, aggressive non-Hodgkin's lymphoma, Burkitt's lymphoma, small cell follicular lymphoma, cutaneous T cell lymphoma (CTCL) and large cell follicular lymphoma.

46. The kit according to any one of claims 40-45, wherein the CD47 blockade drug  
15 enhances the anti-cancer effect of the proteasome inhibitor.

47. The kit according to any one of claims 40-45, wherein the proteasome inhibitor enhances the anti-cancer effect of the CD47 blockade drug.

Figure 1

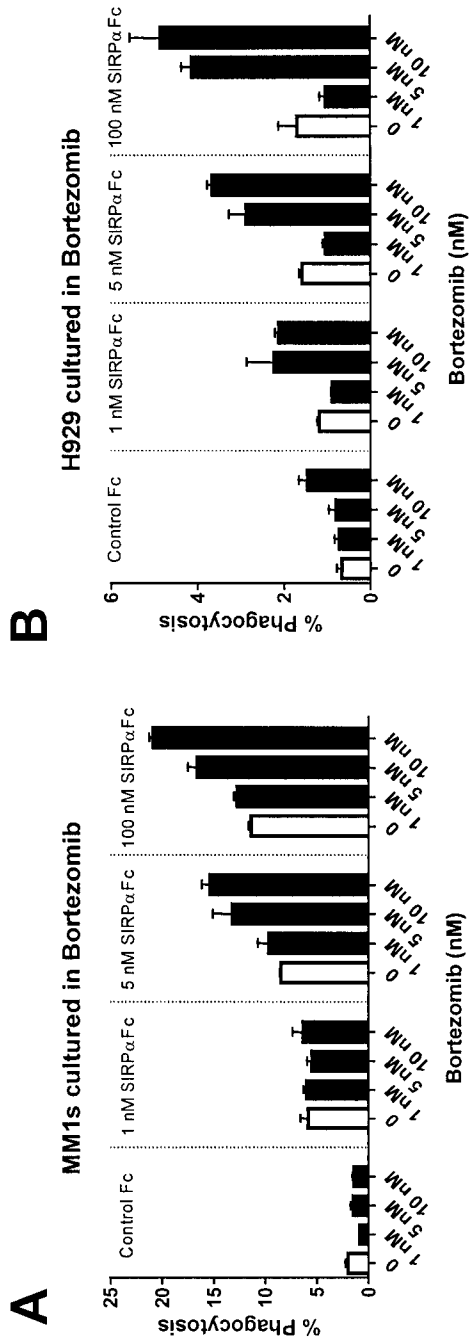


Figure 2

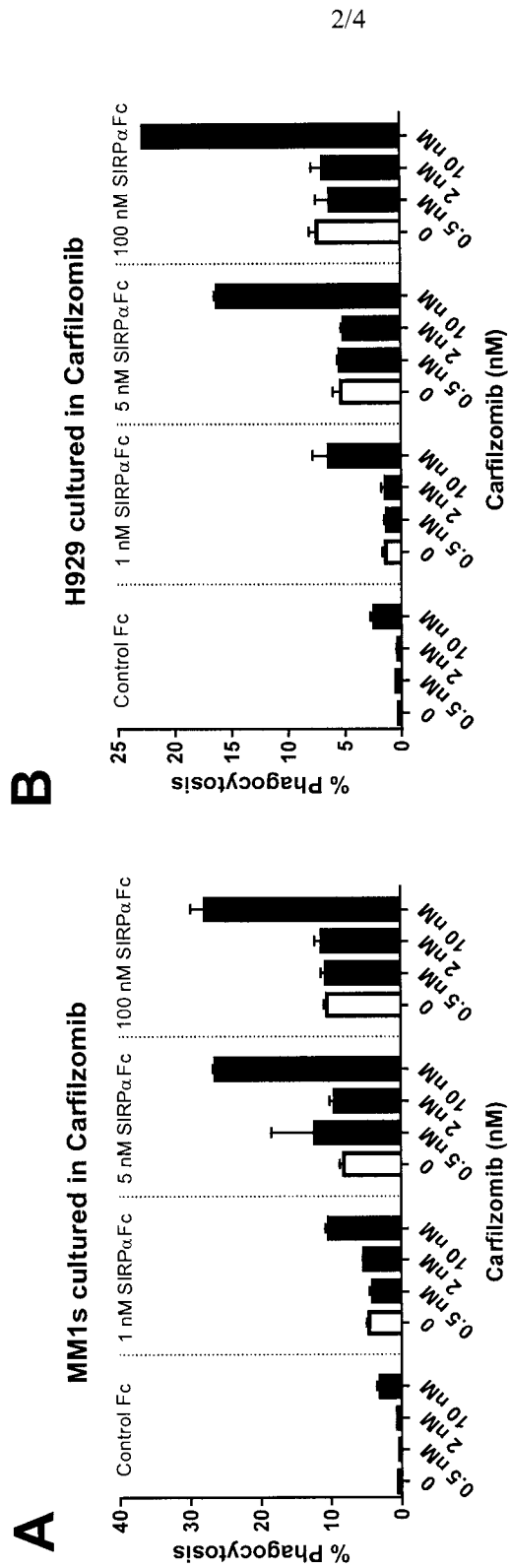


Figure 3

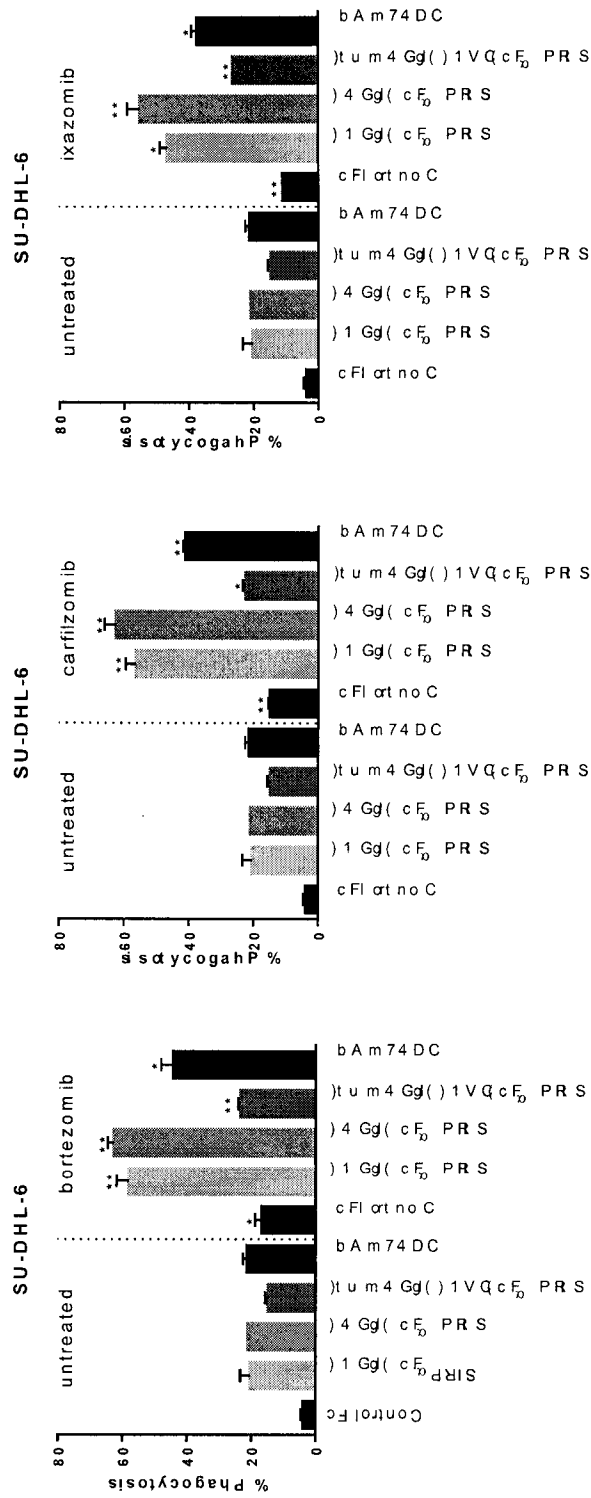
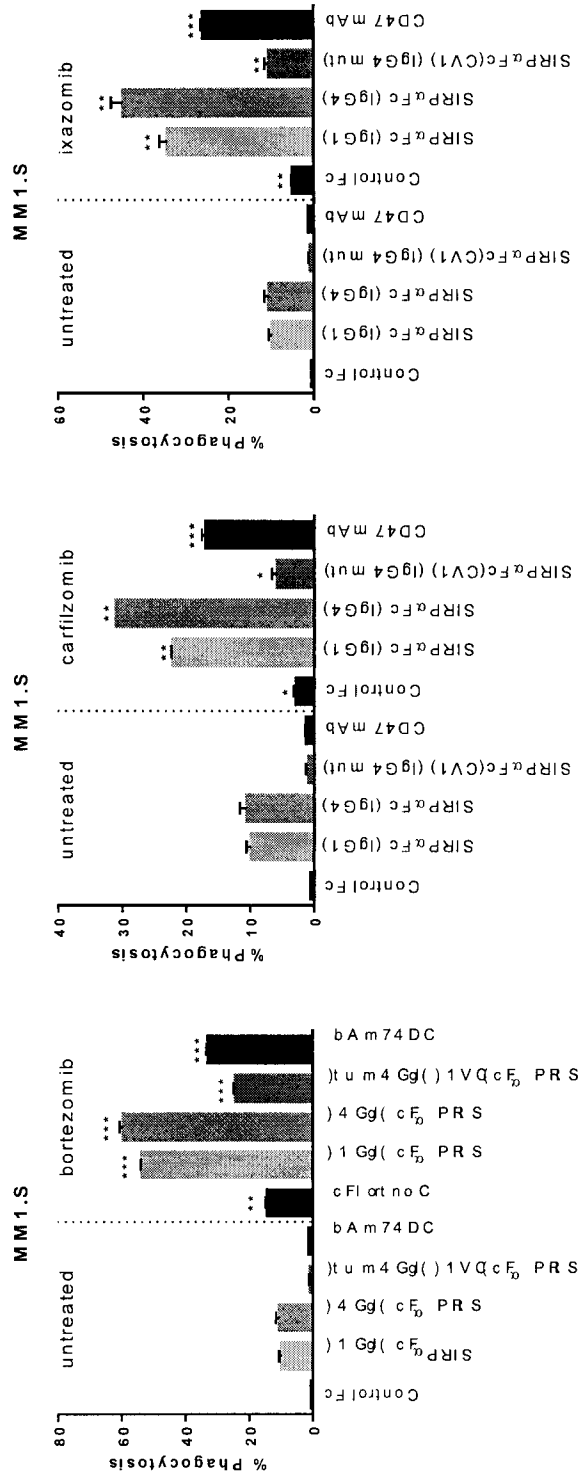


Figure 3 Cont.



## INTERNATIONAL SEARCH REPORT

International application No.

**PCT/CA2017/051300**

<p>A. CLASSIFICATION OF SUBJECT MATTER  IPC: <b>A61K 47/68</b> (2017.01), <b>A61K 31/69</b> (2006.01), <b>A61K 38/05</b> (2006.01), <b>A61K 38/07</b> (2006.01),  <b>A61K 38/17</b> (2006.01), <b>A61P 35/00</b> (2006.01) (more IPCs on the last page)</p> <p>According to International Patent Classification (IPC) or to both national classification and IPC</p>														
<p>B. FIELDS SEARCHED</p> <p>Minimum documentation searched (classification system followed by classification symbols)  keywords used across the whole IPC</p> <p>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched</p> <p>Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)  Databases: Google, Medline CaPlus, Biosis, STN  Keywords: CD47 blockade drug, anti-CD47 antibody, SIRP-alpha Fc proteosome inhibitor, Combination/Synergistic treatment of CD47+ disease, cancer</p>														
<p>C. DOCUMENTS CONSIDERED TO BE RELEVANT</p> <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>Y</td> <td>WO 2016/054555 A2 (CAO, Z. A. et al.) 7 April 2016 (07-04-2016)</td> <td>1-47</td> </tr> <tr> <td>Y</td> <td>WO 2014/094122 A1 (UGER, R. A. et al.) 26 June 2014 (26-06-2014)</td> <td>1-47</td> </tr> <tr> <td>X, P</td> <td>US 9650441 B2 (GROSVELD, F. et al.) 16 May 2017 (16-05-2017)</td> <td>1-47</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	Y	WO 2016/054555 A2 (CAO, Z. A. et al.) 7 April 2016 (07-04-2016)	1-47	Y	WO 2014/094122 A1 (UGER, R. A. et al.) 26 June 2014 (26-06-2014)	1-47	X, P	US 9650441 B2 (GROSVELD, F. et al.) 16 May 2017 (16-05-2017)	1-47
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Y	WO 2016/054555 A2 (CAO, Z. A. et al.) 7 April 2016 (07-04-2016)	1-47												
Y	WO 2014/094122 A1 (UGER, R. A. et al.) 26 June 2014 (26-06-2014)	1-47												
X, P	US 9650441 B2 (GROSVELD, F. et al.) 16 May 2017 (16-05-2017)	1-47												
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.														
<p>* Special categories of cited documents:</p> <p>“A” document defining the general state of the art which is not considered to be of particular relevance</p> <p>“E” earlier application or patent but published on or after the international filing date</p> <p>“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)</p> <p>“O” document referring to an oral disclosure, use, exhibition or other means</p> <p>“P” document published prior to the international filing date but later than the priority date claimed</p>	<p>“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</p> <p>“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</p> <p>“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</p> <p>“&amp;” document member of the same patent family</p>													
<p>Date of the actual completion of the international search  02 January 2018 (02-01-2018)</p>		<p>Date of mailing of the international search report  30 January 2018 (30-01-2018)</p>												
<p>Name and mailing address of the ISA/CA  Canadian Intellectual Property Office  Place du Portage I, C114 - 1st Floor, Box PCT  50 Victoria Street  Gatineau, Quebec K1A 0C9  Facsimile No.: 819-953-2476</p>		<p>Authorized officer    Damiano Conte (819) 639-7784</p>												

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

- a.  forming part of the international application as filed:
- in the form of an Annex C/ST.25 text file.
  - on paper or in the form of an image file.
- b.  furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c.  furnished subsequent to the international filing date for the purposes of international search only:
- in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
  - on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2.  In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.  
**PCT/CA2017/051300**

Patent Document Cited in Search Report	Publication Date	Patent Family Members	Publication Date
WO2014094122	2014-06-26	CA2894245A1	2014-06-26
		AU2013362789A1	2015-07-02
		EP2931752A1	2015-10-21
		CN105073780A	2015-11-18
		US2015329616A1	2015-11-19
		JP2016501896A	2016-01-21
		IN5935/DELNP/2015A	2016-01-29
		HK1217716A1	2017-01-20
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		IN201717015513A	2017-09-15
		JP2017535528A	2017-11-30
		US 9650441 B2	2017-05-16
WO2017053423	2017-03-30		
US9650441	2017-05-16		
US2017204181	2017-07-20		
US9803016	2017-10-31		