a key “don’t eat-me” signal that can turn off engulfment mediated by multiple modalities, including apoptotic cell clearance and FcR mediated phagocytosis. Blocking the CD47 mediated engagement of SIRPα on a phagocyte, or the loss of CD47 expression in knockout mice, can cause removal of live cells and non-aged erythrocytes. Alternatively, blocking SIRPα recognition also allows engulfment of targets that are not normally phagocytosed. Anti-CD47 antibody treatment has also been shown to not only enable macrophage phagocytosis of cancer, but can also initiate an anti-tumor cytotoxic T cell immune response.

[08] Related publications include "Engineered Sirp alpha Variants As Immunotherapeutic Adjuvants To Anticancer Antibodies." Science 341(6141): 88-91; Willingham, S. B., J. P. Volkmer, Et Al. (2012). "The Cd47-Signal Regulatory Protein Alpha (Sirpa) Interaction Is A Therapeutic Target For Human Solid Tumors." Proc Natl Acad Sci U S A 109(17): 6662-6667. Chao, M. P., A. A. Alizadeh, Et Al. (2010). "Anti-Cd47 Antibody Synergizes With Rituximab To Promote Phagocytosis And Eradicate Non-Hodgkin Lymphoma." Cell **142**(5): 699-713.

SUMMARY

[09] Methods are provided for targeting cells for depletion, including without limitation tumor cells, in a regimen comprising contacting the target and effector cells with a combination of two or more agents that modulate immunoregulatory signaling. In some embodiments the contacting is performed in vivo. In some embodiments the contacting is performed in vitro, e.g. to prime immune effector cells, followed by administration to a subject. In some embodiments, the combination of agents provides a synergistic effect relative to the administration of an agent as a monotherapy. In various embodiments, the combination of immunoregulatory agents is administered in a therapeutic regimen that includes conventional treatment, e.g. targeted anti-tumor antibodies, chemotherapy, radiation therapy, surgery, and the like.

[10] A benefit of the present disclosure can be the use of lowered doses of the agents relative to the dose required as a single immunoregulatory agent, or a combination of immunoregulatory agents in the absence of CD47 blockade. A benefit of the present disclosure can also, or alternatively, be a decrease in the length of time required for treatment, relative to the length of time required for treatment as a single immunoregulatory agent, or a combination of immunoregulatory agents in the absence of CD47 blockade. A benefit of the present disclosure can also, or alternatively, be an enhanced response relative to the response observed after treatment with a single immunoregulatory agent, or a combination of immunoregulatory agents in the absence of CD47 blockade.

[11] Immunoregulatory modulating agents include (i) an agent that blockades CD47 activity; and (ii) one or more of an agent that agonizes an immune costimulatory molecule, e.g. CD40, OX40, etc.; and/or (iii) an agent that antagonizes an immune inhibitory molecule, e.g. CTLA-4, PD1, PDL1, *etc*. The active agents are administered within a period of time to produce an additive or synergistic effect on depletion of cancer cells in the host. Administration may be Methods of administration include, without limitation, systemic administration, intra-tumoral administration, etc. Usually the active agent (i) is administered within about a period of about 45 days, about 30 days, about 21 days, about 14 days, about 10 days, about 8 days, about 7 days, about 6 days, about 5 days, about 4 days, about 3 days, about 2 days, about 1 day or substantially the same day as an agent (ii) and/or (iii). In some embodiments an agent (i) is administered prior to an agent (ii) and/or (iii). In some embodiments an agent (i) is administered after an agent (ii) and/or (iii). The agents can be considered to be combined if administration scheduling is such that the serum level of both agents is at a therapeutic level at the same time. Administration may be repeated as necessary for depletion of the cancer cell population.

[11a] In one aspect of the present disclosure, there is provided a use of a combination of (i) an agent that blockades CD47 activity; and (ii) one or more of an agent that agonizes an immune costimulatory molecule and/or (iii) an agent that antagonizes an immune inhibitory molecule, in the manufacture of a medicament for targeting cancer cells for immunodepletion, wherein a population of cells comprising the targeted cells is to be contacted with the medicament, and wherein said combination is in a dose effective to increase depletion of the targeted cells.

[12] In some embodiments, an individual cancer is selected for treatment with a combination therapy of the present disclosure because the cancer is a cancer type that is responsive to a checkpoint inhibitor, e.g. a PD1 antagonist, a PDL1 antagonist, a CTLA4 antagonist, a TIM-3 antagonist, a BTLA antagonist, a VISTA antagonist, a LAG3 antagonist; etc. In some embodiments, such an immunoregulatory agent is a CTLA-4, PD1 or PDL1 antagonist, e.g. avelumab, nivolumab, pembrolizumab, ipilimumab, and the like. In some such embodiments the cancer is, without limitation, melanoma or small cell lung cancer. In some such embodiments, the cancer is a type that has a high neoantigen, or mutagenesis, burden (see Vogelstein et al. (2013) Science 339(6127):1546-1558, herein specifically incorporated by reference). In other embodiments, the cancer with a type with a low neoantigen burden. In some such embodiments, the combination therapy of the present disclosure enhances the activity of the checkpoint inhibitor. In other embodiments, where the individual cancer does not respond to a checkpoint inhibitor alone, the combination therapy provides a therapeutic response.

[13] In some embodiments, an individual cancer is selected for treatment with a combination therapy of the present disclosure because the cancer is a cancer type that is responsive to an immune response agonist, e.g. a CD28 agonist, an OX40 agonist; a GITR agonist, a CD137 agonist, a CD27 agonist, an HVEM agonist, etc. In some embodiments, such an immunoregulatory agent is an OX40, CD137, or GITR agonist e.g. tremelimumab, and the like. In some such embodiments the cancer is, without limitation, melanoma or small cell lung cancer. In some such embodiments, the cancer is a type that has a high neoantigen, or

mutagenesis, burden. In other embodiments, the cancer with a type with a low neoantigen burden. In some such embodiments, the combination therapy of the present disclosure enhances the activity of the agonist. In other embodiments, where the individual cancer does not respond to a agonist alone, the combination therapy provides a therapeutic response.

[14] In some embodiments, an individual cancer is selected for treatment with a combination therapy of the present disclosure because the cancer is a cancer type that is responsive to a chemokine receptor antagonist, e.g. CCR2, CCR4, etc. In some such embodiments the cancer is, without limitation, a lymphoma, including without limitation T cell lymphoma, e.g. cutaneous T cell lymphoma. In some such embodiments, the cancer is a type that has a high neoantigen, or mutagenesis, burden. In other embodiments, the cancer with a type with a low neoantigen burden. In some such embodiments, the combination therapy of the present disclosure enhances the activity of the anti-chemokine receptor antagonist. In other embodiments, where the individual cancer does not respond to the antagonist alone, the combination therapy provides a therapeutic response.

[15] The methods of the present disclosure may further comprise administration of an agent that specifically binds to a target cell or an inhibitory immune cell, e.g. an antibody or biologically active fragment or derivative thereof. The level of depletion of the targeted cell, e.g. a cancer cell, an inhibitory immune cell including without limitation regulatory T cells. A number of antibodies are currently in clinical use for the treatment of cancer, and others are in varying stages of clinical development. Antibodies of interest for the methods of the present disclosure may act through ADCC, and may be selective for tumor cells, although one of skill in the art will recognize that some clinically useful antibodies do act on non-tumor cells, e.g. CD20, etc.

[16] In some embodiments a primer agent is administered prior to administering a therapeutically effective dose of an anti-CD47 agent to the individual. Suitable primer agents include an erythropoiesis-stimulating agent (ESA), and/or a priming dose of an anti-CD47 agent. Following administration of the priming agent, and allowing a period of time effective for an increase in reticulocyte production, a therapeutic dose of an anti-CD47 agent is administered. The therapeutic dose can be administered in number of different ways. In some embodiments, two or more therapeutically effective doses are administered after a primer agent is administered. In some embodiments a therapeutically effective dose of an anti-CD47 agent is administered as two or more doses of escalating concentration, in others the doses are equivalent.

[17] In some embodiments, administration of a combination of agents of the present disclosure is 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® (darbepoetin alfa), Epogen®NF/Procrit®NF (epoetin alfa), Omontys® (peginesatide), Procrit®, etc.

[18] An anti-CD47 agent for use in the methods of the present disclosure interferes with binding between CD47 present on the cancer cell and SIRPα present on a phagocytic cell. Such methods increase phagocytosis of the cancer cell. Suitable anti-CD47 agents include soluble SIRPα polypeptides; soluble CD47; anti-CD47 antibodies, anti-SIRPα antibodies, and the like, where the term antibodies encompasses antibody fragments and variants thereof, as known in the art. In some embodiments the anti-CD47 agent is an anti-CD47 antibody. In some embodiments the anti-CD47 antibody is a non-hemolytic antibody. In some embodiments the antibody comprises a human IgG4 Fc region.

BRIEF DESCRIPTION OF THE FIGURES

[19] Figure 1. Effect of combined CD40 agonist and CD47 antagonist.

[20] Figure 2. (A-C) Effect of combined anti-CTLA-4 and CD47 antagonist.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[21] Methods are provided for the targeted depletion of cancer cells in a subject, where the cancer cells are selectively ablated by immune cell responses to the tumor cells, following contacting with a combination of agents that (i) block CD47 signaling; and (ii) one or more of an agent that agonizes an immune costimulatory molecule, e.g. CD40, OX40, CD28, GITR, CD137, CD27, HVEM, etc.; and/or (iii) an agent that antagonizes an immune inhibitory molecule, e.g. CTLA-4, PD1, PDL1, TIM-3, BTLA, VISTA, LAG-3, *etc*.

[22] To facilitate an understanding of the invention, a number of terms are defined below.

[23] Before the present active agents and methods are described, it is to be understood that this invention is not limited to the particular methodology, products, apparatus and factors described, as such methods, apparatus and formulations may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intened to limit the scope of the present invention which will be limited only by appended claims.

[24] It must be noted that as used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a drug candidate" refers to one or mixtures of such candidates, and reference to "the method" includes reference to equivalent steps and methods known to those skilled in the art, and so forth.

What is Claimed is:

1. Use of a combination of (i) an agent that blockades CD47 activity; and (ii) one or more of an agent that agonizes an immune costimulatory molecule and/or (iii) an agent that antagonizes an immune inhibitory molecule, in the manufacture of a medicament for targeting cancer cells for immunodepletion, wherein a population of cells comprising the targeted cells is to be contacted with the medicament, and wherein said combination is in a dose effective to increase depletion of the targeted cells.

2. The use of claim 1, wherein the combination comprises an antibody that agonizes an immune costimulatory molecule.

3. The use of claim 2, wherein the antibody is an agonist of CD40.

4. The use of claim 2 wherein the antibody is an agonist of OX40.

5. The use of claim 1, wherein the combination comprises an antibody that antagonizes an immune inhibitory molecule.

6. The use of claim 5, wherein the antibody is an antagonist of CTLA4.

7. The use of claim 5, wherein the antibody is an antagonist of PD1 and/or PDL1.

8. The use of claim 5, wherein the antibody is an antagonist of TIM3.

9. The use of claim 5, wherein the antibody is an antagonist of LAG3.

10. The use of any one of claims 1-9, wherein the combination further comprises an antibody that binds to an antigen on the targeted tumor cell.

11. The use of any of claims 1-10, wherein the contacting is performed in vitro.

12. The use of any of claims 1-10, wherein the contacting is to be performed on an individual mammal in vivo.

13. The use of claim 12, wherein the treatment provides for increased overall survival of the individual.

14. The use of claim 13, wherein depletion of the target cells is enhanced relative to the depletion observed with a monotherapy of any single agent administered as a monotherapy.

15. The use of any of claims 1-14, wherein the agent that agent that blockades CD47 activity is an anti-CD47 antibody.

16. The use of claim 15, wherein the anti-CD47 antibody comprises an IgG4 Fc region.

17. The use of claim 16 wherein the antibody is 5F9-G4.

18. The use according to any of claims 1-17, wherein said mammal is a mouse.

19. The use according to any of claims 1-17, wherein said mammal is a human.

20. The use of claim 12, wherein a priming dose of the agent that blockades CD47 activity is to be further administered.

21. The use of claim 12, wherein a priming dose of an erythropoietin stimulating agent is to be further administered.

22. The use of any of claims 1-21, wherein the combination of an agent that (i) an agent that blockades CD47 activity; and (ii) one or more of an agent that agonizes an immune costimulatory molecule and/or (iii) an agent that antagonizes an immune inhibitory molecule, in a dose effective to increase depletion of the targeted cells, provides for a therapeutically effective dose of agents with a clinically significant reduction in immune-related adverse events relative to the dosing required for efficacy in the absence of the anti-CD47 agent.