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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

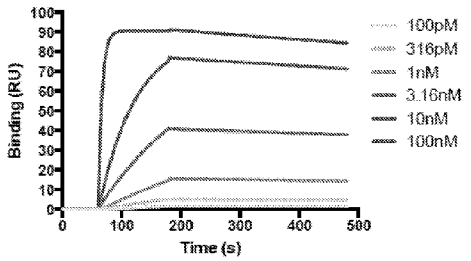
[Continued on next page]

(54) Title: SIRP POLYPEPTIDE COMPOSITIONS AND METHODS OF USE

FIG. 2

Surface plasmon resonance-based measurement of binding affinity and kinetics of HAC-GV3, a fusion of the SIRP-gamma variant GV3 with a PD-1 variant HAC, for human CD47.

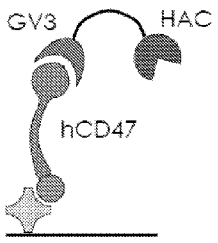
FIG. 2A



KD = 160 pM

$T_{1/2}$ = ~40 min

FIG. 2B



(57) Abstract: Provided herein are SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptides for immunotherapy and/or treatment of cancer, anemia, transplant, asthma, allergy, auto-immune disease, and viral infection.

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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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SIRP POLYPEPTIDE COMPOSITIONS AND METHODS OF USE

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/266,450, filed December 11, 2015 and U.S. Provisional Application No. 62/163,282, filed May 18, 2015, both of which applications are incorporated herein by reference in their entirety.

SEQUENCE LISTING

[0001.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 17, 2016, is named 47815_701_601_SL.txt and is 66,023 bytes in size.

BACKGROUND OF THE INVENTION

[0002] Signal regulatory proteins (SIRPs) constitute a family of cell surface glycoproteins which are expressed on myeloid cells (including macrophages, granulocytes, myeloid dendritic cells, and mast cells), lymphocytes, and neuronal cells and regulate their activity.

SUMMARY OF THE INVENTION

[0003] SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptides and analogs thereof are provided which may be referred within as decoy polypeptides. In one embodiment there is provided a decoy polypeptide comprising a SIRP-gamma, a SIRP-beta or a SIRP-beta2 polypeptide, wherein the polypeptide comprises at least one amino acid modification to increase affinity of the decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide binding to CD47 as compared to the affinity for CD47 of the corresponding wild type SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide. In particular aspects, the polypeptide comprises a SIRP-gamma polypeptide with the sequence EEELQMIQPEKLLLVTVGKTATLHCTVTSLLPVGPVLWFRGVGPGRELIYNQKEGHFPR VTTVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 1). In additional aspects, the SIRP-gamma polypeptide is 90% identical to a wildtype SIRP-gamma polypeptide. In further aspects, the SIRP-gamma polypeptide has an amino acid substitution at M6, V27, L30, L31, V33, V36, L37, V42, E47, Q52, K53, E54, H56, L66, T67, V92, S98 or N101. In additional aspects, the SIRP-gamma polypeptide has a substitution at M6 wherein the substitution is I, L or F. In further aspects, the SIRP-gamma polypeptide has a substitution at V27 wherein the substitution is F, I or L. In additional aspects, the SIRP-gamma polypeptide has a substitution at L30 wherein the substitution is I, V, H, N or D. In further aspects, the SIRP-gamma polypeptide has a substitution at L31 wherein the

substitution is F, I or V. In additional aspects, the SIRP-gamma polypeptide has a substitution at V33 wherein the substitution is I, L, P, T or A. In further aspects, the SIRP-gamma polypeptide has a substitution at V36 wherein the substitution is I. In further aspects, the SIRP-gamma polypeptide has a substitution at L37 wherein the substitution is Q. In further aspects, the SIRP-gamma polypeptide has a substitution at V42 wherein the substitution is A. In additional aspects, the SIRP-gamma polypeptide has a substitution at E47 wherein the substitution is V. In further aspects, the SIRP-gamma polypeptide has a substitution at Q52 wherein the substitution is P, L, V, A or E. In further aspects, the SIRP-gamma polypeptide has a substitution at K53 wherein the substitution is R. In additional aspects, the SIRP-gamma polypeptide has a substitution at E54 wherein the substitution is D, K, N, Q or H. In further aspects, the SIRP-gamma polypeptide has a substitution at H56 wherein the substitution is P or R. In additional aspects, the SIRP-gamma polypeptide has a substitution at L66 wherein the substitution is I, V, P, T, A, R, S or G. In further aspects, the SIRP-gamma polypeptide has a substitution at T67 wherein the substitution is I, N, F, S, Y, V, A or D. In additional aspects, the SIRP-gamma polypeptide has a substitution at V92 wherein the substitution is I. In further aspects, the SIRP-gamma polypeptide has a substitution at S98 wherein the substitution is R, N, K, T, I or M. In additional aspects, the SIRP-gamma polypeptide is substituted at N101 wherein the substitution is K, D, E, H or Q. In further aspects, SIRP-gamma polypeptide has the sequence EEELQX$_1$IQPEKLLLVTVGKTATLHCTX$_2$TSX$_3$X$_4$PX$_5$GPX$_6$X$_7$WFRGX$_8$GPGRX$_9$LIYNX$_{10}$X$_{11}$X$_{12}$GX$_{13}$FPRVTTVSDX$_{14}$X$_{15}$KRNNMDFSIRISSITPADVGTYYCX$_{16}$KFRKGX$_{17}$PEX$_{18}$VEFKSGPGTEMALGAKPS (SEQ ID NO: 2), wherein X$_1$ is M, I, L or F; X$_2$ is F, I, L or V; X$_3$ is L, I, V, H, N or D; X$_4$ is F, I, L or V; X$_5$ is V, I, L, P, T or A; X$_6$ is V or I; X$_7$ is L or Q; X$_8$ is V or A; X$_9$ is E or V; X$_{10}$ is Q, P, L, V, A or E; X$_{11}$ is K or R; X$_{12}$ is E, D, K, N, Q or H; X$_{13}$ is H, P or R; X$_{14}$ is L, I, V, P, T, A, R, S or G; X$_{15}$ is T, I, N, F, S, Y, V, A or D; X$_{16}$ is V or I; X$_{17}$ is S, R, N, K, T, I or M; and X$_{18}$ is N, K, D, E, H or Q. In additional aspects, the SIRP-gamma polypeptide has the sequence EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPVLWFRGVGPGRVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRISSITPADVGTYYCIKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 3). In additional aspects, the SIRP-gamma polypeptide has the sequence EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQRDGPFPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 4). In additional aspects, the SIRP-gamma polypeptide has the sequence EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGPFPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 5). In additional aspects, the SIRP-gamma polypeptide has the sequence

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 6). In further aspects, the SIRP-gamma polypeptide has the sequence
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGTPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 7). In additional aspects, the SIRP-gamma polypeptide has one of the following sequences:
HLib1:
EEELQIIQPEKLLLVTVGKTATLHCTITSHFPVGPIQWFRGVGPGRVLIYNQKDGHFPRV
TTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 8);
HLib2:
EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 9);
HLib3:
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPVLWFRGVGPGRVLIYNQRQGPFPRVT
TVSDTTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 10);
HLib4:
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRELIYNAREGRFPRVT
TVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 11);
HMLib1:
EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRQGPFPRVT
TVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFRKGSPDDVEFKSGAGTELSVRAKPS
(SEQ ID NO: 12);
HMLib2:
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPVLWFRGVGPGRVLIYNQRQGPFPRVT
TVSDTTKRNNMDFSIRISSITPADVGTYYCIKFRKGSPENVEFKSGPGTEMALGAKPS
(SEQ ID NO: 3);
HMLib3:
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQREGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 13);

HMLib4:

EEELQMIQPEKLLLVTVGKTATLHCTVTSLLPVGPVLWFRGVGPGRELIYNQKEGHFPR VTTVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 42);

HMLib5:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 14);

HMLib6:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTXWH (SEQ ID NO: 15), wherein X is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, or V;

HMLib7:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQKDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGTPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 16);

MLib1:

EEELQIIQPEKLLLVTVGKTATLHCTITSLLPVGPIQWFRGVGPGRELIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 17);

MLib2:

EEELQIIQPEKLLLVTVGKTATLHCTLTSLLPVGPILWFRGVGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGNPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 18);

MLib3:

EEELQLIQPEKLLLVTVGKTATLHCTITSLFPPGPIQWFRGVGPGRVLIYNQKDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGIPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 19);

MLib4:

EEELQIIQPEKLLLVTVGKTATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 20);

MLib5:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPIGPILWFRGVGPGRVLIYNQKDGPFPRVTT
VSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 21);
MLib6:
EEELQMIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRV
TTVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 22);
MLib7:
EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 23);
MLib8:
EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQKDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGTPEDVEFKSGPGTEMALXAKPS
(SEQ ID NO: 24); or
GV1.2:
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQREGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 13).

[0004] In additional embodiments, the decoy polypeptide comprises a SIRP-beta polypeptide with the sequence
EDELQVIQPEKSVSVAAGESATLRCAMTSLIPVGPIMWFRGAGAGRELIYNQKEGHFPR
VTTVSELTKRNNLDFSISISNITPADAGTYYCVKFRKGSPDDVEFKSGAGTELSVRAKPS
(SEQ ID NO: 25). In particular aspects, the SIRP-beta polypeptide is at least 90% identical to a wildtype SIRP-beta polypeptide. In further aspects, the SIRP-beta polypeptide has an amino acid substitution at I6, I27, F31, Q37, V47, R53, Q54, P56, T66 or I92. In additional aspects, the SIRP-beta polypeptide has a substitution at V6 wherein the substitution is I. In further aspects, the SIRP-beta polypeptide has a substitution at M27 wherein the substitution is I. In further aspects, the SIRP-beta polypeptide has a substitution at I31 wherein the substitution is F. In additional aspects, the SIRP-beta polypeptide has a substitution at M37 wherein the substitution is Q. In further aspects, the SIRP-beta polypeptide has a substitution at E47 wherein the substitution is V. In additional aspects, the SIRP-beta polypeptide has a substitution at K53 wherein the substitution is R. In further aspects, the SIRP-beta polypeptide has a substitution at E54 wherein the substitution is Q. In additional aspects, the SIRP-beta polypeptide has a substitution at H56 wherein the substitution is P. In further aspects, the SIRP-beta polypeptide

has a substitution at L66 wherein the substitution is T. In additional aspects, the SIRP-beta polypeptide has a substitution at V92 wherein the substitution is I. In further aspects, the SIRP-beta polypeptide has the sequence EDELQIIQPEKSVSVAAGESATLRCAITSLFPVGPIQWFRGAGAGRVLIYNQRQGPFPRVT TVSETTKRNNLDFSISISNITPADAGTYYCIKFRKGSPDDVEFKSGAGTELSVRAKPS (SEQ ID NO: 26). In further aspects, the SIRP-beta polypeptide has the sequence EDELQX_1IQPEKSVSVAAGESATLRCAX_2TSLX_3PVGPIX_4WFRGAGAGRX_5LIYNQX_6X_7G X_8FPRVTTVSEX_9TKRNNLDFSISISNITPADAGTYYCX_{10}KFRKGSPDDVEFKSGAGTELS VRAKPS (SEQ ID NO: 45) wherein X_1 is V or I; X_2 is M or I; X_3 is I or F; X_4 is M or Q; X_5 is E or V; X_6 is K or R; X_7 is E or Q; X_8 is H or P; X_9 is L or T; and X_{10} is V or I.

[0005] In additional embodiments, the decoy comprises a SIRP-beta2 polypeptide with the sequence EEELQVIQPDKSISVAAGESATLHCTVTSLIPVGPIQWFRGAGPGRELIYNQKEGHFPRVT TVSDLTKRNNMDFSIRISNITPADAGTYYCVKFRKGSPDHVEFKSGAGTELSVRAKPS (SEQ ID NO: 27). In particular aspects, the SIRP-beta2 polypeptide is at least 90% identical to a wildtype SIRP-beta2 polypeptide. In additional aspects, the SIRP-beta2 polypeptide has an amino acid substitution at I6, I27, F31, V47, R53, Q54, P56, T66, I92 or D101. In additional aspects, the SIRP-beta2 polypeptide is substituted at V6 wherein the substitution is I. In further aspects, the SIRP-beta2 polypeptide is substituted at V27 wherein the substitution is I. In additional aspects, the SIRP-beta2 polypeptide is substituted at I31 wherein the substitution is F. In further aspects, the SIRP-beta2 polypeptide is substituted at E47 wherein the substitution is V. In additional aspects, the SIRP-beta2 polypeptide is substituted at K53 wherein the substitution is R. In further aspects, the SIRP-beta2 polypeptide is substituted at E54 wherein the substitution is Q. In additional aspects, the SIRP-beta2 polypeptide is substituted at H56 wherein the substitution is P. In further aspects, the SIRP-beta2 polypeptide is substituted at L66 wherein the substitution is T. In further aspects, the SIRP-beta2 polypeptide is substituted at V92 wherein the substitution is I. In additional aspects the SIRP-beta2 polypeptide is substituted at H101 wherein the substitution is D. In further aspects, the SIRP-beta 2 polypeptide has the sequence EEELQIIQPDKSISVAAGESATLHCTITSLFPVGPIQWFRGAGPGRVLIYNQRQGPFPRVTT VSDTTKRNNMDFSIRISNITPADAGTYYCIKFRKGSPDDVEFKSGAGTELSVRAKPS (SEQ ID NO: 28). In further aspects, the SIRP-beta2 polypeptide has the sequence EEELQX_1IQPDKSISVAAGESATLHCTX_2TSLX_3PVGPIQWFRGAGPGRX_4LIYNQX_5X_6GX_7 FPRVTTVSDX_8TKRNNMDFSIRISNITPADAGTYYCX_9KFRKGSPDX_{10}VEFKSGAGTELSV

RAKPS (SEQ ID NO: 46) wherein X_1 is V or I; X_2 is V or I; X_3 is I or F; X_4 is E or V; X_5 is K or R; X_6 is E or Q; X_7 is H or P; X_8 is L or T; X_9 is V or I; and X_{10} is H or D.

[0006] In additional embodiments, there is provided a decoy polypeptide selected from a SIRP-gamma, SIRP-beta, and SIRP-beta2 polypeptide wherein the SIRP-gamma polypeptide has the sequence

EEELQX_1IQPEKLLLVTVGKTATLHCTX_2TSX_3X_4PX_5GPX_6X_7WFRGX_8GPGRX_9LIYN$X_{10}X_{11}X_{12}$GX_{13}FPRVTTVSD$X_{14}X_{15}$KRNNMDFSIRISSITPADVGTYYCX_{16}KFRKGX_{17}PEX_{18}VEFKSGPGTEMALGAKPS (SEQ ID NO: 2), wherein X_1 is M, I, L or F; X_2 is F, I, L or V; X_3 is L, I, V, H, N or D; X_4 is F, I, L or V; X_5 is V, I, L, P, T or A; X_6 is V or I; X_7 is L or Q; X_8 is V or A; X_9 is E or V; X_{10} is Q, P, L, V, A or E; X_{11} is K or R; X_{12} is E, D, K, N, Q or H; X_{13} is H, P or R; X_{14} is L, I, V, P, T, A, R, S or G; X_{15} is T, I, N, F, S, Y, V, A or D; X_{16} is V or I; X_{17} is S, R, N, K, T, I or M; and X_{18} is N, K, D, E, H or Q,

wherein the SIRP-beta polypeptide has the sequence the sequence

EDELQX_1IQPEKSVSVAAGESATLRCAX_2TSLX_3PVGPIX_4WFRGAGAGRX_5LIYNQX_6X_7GX_8FPRVTTVSEX_9TKRNNLDFSISISNITPADAGTYYCX_{10}KFRKGSPDDVEFKSGAGTELSVRAKPS (SEQ ID NO: 45) wherein X_1 is V or I; X_2 is M or I; X_3 is I or F; X_4 is M or Q; X_5 is E or V; X_6 is K or R; X_7 is E or Q; X_8 is H or P; X_9 is L or T; and X_{10} is V or I; and

wherein the SIRP-beta2 polypeptide has the sequence

EEELQX_1IQPDKSISVAAGESATLHCTX_2TSLX_3PVGPIQWFRGAGPGRX_4LIYNQX_5X_6GX_7FPRVTTVSDX_8TKRNNMDFSIRISNITPADAGTYYCX_9KFRKGSPDX_{10}VEFKSGAGTELSVRAKPS (SEQ ID NO: 46) wherein X_1 is V or I; X_2 is V or I; X_3 is I or F; X_4 is E or V; X_5 is K or R; X_6 is E or Q; X_7 is H or P; X_8 is L or T; X_9 is V or I; and X_{10} is H or D.

[0007] In additional embodiments, the SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide blocks binding of CD47 to a ligand. In particular aspects, the ligand is SIRP-alpha, SIRPgamma, or thrombospoindin-1.

[0008] In additional embodiments, the SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide binds to a cell. In particular aspects, the cell is a tumor cell, virally infected cell, bacterially infected cell, damaged red blood cell, arterial plaque cell, fibrotic tissue cell, a healthy normal cell such as hematopoietic stem cell, a healthy myeloid or lymphoid precursor cell, or a healthy differentiated hematopoietic cell type such as T, B, plasma, or NK cell.

[0009] In additional embodiments the SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide enables phagocytosis or ADCC of a tumor cell, virally infected cell, bacterially infected cell, damaged red blood cell, arterial plaque cell, fibrotic tissue cell, healthy normal cell such as hematopoietic stem cell, healthy myeloid or lymphoid precursor cell or healthy differentiated hematopoietic cell type such as T, B, plasma, or NK cells.

[0010] In additional embodiments, the SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide is fused to an immunoglobulin Fc sequence. In additional embodiments, the SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide is multimeric. In additional embodiments, the SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide is monomeric. In additional embodiments the decoy polypeptide further comprises a detectable label.

[0011] In additional embodiments, there is provided a composition comprising the decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide as described above and a pharmaceutically acceptable carrier or excipient. In particular aspects, the composition further comprises an additional drug. In particular aspects, the drug comprises one or more of a chemotherapeutic agent, kinase inhibitor, proteasome inhibitor, or inhibitor of viral DNA or RNA polymerase. In additional embodiments, the composition further comprises a monoclonal antibody. In particular aspects, the monoclonal antibody binds an antigen on a cancer cell (e.g., myeloma), an immune cell, a pathogen-infected cell, or a hematopoietic stem cell. In additional aspects, the antigen on a cancer cell comprises EGFR, Her2/neu, CD19, CD20, CD22, CD25, CD30, CD33, CD38, CD45, CD47, CD56, CD70, CD117, or EpCAM. In further aspects, the antigen on the immune cell comprises M1prime, CD2, CD3, CD4, CD5, CD8, CD19, CD20, CD22, CD25, CD38, CD56, PD-1, PD-L1, CTLA4, BTLA, TIM3, LAG3, OX40, GITR or CD137 (4-1BB). In additional aspects, the antigen on a pathogen-infected cell comprises cytomegalovirus (CMV) proteins, including UL-18, UL11, pp65, gB, and pp150. In additional aspects, the antigen on a pathogen-infected cell comprises HIV envelope proteins, including Gp41, Gp120, V1V2 glycan, and V3 glycan, and influenza hemagglutinin. In further aspects, the antigen on the hematopoietic stem cell comprises CD11, CD45, CD117 or Sca1.

[0012] In additional embodiments, there is provided a decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide as described above with increased persistence. In some aspects, increased persistence of the decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptides results in increased cell-surface half-life compared to other SIRP polypeptides.

[0013] In additional embodiments, there is provided a decoy SIRP-gamma, SIRP-beta, or SIRP-beta2 polypeptide as described above with increased occupancy or receptor occupancy. In some aspects, increased occupancy of decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptides results in increased binding of the decoy SIRP-gamma, SIRP-beta, or SIRP-beta2 polypeptides to one or more target cells.

[0014] In additional embodiments, there is provided an isolated nucleic acid encoding any one of the above decoy polypeptides. In additional embodiments, there is provided a cell expressing any one of the above decoy polypeptides.

[0015] In additional embodiments, there is provided a method of modulating phagocytosis or ADCC of a cell expressing CD47, the method comprising contacting the cell with any one of the above decoy polypeptides or the compositions. In additional embodiments, there is provided a method of treating a subject in need thereof comprising administering an effective amount of any one of the above decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptides or compositions. In particular aspects, the subject has cancer, anemia, viral infection, bacterial infection, auto-immune disease, asthma, allergy, transplant rejection, atherosclerosis, or fibrosis.

[0016] In additional embodiments, there is provided any one of the above decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptides or compositions for use in treating cancer, viral infection, bacterial infection, auto-immune disease, asthma, allergy, transplant rejection, atherosclerosis, or fibrosis. In additional embodiments there is provided any one of the above decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptides or compositions for use in preconditioning for a hematopoietic stem cell transplant.

[0017] In additional embodiments, there is provided a method of visualizing a cell expressing CD47, the method comprising contacting a population of cells with any one of the above decoy polypeptides and a detectable label. In particular aspects, the cell is a tumor cell, virally infected cell, bacterially infected cell, autoreactive T or B cell, damaged red blood cell, arterial plaque cell, fibrotic tissue cell, healthy normal cell such as hematopoietic stem cell, a healthy myeloid or lymphoid precursor cell or healthy differentiated hematopoietic cell type such as T, B, plasma, or NK cell. In additional aspects, the contact is in vivo. In further aspects, the contact is in vitro.

[0018] In additional embodiments, there is provided a method of purifying a cell expressing CD47, the method comprising contacting a population of cells with any one of the above decoy polypeptides and a detectable label and purifying cells bound to the detectable label.

INCORPORATION BY REFERENCE

[0019] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

[0020] **FIGS. 1A and 1B** show the surface plasmon resonance-based measurement of binding affinity and kinetics of SIRP-gamma variant GV3 for human CD47. **FIG. 1A** shows concentrations of the SIRP-gamma variant GV3 used included 100 pM, 316 pM, 1 nM, 3.16 nM, 10 nM, and 100 nM. Using a 1:1 Langmuir binding model, the calculated dissociation constant K_d was 92 pM. The calculated dissociation half-life $T_{1/2}$ was about 44 minutes. **FIG. 1B**

shows a graphic representation of a biotinylated human CD47 bound to the SIRP-gamma variant GV3.

[0021] **FIGS. 2A and 2B** show the surface plasmon resonance-based measurement of binding affinity and kinetics of HAC-GV3, a fusion protein of SIRP-gamma variant GV3 with PD-1 variant HAC, for human CD47. **FIG. 2A** shows the concentrations of the HAC-GV3 fusion protein used included 100 pM, 316 pM, 1 nM, 3.16 nM, 10 nM, and 100 nM. Using a 1:1 Langmuir binding model, the calculated dissociation constant K_d was 160 pM. The calculated dissociation half-life $T_{1/2}$ was about 40 minutes. **FIG. 2B** shows a graphic representation of a biotinylated human CD47 bound to the fusion protein comprising SIRP-gamma variant GV3 and HAC.

[0022] **FIGS. 3A and 3B** show the surface plasmon resonance-based measurement of binding affinity and kinetics of HAC-GV3, a fusion protein of SIRP-gamma variant GV3 with PD-1 variant HAC, for human PD-L1. **FIG. 3A** shows the concentrations of the HAC-GV3 fusion protein used included 100 pM, 316 pM, 1 nM, 3.16 nM, 10 nM, and 100 nM. Using a 1:1 Langmuir binding model, the calculated dissociation constant K_d was 134 pM. The calculated dissociation half-life $T_{1/2}$ was about 38 minutes. **FIG. 3B** shows a graphic representation of a biotinylated human PD-L1 bound to HAC-GV3.

[0023] **FIGS. 4A and 4B** show the surface plasmon resonance-based measurement of binding affinity and kinetics of simultaneous binding of both human CD47 and human PD-L1 by HAC-GV3. **FIG. 4A** shows that in comparison to human GV3 alone, the binding curve for fusion protein HAC-GV3 showed a first peak for binding to biotinylated CD47 and a second peak for binding to PD-L1. **FIG. 4B** shows a graphic representation of a biotinylated human CD47 bound to the GV3 portion of the HAC-GV3 fusion protein, wherein the HAC portion is bound to PD-L1.

[0024] **FIG. 5** shows the flow cytometry-based measurement of blockade of CD47/SIRP-alpha interactions on the surface of CD47+ GFP-luciferase+ DLD1-Tg cells by GV3 and HAC-GV3. 50 nM of biotinylated SIRP-alpha tetramers were combined with titrating concentrations of either GV3 or HAC-GV3 in the presence of the CD47+ cells. Percent median fluorescence intensity (% MFT) as a readout of SIRP-alpha binding is plotted against log concentration in M.

[0025] **FIG. 6** shows the flow cytometry-based measurement of blockade of CD47/SIRP-alpha interactions on the surface of CD47+ PD-L1+ GFP-luciferase+ DLD1-Tg cells by GV3 and HAC-GV3. 50 nM of biotinylated SIRP-alpha tetramers were combined with titrating concentrations of either GV3 or HAC-GV3 in the presence of the CD47+ PD-L1+ cells. Percent median fluorescence intensity (% MFT) as a readout of SIRP-alpha binding is plotted against log concentration in M.

[0026] **FIG. 7** shows the flow cytometry-based measurement of blockade of PD-1/PD-L1 interactions on the surface of human PD-L1+ yeast cells by HAC and HAC-GV3. 100 nM of biotinylated PD-1 tetramers were combined with titrating concentrations of either HAC or HAC-GV3 in the presence of the hPD-L1+ yeast cells. Percent median fluorescence intensity (% MFT) as a readout of PD-1 binding is plotted against log concentration in M.

[0027] **FIG. 8** shows the flow cytometry-based measurement of blockade of PD-1/PD-L1 interactions on the surface of CD47+ PD-L1+ GFP-luciferase+ DLD1-Tg cells by HAC and HAC-GV3. 100 nM of biotinylated PD-1 tetramers were combined with titrating concentrations of either HAC or HAC-GV3 in the presence of the CD47+ PD-L1+ cells. Percent median fluorescence intensity (% MFT) as a readout of PD-1 binding is plotted against log concentration in M.

[0028] **FIG. 9** shows the FACS-based measurement of phagocytosis by donor-derived human macrophages against the human colon cancer cell line DLD1, indicating that GV3 microbody and HAC-GV3 potentiate phagocytosis across a wide range of opsonizing antibody concentrations. Assessment of phagocytosis was performed by co-culture of 100,000 target cells and 50,000 macrophages for two hours in ultra-low attachment 96 well U-bottom plates (Corning) in IMDM+GlutaMax (Life Technologies) without antibiotics or serum added. Macrophages were generated by magnetic bead-based purification of primary human monocytes using the CD14+ Whole Blood isolation kit (Miltenyi) followed by 7 days of culture with human serum (Gemini); on day 7, these cells were harvested from plates using TrypLE Express (Life Technologies). Macrophages were marked with Calcein AM red/orange cell stain (Life Technologies) according to manufacturer indications. Target cells were engineered to stably express green fluorescent protein. A dimerized microbody form of HGV3 (GV3mb) or HAC-GV3 fusion protein was added to reaction wells at a saturating concentration of 10nM, while the opsonizing antibody Cetuximab was titrated across three logs of concentration. Error bars represent the standard deviation of duplicate experiments. Reactions were run on an LSRFortessa Analyzer outfitted with a high-throughput auto-sampler (BD Biosciences). Phagocytosis was evaluated as GFP+ macrophages expressed as a percentage of the total macrophages, as analyzed using FlowJo v.9.4.10 (Tree Star) and was normalized as indicated in the figure legends.

[0029] **FIG. 10** shows the experimental outline for NSG mice engrafted with human leukemia cells enable approximation of human RO kinetics in vivo

[0030] **FIGS. 11A-D** show HAC-GV3 achieves superior receptor occupancy and persistence in tissue as compared to GV3. **FIG. 11A** shows receptor occupancy and persistence in total splenic cells. **FIG. 11B** shows receptor occupancy and persistence in splenocytes. **FIG. 11C**

shows receptor occupancy and persistence in human CD47+PDL1+ cells. **FIG. 11D** shows receptor occupancy and persistence in B16-F0 melanoma cells.

DETAILED DESCRIPTION OF THE INVENTION

[0031] SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptides and analogs thereof are provided which may be referred within as decoy polypeptides. The decoy polypeptides are variants of the wildtype human SIRP-gamma, SIRP-beta or SIRP-beta2 proteins. In one embodiment, provided herein is a soluble SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide, wherein the polypeptide lacks a transmembrane domain and comprises at least one amino acid change relative to the wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 sequence, and wherein the amino acid change increases the affinity of the SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide binding to CD47, for example by decreasing the off-rate by at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 500-fold, or more.

[0032] Signal regulatory proteins (SIRPs) constitute a family of cell surface glycoproteins which are expressed on myeloid (including macrophages, granulocytes, myeloid dendritic cells, and mast cells) and neuronal cells. SIRPs constitute a diverse multigene family of immune receptors encompassing inhibitory, activating, nonsignaling and soluble members. CD47, a broadly expressed transmembrane glycoprotein, functions as a cellular ligand for SIRPα and binds to the NH2-terminal extracellular terminus of SIRPα. SIRPα's role has been best documented in respect of its inhibitory role in the phagocytosis of host cells by macrophages and antibody-directed cellular cytotoxicity (ADCC) by neutrophils. In particular, the binding of SIRPα on myeloid cells by CD47 expressed on target cells, generates an inhibitory signal that negatively regulates phagocytosis and ADCC. Agents that bind to either CD47 or to SIRPα and antagonize the CD47:SIRPα interaction act to active macrophage phagocytosis and neutrophil ADCC, particularly towards antibody-opsonized cells (Majeti et al Cell 2009, Chao et al Cell 2010, Zhang et al, Weiskopf et al Science 2013). The agents include monoclonal antibodies, and soluble CD47 and SIRPα receptor "decoys." CD47 is also a ligand for SIRP-gamma, a gene distinct from SIRPα that is expressed on lymphocytes of unclear function. SIRP-beta and SIRP-beta2 are also distinct genes from SIRPα, and despite their similarity in sequence and in structure to SIRPα, they do not naturally bind CD47. However, they can be made to do so through mutation (Hatherly et al, Molecular Cell 2008). In principle, decoy receptors based-off a SIRP-gamma, SIRP-beta, or SIRP-beta2 scaffold could also antagonize the SIRPα-CD47 interaction to increase myeloid cell phagocytosis or ADCC. These decoys would enjoy a major advantage over SIRPα-based decoys as CD47-targeting therapeutics, as the SIRPα ectodomain is highly polymorphic between individuals, thus increasing the likelihood of immunogenicity if

the SIRPα was administered as a recombinant therapeutic. By contrast, the ectodomains of SIRP-gamma, SIRP-beta, and SIRP-beta2 are not widely polymorphic, thus avoiding unnecessary immunogenic sequences.

[0033] In some embodiments, SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptides described herein stimulate phagocytosis or ADCC by myeloid cells (e.g., macrophages, monocytes, dendritic cells, neutrophils, etc.) to eliminate pathogenic cells (e.g., tumor cells, virally or bacterially infected cells, autoreactive T cells, etc). In some of such embodiments, cells are eliminated selectively, thereby reducing the potential for toxic side effects. In other embodiments, the same polypeptides could be used to enhance the elimination of endogenous cells for therapeutic effect, such as B or T lymphocytes in autoimmune disease, asthma, and allergy, or hematopoietic stem cells (HSCs) for stem cell transplantation.

[0034] In some embodiments, SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptides described herein have increased occupancy or receptor occupancy compared to other SIRP polypeptides known in the art. In some embodiments, SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptides described herein have increased persistence compared to other SIRP polypeptides known in the art. Occupancy or receptor occupancy, as used herein, refers to binding to a target cell, target receptor, target protein, or target tissue. Persistence, as used herein, refers to both serum half-life or cell binding half-life of the polypeptides when administered to an individual, subject, or patient.

SIRP Polypeptides and SIRP Polypeptide Variants

[0035] The signal regulatory proteins (SIRP, CD172) are a family of membrane proteins involved in leukocyte function. The SIRP protein family includes Signal-Regulatory protein Gamma (SIRP-gamma, CD172g), Signal Regulatory Protein Beta (SIRP-beta, Signal Regulatory Protein Beta 1, CD172b, SIRP-beta1) and Signal Regulatory Protein Beta 2 (SIRP-beta2, PTPN1L).

[0036] The amino acid sequence of human SIRP-gamma is available in the SWISS-PROT database as Q9P1W8. SIRP-gamma includes three isoforms. The amino acid sequence of human SIRP-beta is available in the SWISS-PROT database as O00241 and Q5TFQ8. SIRP-beta includes three isoforms. The amino acid sequence of SIRP-beta2 is available in the SWISS-PROT database as Q5JXA9. SIRP-beta2 includes three isoforms.

[0037] Contemplated within the scope of embodiments presented herein are variants of the SIRP-gamma, SIRP-beta and SIRP-beta2 polypeptides that act as decoys for CD47 and activate phagocytosis. In some embodiments, a decoy polypeptide comprises a SIRP-gamma, a SIRP-beta or a SIRP-beta2 polypeptide, wherein the polypeptide comprises at least one amino acid modification to increase affinity of the decoy SIRP-gamma, SIRP-beta and SIRP-beta2

polypeptide binding to CD47 as compared to the affinity for CD47 of the corresponding wild type SIRP-gamma, SIRP-beta and SIRP-beta2 polypeptide.

[0038] As used herein, "sequence substantially identical to a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide sequence" means that, in one embodiment, a sequence is at least 80% identical to a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide sequence. In other embodiments, "sequence substantially identical to a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide sequence" means that, a sequence is at least 85% identical to a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide sequence. In other embodiments, "sequence substantially identical to a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide sequence" means that, a sequence is at least 90% identical to a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide sequence. In other embodiments, "sequence substantially identical to a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide sequence" means that, a sequence is at least 95% identical to a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide sequence.

[0039] In some embodiments, the decoy polypeptides described herein incorporate high affinity homologues or variants of SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptides (e.g., variants of SIRP-gamma, SIRP-beta or SIRP-beta2). Accordingly, the embodiments presented herein encompass the use of a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide having a sequence that is similar to, but not identical to, the amino acid sequence of wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide. Thus, also contemplated within the scope of embodiments provided herein is the use of a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide that has a sequence identity of 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, or 80% compared to the sequence of wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide, and which binds with high affinity to CD47.

[0040] In some embodiments, a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide variant is modified to contain conservative variations so as to change non-critical residues or residues in non-critical regions. Amino acids that are not critical are identified by known methods, such as site-directed mutagenesis, crystallization, nuclear magnetic resonance, photoaffinity labeling, or alanine-scanning mutagenesis (Cunningham et al., Science, 244:1081-1085 (1989); Smith et al., J. Mol. Biol., 224:899-904 (1992); de Vos et al., Science, 255:306-312 (1992)). Modified proteins are tested for activity or ability to bind to CD47 via methods such as in vitro activity or in vivo activity.

[0041] In some embodiments, a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide variant incorporates 1, 2, 3, 4, 5 or more amino acid substitutions that improve SIRP-gamma, SIRP-beta

or SIRP-beta2 polypeptide stability or with a different hydrophobic amino acid that improves SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide stability against oxidation, or with a different amino acid that improves SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide against protease. Thus, a "variant" SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide differs in amino acid sequence from the sequence represented by a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide by one or more substitutions, deletions, insertions, inversions, truncations, modifications, or a combination thereof. Such a variant optionally contains amino acid substitutions that substitute a given amino acid with another amino acid of similar characteristics. Conservative substitutions include, among the aliphatic amino acids, interchange of alanine, valine, leucine, and isoleucine; interchange of the hydroxyl residues serine and threonine, exchange of the acidic residues aspartate and glutamate, substitution between the amide residues asparagine and glutamine, exchange of the basic residues lysine and arginine, and replacements among the aromatic residues phenylalanine and tyrosine. See Bowie et al., Science, 247:1306-1310 (1990).

[0042] The SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide is a variant, as described in detail below, in ways in which the variant retains or improves the characteristic of CD47 binding.

[0043] In some embodiments, a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide variant is a fragment of the SIRP-gamma, SIRP-beta or SIRP-beta2 protein. In some embodiments, SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide variants include proteolytic cleavage-resistant SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide fragments or SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide fragments containing one or more non-natural amino acids, such as D-amino acids. Such derivatives would have the benefit of increased circulating half-life, while retaining the beneficial characteristic of CD47 binding.

[0044] In another embodiment, a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide variant comprises a decoy polypeptide. In certain embodiments, the decoy polypeptide comprises a SIRP-gamma polypeptide with the sequence EEELQMIQPEKLLLVTVGKTATLHCTVTSLLPVGPVLWFRGVGPGRELIYNQKEGHFPR VTTVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 1), or a polypeptide substantially identical to a wildtype SIRP-gamma sequence. In particular aspects, the SIRP-gamma polypeptide has at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen or eighteen amino acid substitutions at M6, V27, L30, L31, V33, V36, L37, V42, E47, Q52, K53, E54, H56, L66, T67, V92, S98 or N101. In further aspects, the SIRP-gamma polypeptide has a substitution at M6 wherein the substitution is I, L or F. In additional aspects, the SIRP-gamma

polypeptide has a substitution at V27 wherein the substitution is F, I or L. In further aspects, the SIRP-gamma polypeptide has a substitution at L30 wherein the substitution is I, V, H, N or D. In additional aspects, the SIRP-gamma polypeptide has a substitution at L31 wherein the substitution is F, I or V. In further aspects, the SIRP-gamma polypeptide has a substitution at V33 wherein the substitution is I, L, P, T or A. In additional aspects, the SIRP-gamma polypeptide has a substitution at V36 wherein the substitution is I. In additional aspects, the SIRP-gamma polypeptide has a substitution at L37 wherein the substitution is Q. In additional aspects, the SIRP-gamma polypeptide has a substitution at V42 wherein the substitution is A. In further aspects, the SIRP-gamma polypeptide has a substitution at E47 wherein the substitution is V. In additional aspects, the SIRP-gamma polypeptide has a substitution at Q52 wherein the substitution is P, L, V, A or E. In additional aspects the SIRP-gamma polypeptide has a substitution at K53 wherein the substitution is R. In further aspects, the SIRP-gamma polypeptide has a substitution at E54 wherein the substitution is D, K, N, Q or H. In further aspects, the SIRP-gamma polypeptide has a substitution at H56 wherein the substitution is P or R. In additional aspects, the SIRP-gamma polypeptide has a substitution at L66 wherein the substitution is I, V, P, T, A, R, S or G. In further aspects, the SIRP-gamma polypeptide has a substitution at T67 wherein the substitution is I, N, F, S, Y, V, A or D. In further aspects, the SIRP-gamma polypeptide has a substitution at V92 wherein the substitution is I. In additional aspects, the SIRP-gamma polypeptide has a substitution at S98 wherein the substitution is R, N, K, T, I or M. In further aspects, the SIRP-gamma polypeptide is substituted at N101 wherein the substitution is K, D, E, H or Q. In additional aspects, the SIRP-gamma polypeptide has the sequence

EEELQX_1IQPEKLLLVTVGKTATLHCTX_2TS$X_3$$X_4PX_5GPX_6$$X_7$WFRG$X_8$GPGR$X_9$LIYN$X_{10}X_{11}$$X_{12}GX_{13}$FPRVTTVSD$X_{14}$$X_{15}$KRNNMDFSIRISSITPADVGTYYC$X_{16}$KFRKG$X_{17}PEX_{18}$VEF KSGPGTEMALGAKPS (SEQ ID NO: 2), wherein X_1 is M, I, L or F; X_2 is F, I, L or V; X_3 is L, I, V, H, N or D; X_4 is F, I, L or V; X_5 is V, I, L, P, T or A; X_6 is V or I; X_7 is L or Q; X_8 is V or A; X_9 is E or V; X_{10} is Q, P, L, V, A or E; X_{11} is K or R; X_{12} is E, D, K, N, Q or H; X_{13} is H, P or R; X_{14} is L, I, V, P, T, A, R, S or G; X_{15} is T, I, N, F, S, Y, V, A or D; X_{16} is V or I; X_{17} is S, R, N, K, T, I or M; and X_{18} is N, K, D, E, H or Q. In additional aspects the decoy polypeptide has the sequence

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPVLWFRGVGPGRVLIYNQRQGPFPRVT TVSDTTKRNNMDFSIRISSITPADVGTYYCIKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 3). In further aspects, the SIRP-gamma polypeptide has the sequence

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS

(SEQ ID NO: 4). In additional aspects, the SIRP-gamma polypeptide has the sequence
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 5). In additional aspects, the SIRP-gamma polypeptide has the sequence
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 6). In further aspects, the SIRP-gamma polypeptide has the sequence
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGTPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 7). In additional aspects, the SIRP-gamma polypeptide has one of the following sequences:
HLib1:
EEELQIIQPEKLLLVTVGKTATLHCTITSHFPVGPIQWFRGVGPGRVLIYNQKDGHFPRV
TTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 8);
HLib2:
EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 9);
HLib3:
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPVLWFRGVGPGRVLIYNQRQGPFPRVT
TVSDTTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 10);
HLib4:
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRELIYNAREGRFPRVT
TVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 11);
HMLib1:
EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRQGPFPRVT
TVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFRKGSPDDVEFKSGAGTELSVRAKPS
(SEQ ID NO: 12);
HMLib2:
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPVLWFRGVGPGRVLIYNQRQGPFPRVT
TVSDTTKRNNMDFSIRISSITPADVGTYYCIKFRKGSPENVEFKSGPGTEMALGAKPS
(SEQ ID NO: 3);

HMLib3:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQREGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 13);

HMLib4:

EEELQMIQPEKLLLVTVGKTATLHCTVTSLLPVGPVLWFRGVGPGRELIYNQKEGHFPR VTTVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 42);

HMLib5:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 14);

HMLib6:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTXWH (SEQ ID NO: 15), wherein X is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, or V;

HMLib7:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQKDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGTPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 16);

MLib1:

EEELQIIQPEKLLLVTVGKTATLHCTITSLLPVGPIQWFRGVGPGRELIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 17);

MLib2:

EEELQIIQPEKLLLVTVGKTATLHCTLTSLLPVGPILWFRGVGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGNPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 18);

MLib3:

EEELQLIQPEKLLLVTVGKTATLHCTITSLFPPGPIQWFRGVGPGRVLIYNQKDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGIPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 19);

MLib4:

EEELQIIQPEKLLLVTVGKTATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 20);
MLib5:
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPIGPILWFRGVGPGRVLIYNQKDGPFPRVTT
VSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 21);
MLib6:
EEELQMIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRV
TTVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 22);
MLib7:
EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 23);
MLib8:
EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQKDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGTPEDVEFKSGPGTEMALXAKPS
(SEQ ID NO: 24); or
GV1.2:
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQREGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 13).

[0045] In additional embodiments the decoy polypeptide comprises a SIRP-beta polypeptide with the sequence
EDELQVIQPEKSVSVAAGESATLRCAMTSLIPVGPIMWFRGAGAGRELIYNQKEGHFPR
VTTVSELTKRNNLDFSISISNITPADAGTYYCVKFRKGSPDDVEFKSGAGTELSVRAKPS
(SEQ ID NO: 25). In particular aspects, the SIRP-beta polypeptide is at least 90% identical to a wildtype SIRP-beta polypeptide. In additional aspects, the SIRP-beta polypeptide has amino acid substitutions at V6, M27, I31, M37, E47, K53, E54, H56, L66 or V92. In further aspects, the SIRP-beta polypeptide has a substitution at V6 wherein the substitution is I. In additional aspects, the SIRP-beta polypeptide has a substitution at M27 wherein the substitution is I. In further aspects, the SIRP-beta polypeptide has a substitution at I31 wherein the substitution is F. In additional aspects, the SIRP-beta polypeptide has a substitution at M37 wherein the substitution is Q. In further aspects, the SIRP-beta polypeptide has a substitution at E47 wherein

the substitution is V. In additional aspects, the SIRP-beta polypeptide has a substitution at K53 wherein the substitution is R. In further aspects, the SIRP-beta polypeptide has a substitution at E54 wherein the substitution is Q. In additional aspects, the SIRP-beta polypeptide has a substitution at H56 wherein the substitution is P. In further aspects, the SIRP-beta polypeptide has a substitution at L66 wherein the substitution is T. In additional aspects, the SIRP-beta polypeptide has a substitution at V92 wherein the substitution is I. In further aspects, the SIRP-beta polypeptide has the sequence EDELQIIQPEKSVSVAAGESATLRCAITSLFPVGPIQWFRGAGAGRVLIYNQRQGPFPRVT TVSETTKRNNLDFSISISNITPADAGTYYCIKFRKGSPDDVEFKSGAGTELSVRAKPS (SEQ ID NO: 26). In further aspects, the SIRP-beta polypeptide has the sequence EDELQX_1IQPEKSVSVAAGESATLRCAX_2TSLX_3PVGPIX_4WFRGAGAGRX_5LIYNQ$X_6$$X_7$G X_8FPRVTTVSEX_9TKRNNLDFSISISNITPADAGTYYCX_{10}KFRKGSPDDVEFKSGAGTELS VRAKPS (SEQ ID NO: 45) wherein X_1 is V or I; X_2 is M or I; X_3 is I or F; X_4 is M or Q; X_5 is E or V; X_6 is K or R; X_7 is E or Q; X_8 is H or P; X_9 is L or T; and X_{10} is V or I.

[0046] In further embodiments, the decoy polypeptide comprises a SIRP-beta2 polypeptide with the sequence EEELQVIQPDKSISVAAGESATLHCTVTSLIPVGPIQWFRGAGPGRELIYNQKEGHFPRVT TVSDLTKRNNMDFSIRISNITPADAGTYYCVKFRKGSPDHVEFKSGAGTELSVRAKPS (SEQ ID NO: 27). In particular aspects, the SIRP-beta2 polypeptide is at least 90% identical to a wildtype SIRP-beta2 polypeptide. In additional aspects, the SIRP-beta2 polypeptide has amino acid substitutions at V6, V27, I31, E47, K53, E54, H56, L66, V92 or H101. In further aspects, the SIRP-beta2 polypeptide is substituted at V6 wherein the substitution is I. In additional aspects, the SIRP-beta2 polypeptide is substituted at V27 wherein the substitution is I. In further aspects, the SIRP-beta2 polypeptide is substituted at I31 wherein the substitution is F. In additional aspects, the SIRP-beta2 polypeptide is substituted at E47 wherein the substitution is V. In further aspects, the SIRP-beta2 polypeptide is substituted at K53 wherein the substitution is R. In additional aspects, the SIRP-beta2 polypeptide is substituted at E54 wherein the substitution is Q. In further aspects, the SIRP-beta2 polypeptide is substituted at H56 wherein the substitution is P. In additional aspects, the SIRP-beta2 polypeptide is substituted at L66 wherein the substitution is T. In further aspects, the SIRP-beta2 polypeptide is substituted at V92 wherein the substitution is I. In additional aspects, the SIRP-beta2 polypeptide is substituted at H101 wherein the substitution is D. In further aspects, the SIRP-beta 2 polypeptide has the sequence EEELQIIQPDKSISVAAGESATLHCTITSLFPVGPIQWFRGAGPGRVLIYNQRQGPFPRVTT VSDTTKRNNMDFSIRISNITPADAGTYYCIKFRKGSPDDVEFKSGAGTELSVRAKPS

(SEQ ID NO: 28). In further aspects, the SIRP-beta2 polypeptide has the sequence EEELQX_1IQPDKSISVAAGESATLHCTX_2TSLX_3PVGPIQWFRGAGPGRX_4LIYNQX_5X_6GX_7FPRVTTVSDX_8TKRNNMDFSIRISNITPADAGTYYCX_9KFRKGSPDX_{10}VEFKSGAGTELSVRAKPS (SEQ ID NO: 46) wherein X_1 is V or I; X_2 is V or I; X_3 is I or F; X_4 is E or V; X_5 is K or R; X_6 is E or Q; X_7 is H or P; X_8 is L or T; X_9 is V or I; and X_{10} is H or D.

[0047] In some embodiments, the decoy polypeptide blocks binding of CD47 to a binding partner or ligand. In particular aspects, the binding partner or ligand comprises one or more of SIRPalpha (SIRPA), SIRPgamma (SIRPG), or thrombospoindin-1 (TSP-1, THBS1).

[0048] In some embodiments, the decoy polypeptide binds to a cell. In particular aspects, binding of the decoy polypeptide to the cell, activates, enables, induces, or causes phagocytosis of the cell by a phagocyte, such as a professional phagocyte (e.g. monocytes, macrophages, neutrophils, dendritic cells or mast cells), a non-professional phagocyte (e.g. epithelial cells, endothelial cells, fibroblasts or mesenchymal cells) or both. In additional aspects, the cell is a tumor cell, virally infected cell, bacterially infected cell, self-reactive cell, such as a self-reactive T cell or B cell, or other undesirable or pathogenic cells or tissues in the body, such as damaged red blood cells, arterial plaques, or fibrotic tissue. In additional aspects, the cell is a healthy normal cell such as hematopoietic stem cell, a healthy myeloid or lymphoid precursor cell, or a healthy differentiated hematopoietic cell type such as T, B, plasma, or NK cells.

[0049] In some embodiments, the decoy polypeptide is a chimera or a fusion protein. In particular aspects, the decoy polypeptide is fused to an immunoglobulin Fc sequence, a green fluorescent protein, a red fluorescent protein, a biotin, a HIS tag, a MYC tag, a FLAG, or other polypeptide sequence. In some embodiments, the decoy polypeptide is fused to a wild type subunit of PD-1 (PDCD1), PD-L1 (CD274), PD-L2 (PDCD1LG2), CTLA4, TIM3 (HAVCR2), CEACAM1, LAG3, BTLA, TNFRSF14, TIGIT, PVR, LIGHT, IL2, IL12A, IL15, IL10, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LILRA1, LILRA2, LILRA3, LILRA4, LILRA5, LILRA6, CD40, CD40L, OX40, OX40L, CD137 (4-1BB, TNFRSF9), TNFSF9 (4-1BBL), B7-H4 (VCTN1), SIRPA, CD47, CD33, CD44, C5, C3, or other proteins. In some embodiments, the decoy polypeptide is fused to an subunit that is a variant of PD-1 (PDCD1), PD-L1 (CD274), PD-L2 (PDCD1LG2), CTLA4, TIM3 (HAVCR2), CEACAM1, LAG3, BTLA, TNFRSF14, TIGIT, PVR, LIGHT, IL2, IL12A, IL15, IL10, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LILRA1, LILRA2, LILRA3, LILRA4, LILRA5, LILRA6, CD40, CD40L, OX40, OX40L, CD137 (4-1BB, TNFRSF9), TNFSF9 (4-1BBL), B7-H4 (VCTN1), SIRPA, CD47, CD33, CD44, C5, C3, or other immune regulatory proteins, engineered for high affinity binding to their respective ligands. In some embodiments, the decoy polypeptide is fused to an subunit that is a variant of PD-1 (PDCD1), PD-L1 (CD274), PD-L2 (PDCD1LG2), CTLA4,

TIM3 (HAVCR2), CEACAM1, LAG3, BTLA, TNFRSF14, TIGIT, PVR, LIGHT, IL2, IL12A, IL15, IL10, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LILRA1, LILRA2, LILRA3, LILRA4, LILRA5, LILRA6, CD40, CD40L, OX40, OX40L, CD137 (4-1BB, TNFRSF9), TNFSF9 (4-1BBL), B7-H4 (VCTN1), SIRPA, CD47, CD33, CD44, C5, C3, or other proteins, engineered for reduced affinity binding to their respective ligands. In some embodiments, the decoy polypeptide is fused to an subunit that is a variant of PD-1 (PDCD1), PD-L1 (CD274), PD-L2 (PDCD1LG2), CTLA4, TIM3 (HAVCR2), CEACAM1, LAG3, BTLA, TNFRSF14, TIGIT, PVR, LIGHT, IL2, IL12A, IL15, IL10, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LILRA1, LILRA2, LILRA3, LILRA4, LILRA5, LILRA6, CD40, CD40L, OX40, OX40L, CD137 (4-1BB, TNFRSF9), TNFSF9 (4-1BBL), B7-H4 (VCTN1), SIRPA, CD47, CD33, CD44, C5, C3, or other proteins, engineered for altered binding affinity to additional ligands besides their natural ligands. In additional aspects, the decoy polypeptide is fused to or administered in combination with a monoclonal antibody such as an anti-CD20 antibody, an anti-EGFR antibody, an anti-Her2/Neu (ERBB2) antibody, an anti-EPCAM antibody, an anti-GL2 antibody, anti-GD2, anti-GD3, anti-CD2, anti-CD3, anti-CD4, anti-CD8, anti-CD19, anti-CD22, anti-CD30, anti-CD33, anti-CD45, anti-CD47, anti-CD52, anti-CD56, anti-CD70, anti-CD117, an anti-SIRPA antibody, an anti-CD47 antibody, an anti-LILRB1 antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, or any antibody designed to bind to a tumor cell, a virally- or bacterially-infected cell, immune cell, or healthy normal cell, or to a cytokine, chemokine, or hormone of any kind.

[0050] In some embodiments decoy polypeptide comprises a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide in multimeric form. In particular aspects, the decoy polypeptide comprises a dimer, a trimer, a tetramer, a pentamer or other multimer. In other embodiments, the decoy polypeptide comprises a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide in monomeric form.

[0051] In some embodiments, the decoy polypeptide comprises a detectable label. In particular aspects, the detectable label comprises an enzymatic label such as horseradish peroxidase (HRP), alkaline phosphatase (AP) or glucose oxidase. In additional aspects, the detectable label comprises a fluorescent label such as Alexa Fluor® 350, Alexa Fluor® 405, Alexa Fluor® 488, Alexa Fluor® 532, Alexa Fluor® 546, Alexa Fluor® 555, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 647, Alexa Fluor® 680, Alexa Fluor® 750, BODIPY® FL, Coumarin, Cy®3, Cy®5, Fluorescein (FITC), Oregon Green®, Pacific Blue™, Pacific Green™, Pacific Orange™, Tetramethylrhodamine (TRITC), Texas Red® or other fluorescent label. In further aspects, the detectable label comprises a chelating group, such as Cyclen, Cyclam, DO2A, DOTP, DOTMA, TETA, DOTAM, CB-T2A, DOTA or NOTA. In further

aspects, the detectable label comprises a radioactive isotope such as ^{32}P, ^{33}P, ^{3}H, ^{14}C, ^{125}I, ^{18}F, ^{68}Ga, ^{64}Cu, ^{89}Zr, ^{11}C, ^{13}N, ^{15}O, ^{62}Cu, ^{124}I, ^{76}Br, ^{82}Rb or other radioactive isotope.

[0052] In additional embodiments, the decoy polypeptide is a fusion or chimeric polypeptide. In additional embodiments, the decoy polypeptide comprises a SIRP-gamma polypeptide sequence fused to a polypeptide sequence comprising an immune checkpoint inhibitor, a co-stimulatory molecule, or a cytokine or an attenuated cytokine, wherein the sequences are connected by a Gly-Ser linker of varying length and composition. For example, the linker sequence comprises the sequence GGGGSGGGGS (SEQ ID NO: 29). The order of the two polypeptide sequences at the N- or C-terminus could also be varied. In additional embodiments, the decoy polypeptide is a multi-specific high-affinity SIRP-gamma agent. In further embodiments, the decoy polypeptide comprises one of the following sequences:

1) Fusion to immune checkpoint inhibitors

    a. PD-1/PD-L1 antagonist

Example: HAC-GV3 (high-affinity PD-1 decoy fused to GV3)
DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFHVVWHRESPSGQTDTLA
AFPEDRSQPGQDARFRVTQLPNGRDFHMSVVRARRNDSGTYVCGVISLAPKIQI
KESLRAELRVTERGGGGSGGGGSEEELQIIQPEKLLLVTVGKTATLHCTITSLFPV
GPIQWFRGVGPGRVLIYNQKDGHFPRVTTVSDGTKRNNMDFSIRISSITPADVGT
YYCVKFRKGSPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 30);

    b. BTLA/CD160 antagonist

Example: GV3-BTLA decoy
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH
FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE
MALGAKPSGGGGSGGGGSWNIHGKESCDVQLYIKRQSEHSILAGDPFELECPVK
YCANRPHVTWCKLNGTTCVKLEDRQTSWKEEKNISFFILHFEPVLPNDNGSYRC
SANFQSNLIESHSTTLYVTDVK (SEQ ID NO: 31);

    c. Phosphatidylserine antagonist

Example: GV3-MFGE8 decoy
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH
FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE
MALGAKPSGGGGSGGGGSELNGCANPLGLKNNSIPDKQITASSSYKTWGLHLFS
WNPSYARLDKQGNFNAWVAGSYGNDQWLQVDLGSSKEVTGIITQGARNFGSV
QFVASYKVAYSNDSANWTEYQDPRTGSSKIFPGNWDNHSHKKNLFETPILARYV
RILPVAWHNRIALRLELLGC (SEQ ID NO: 32);

Example: GV3-Tim1 decoy

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSVAGSVKVGGEAGPSVTLPCHYSGAVTSMCWNRGS CSLFTCQNGIVWTNGTHVTYRKDTRYKLLGDLSRRDVSLTIENTAVSDSGVYCC RVEHRGWFNDMKITVSLEIVPPKVTT (SEQ ID NO: 33);

Example: GV3-Tim3 decoy

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSSEVEYRAEVGQNAYLPCFYTPAAPGNLVPVCWGK GACPVFECGNVVLRTDERDVNYWTSRYWLNGDFRKGDVSLTIENVTLADSGIY CCRIQIPGIMNDEKFNLKLVIKPAKVTPA (SEQ ID NO: 34);

Example: GV3-Tim4 decoy

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSTSETVVTEVLGHRVTLPCLYSSWSHNSNSMCWGK DQCPYSGCKEALIRTDGMRVTSRKSAKYRLQGTIPRGDVSLTILNPSESDSGVYC CRIEVPGWFNDVKINVRLNLQRASTTTDEKFNLKLVIKPAKVTPA (SEQ ID NO: 35);

2) Fusion to co-stimulatory molecules

   a. CD40 agonist

Example: GV3-CD40L

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSGDQNPQIAAHVISEASSKTTSVLQWAEKGYYTMSN NLVTLENGKQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILL RAANTHSSAKPCGQQSIHLGGVFELQPGASVFVNVTDPSQVSHGTGFTSFGLLKL (SEQ ID NO: 36);

   b. 41BB (CD137) agonist

Example: GV3-41BBL

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDP GLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQMELRRVVAGEGSGSVSLALH LMPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEA RARHAWQLTQGATVLGLFRVTPEIPA (SEQ ID NO: 37);

3) Fusion to cytokines and attenuated cytokines

Example: GV3-IL2

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINV IVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT (SEQ ID NO: 38);

Example: GV3-IL2 (an "attenuated" cytokine with mutations F42A/D20T)

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLTLQMILNGINNYKNPKL TRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINV IVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT (SEQ ID NO: 39).

SIRP Polypeptide Modifications

[0053] SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide variants encompass derivatives or analogs in which (i) an amino acid is substituted with an amino acid residue that is not one encoded by the genetic code, (ii) the mature polypeptide is fused with another compound such as polyethylene glycol, or (iii) additional amino acids are fused to the SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide, such as a leader or secretory sequence or a sequence for purification of the polypeptide.

[0054] Typical modifications include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

[0055] Modifications are made anywhere in a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Certain common peptide modifications that are useful for modification of SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide include glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, and ADP-ribosylation.

[0056] Moreover, one or more amino acids of the core sequence is altered, in a conservative manner such that the requisite CD47 binding is maintained or increased. Typical substitutions are made among the following groups of amino acids: (a) G, A, V, L and I; (b) G and P; (c) S, C, T, M; (d) F, Y, and W; (e) H, K and R; and (f) D, E, N, and Q. Other substitutions include the following groups: (i) S and T; (ii) P and G; a (iii) A, V, L and I.

[0057] Also contemplated within the scope of embodiments provided herein are modifications of the SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide wherein the polypeptide is joined to another polypeptide with which it is not normally associated (e.g., Glutathione S-transferase (GST)-fusion protein, beta-galactosidase fusions, yeast two-hybrid GAL fusions, poly-His fusions, Ig fusions and the like). Thus, a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide is optionally operatively linked, at either its N-terminus or C-terminus, to a heterologous polypeptide having an amino acid sequence not substantially homologous to the SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide. "Operatively linked" indicates that SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide and the heterologous polypeptide are both in-frame. Such a fusion protein may alter (e.g., enhances, dampens) the ability of the SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide, or a functional variant thereof, to bind CD47. Such a fusion protein may also alter (enhances, dampens) the pharmacokinetic half-life of the SIRP-gamma, SIRP-beta, or SIRP-beta2 polypeptide in a human patient or experimental animal. The fusion protein may also alter the activity that the SIRP-gamma, SIRP-beta, or SIRP-beta2 polypeptide imparts on myeloid cell activity including phagocytosis and ADCC.

Affinity

[0058] As a subject SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide has an increased affinity for CD47 as compared to the affinity for CD47 of a wild type SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide, and/or as compared to the affinity for CD47 of a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide that does not have an amino acid change relative to a corresponding sequence of a wild type SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide (e.g., a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide, as defined above).

[0059] In some embodiments, the SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide has a Kd of 1 x 10^{-7} M or less (e.g., 10^{-8} M or less, 10^{-9} M or less, 10^{-10} M or less, 10^{-11} M or less, 10^{-12} M or less, 10^{-13} M or less, 10^{-14} M or less, 10^{-15} M or less, or 10^{-16} M or less) affinity for CD47. In some cases, the SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide has an affinity for CD47 in a range of from 1 fM to 1 μM (e.g., from 1 fM to 800 nM, from 10 fM to 500 nM, from 100 fM to 100 nM, from 500 fM to 50 nM, from 800 fM to 50 nM, from 1 pM to 50 nM, from 10 pM to 50 nM, from 50 pM to 50 nM, from 100 pM to 50 nM, from 500 fM to 100 nM, from 800 fM to 100 nM, from 1 pM to 100 nM, from 10 pM to 100 nM, from 50 pM to

100 nM, or from 100 pM to 100 nM). In some cases, SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide binds to CD47 with an affinity of 1 μM or greater (e.g., 800 nM or greater, 500 nM or greater, 200 nM or greater, 100 nM or greater, 50 nM or greater, 10 nM or greater, 1 nM or greater, 900 pM or greater, 750 pM or greater, 500 pM or greater, 200 pM or greater, 100 pM or greater, 10 pM or greater, 1 pM or greater, etc., where the affinity increases with decreasing values).

[0060] In some embodiments, the SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide has an affinity for CD47 that is 2-fold or more (e.g., 5-fold or more, 10-fold or more, 100-fold or more, 500-fold or more, 1000-fold or more, 5000-fold or more, 10^4-fold or more, 10^5-fold or more, 10^6-fold or more, 10^7-fold or more, 10^8-fold or more, etc.) greater than the affinity for CD47 of a wild type SIRP-gamma, SIRP-beta or SIRP-beta2 protein; and/or 2-fold or more (e.g., 5-fold or more, 10-fold or more, 100-fold or more, 500-fold or more, 1000-fold or more, 5000-fold or more, 104-fold or more, 10^5-fold or more, 10^6-fold or more, 10^7-fold or more, 10^8-fold or more, etc.) greater than the affinity for CD47 of a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide that does not have an amino acid change relative to a corresponding sequence of a wild type SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide (e.g., a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide, as defined above).

[0061] In some embodiments, the SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide has a dissociation half-life for CD47 that is 2-fold or more (e.g., 5-fold or more, 10-fold or more, 100-fold or more, 500-fold or more, 1000-fold or more, 5000-fold or more, 10^4-fold or more, 10^5-fold or more, 10^6-fold or more, 10^7-fold or more, 10^8-fold or more, etc.) greater than the dissociation half-life for CD47 of a wild type SIRP-gamma, SIRP-beta or SIRP-beta2 protein; and/or 2-fold or more (e.g., 5-fold or more, 10-fold or more, 100-fold or more, 500-fold or more, 1000-fold or more, 5000-fold or more, 10^4-fold or more, 10^5-fold or more, 10^6-fold or more, 10^7-fold or more, 10^8-fold or more, etc.) greater than the dissociation half-life for CD47 of a SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide that does not have an amino acid change relative to a corresponding sequence of a wild type SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide (e.g., a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide, as defined above). For example, in some cases, a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide (as defined above) has a dissociation half-life for CD47 of less than 1 second, while a subject SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide can have a dissociation half-life of 5 seconds or more (e.g., 30 seconds or more, 1 minute or more, 5 minutes or more, 10 minutes or more, 20 minutes or more, 30 minutes or more, 40 minutes or more, etc.). For example, the amino acid substitution of a subject SIRP-gamma, SIRP-beta or

SIRP-beta2 decoy polypeptide can increase the affinity by decreasing the off-rate by at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 500-fold, or more.

[0062] The affinity to bind to CD47 can be determined, for example, by the ability of SIRP-gamma, SIRP-beta or SIRP-beta2decoy polypeptide to bind to CD47 coated on an assay plate; displayed on a microbial cell surface; in solution; etc. The binding activity of SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptides of the present disclosure to CD47 can be assayed by immobilizing the ligand (e.g., CD47) or the SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide, to a bead, substrate, cell, etc. Agents can be added in an appropriate buffer and the binding partners incubated for a period of time at a given temperature. After washes to remove unbound material, the bound protein can be released with, for example, SDS, buffers with a high pH, and the like and analyzed, for example, by Surface Plasmon Resonance (SPR).

[0063] Binding can also be determined by, for example, measuring the ability of a unlabeled SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide to compete with a labeled SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide (e.g., a labeled wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide, as defined above) for binding to CD47. Accordingly, relative biding can be assessed by comparing the results using a candidate unlabeled SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide to results using an unlabeled wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide (as defined above, a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide that does not have an amino acid change relative to the corresponding sequence of a wild type SIRP-gamma, SIRP-beta or SIRP-beta2).

[0064] Any convenient method can be used to generate a subject SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide. As one example non-limiting example, mutagenesis can be performed (beginning with a wildtype SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide, or beginning with a SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide for the purpose of generating a polypeptide with even greater affinity) to generate collections of mutated SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptides. Mutagenesis can be targeted to produce changes at particular amino acids, or mutagenesis can be random. The mutated SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptides can then be screen for their ability to bind a CD47 protein. For example, a CD47 protein (or a variant of a CD47 protein, e.g., a version lacking a transmembrane domain) can be labeled (e.g., with a direct label such as a radioisotope, a fluorescent moiety, etc.; or with an indirect label such as an antigen, an affinity tag, biotin, etc.) and then can be used to contact the candidate SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptides (e.g., where the candidate SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptides can be attached to a solid surface or displayed on the membrane of a cell, e.g., a yeast cell). By varying the concentration of CD47 used, one can identify high-affinity SIRP-

gamma, SIRP-beta or SIRP-beta2 decoy polypeptides from among the candidates (i.e., from among the collection of mutated SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptides).

Methods of treatment

[0065] Provided herein, in some embodiments, are methods and uses for stimulating the immune system of an individual in need thereof comprising administration of a decoy polypeptide described herein (e.g., a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide variant). In some instances, the administration of decoy polypeptide described herein induces and/or sustains phagocytosis of a cell expressing CD47. In other instances, the administration of a decoy polypeptide described herein induces and/or sustains phagocytosis of a cell not expressing CD47. In some instances, the cell is a cancer cell, a virally infected cell, a bacterially infected cell, an autoreactive T or B cell, damaged red blood cells, arterial plaques, or fibrotic tissue. In additional instances, the cell is a healthy normal cell such as hematopoietic stem cell, a healthy myeloid or lymphoid precursor cell, or a healthy differentiated hematopoietic cell type such as T, B, plasma, or NK cells.

[0066] Also provided herein are methods and uses for treatment of cancer in an individual in need thereof comprising administration of a decoy polypeptide described herein. In some of such embodiments, the individual is suffering from cancer. In other embodiments, the individual is suspected to be suffering from cancer. In yet other embodiments, the individual is pre-disposed to cancer (e.g., an individual pre-disposed to breast cancer). In certain embodiments, the cancer is selected from the group consisting of breast cancer, lung cancer, adenocarcinoma of the lung, squamous cell lung cancer, small cell lung cancer, non-small cell lung cancer, head and neck cancer, brain cancer, abdominal cancer, colon cancer, colorectal cancer, esophageal cancer, parapharyngeal cancer, gastrointestinal cancer, glioma, liver cancer, oral cancer, tongue cancer, neuroblastoma, osteosarcoma, ovarian cancer, renal cancer, urinary bladder cancer, urinary tract cancer, pancreatic cancer, retinoblastoma, cervical cancer, uterine cancer, Wilm's tumor, multiple myeloma, skin cancer, lymphoma, leukemia, blood cancer, thyroid cancer, bone cancer, adenocystic tumor, chondrosarcoma, pancreatic islet cell tumor, neuroendocrine tumor, prostate cancer, ovarian cancer, glioblastoma, endometrial carcinoma, endometrial cancer, leiomyosarcoma, gall bladder cancer, hepatocellular cancer, hematological cancer, multiple myeloma, acute myelogenous leukemia, acute/chronic lymphoblastic leukemia, hairy-cell leukemia, follicular lymphoma, multiple myeloma, plasmacytoma, diffuse large B-cell lymphoma. In some embodiments, the cancer is a hematological cancer. In particular aspects, the cancer is multiple myeloma, acute/chronic myelogenous leukemia, acute/chronic lymphoblastic leukemia, hairy-cell leukemia, follicular lymphoma, multiple myeloma, plasmacytoma or diffuse large B-cell lymphoma.

[0067] In some embodiments, the cancer is associated with expression of CD47 including but not limited to Acute myeloid leukemia (AML), Acute leukocytic leukemia (ALL), Hodgkin's lymphoma (HL), Non-Hodgkin's B cell lymphoma (NHBCL), Chronic leukocytic leukemia (B-CLL), Multiple myeloma (MM), pancreatic adenocarcinoma, pancreatic neuroendocrine tumor (PanNET), glioma, medulloblastoma, astrocytoma, prostate cancer, osteosarcoma, small cell lung carcinoma (SCLC), non-small cell lung carcinoma (NSCLC), melanoma, squamous cell head and neck carcinoma, prostate carcinoma, ovarian cancer, breast cancer, colon cancer, renal cancer, and bladder cancer. In some embodiments, the cancer is associated with solid tumors. In certain instances, the solid tumors are advanced, e.g., stage 3 or 4. In certain instances, the solid tumors are histologically associated with the expression of the CD47.

[0068] As used herein, in some embodiments, "treatment" or "treating" or "treated" refers to therapeutic treatment wherein the object is to slow (lessen) an undesired physiological condition, disorder or disease, or to obtain beneficial or desired clinical results. For the purposes described herein, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of the condition, disorder or disease; stabilization (*i.e.*, not worsening) of the state of the condition, disorder or disease; delay in onset or slowing of the progression of the condition, disorder or disease; amelioration of the condition, disorder or disease state; and remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of the condition, disorder or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment. In other embodiments, "treatment" or "treating" or "treated" refers to prophylactic measures, wherein the object is to delay onset of or reduce severity of an undesired physiological condition, disorder or disease, such as, for example is a person who is predisposed to a disease (e.g., an individual who carries a genetic marker for a disease such as breast cancer).

[0069] The methods of treatment described herein treat various stages of cancer including stages which are locally advanced, metastatic and/or recurrent. In cancer staging, locally advanced is generally defined as cancer that has spread from a localized area to nearby tissues and/or lymph nodes. In the Roman numeral staging system, locally advanced usually is classified in Stage II or III. Cancer which is metastatic is a stage where the cancer spreads throughout the body to distant tissues and organs (stage IV). Cancer designated as recurrent generally is defined as the cancer has recurred, usually after a period of time, after being in remission or after a tumor has visibly been eliminated. Recurrence can either be local, i.e., appearing in the same location as the original, or distant, i.e., appearing in a different part of the

body. In certain instances, a cancer treatable by combination therapies described herein is unresectable, or unable to be removed by surgery.

[0070] In some of such embodiments, the methods of treatment described herein provide adjunct therapy to any other cancer therapy prescribed for an individual. Accordingly, in some embodiments, decoy polypeptides described herein are administered in combination with treatment with any other anti-cancer agent including and not limited to methotrexate (RHEUMATREX®, Amethopterin) cyclophosphamide (CYTOXAN®), thalidomide (THALIDOMID®), acridine carboxamide, actimid®, actinomycin, 17-N-allylamino-17-demethoxygeldanamycin, aminopterin, amsacrine, anthracycline, antineoplastic, antineoplaston, 5-azacytidine, azathioprine, BL22, bendamustine, biricodar, bleomycin, bortezomib, bryostatin, busulfan, calyculin, camptothecin, capecitabine, carboplatin, cetuximab, chlorambucil, cisplatin, cladribine, clofarabine, cytarabine, dacarbazine, dasatinib, daunorubicin, decitabine, dichloroacetic acid, discodermolide, docetaxel, doxorubicin, epirubicin, epothilone, eribulin, estramustine, etoposide, exatecan, exisulind, ferruginol, floxuridine, fludarabine, fluorouracil, fosfestrol, fotemustine, ganciclovir, gemcitabine, hydroxyurea, IT-101, idarubicin, ifosfamide, imiquimod, irinotecan, irofulven, ixabepilone, laniquidar, lapatinib, lenalidomide, lomustine, lurtotecan, mafosfamide, masoprocol, mechlorethamine, melphalan, mercaptopurine, mitomycin, mitotane, mitoxantrone, nelarabine, nilotinib, oblimersen, oxaliplatin, PAC-1, paclitaxel, pemetrexed, pentostatin, pipobroman, pixantrone, plicamycin, procarbazine, proteasome inhibitors (e.g., bortezomib), raltitrexed, rebeccamycin, revlimid®, rubitecan, SN-38, salinosporamide A, satraplatin, streptozotocin, swainsonine, tariquidar, taxane, tegafur-uracil, temozolomide, testolactone, thioTEPA, tioguanine, topotecan, trabectedin, tretinoin, triplatin tetranitrate, tris(2-chloroethyl)amine, troxacitabine, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine, vorinostat, zosuquidar, or the like.

[0071] In additional embodiments, the methods of treatment described herein i.e., decoy polypeptide formulations described herein are administered in combination with monoclonal antibodies such as 3F8, 8H9, Abagovomab, Abciximab, Abituzumab, Abrilumab, Actoxumab, Adalimumab, Adecatumumab, Aducanumab, Afelimomab, Afutuzumab, Alacizumab pegol, ALD518, Alemtuzumab, Alirocumab, Altumomab pentetate, Amatuximab, Anatumomab mafenatox, Anetumab ravtansine, Anifrolumab, Anrukinzumab (IMA-638), Apolizumab, Arcitumomab, Ascrinvacumab, Aselizumab, Atezolizumab, Atinumab, Atlizumab (tocilizumab), Atorolimumab, Bapineuzumab, Basiliximab, Bavituximab, Bectumomab, Begelomab, Belimumab, Benralizumab, Bertilimumab, Besilesomab, Bevacizumab, Bezlotoxumab, Biciromab, Bimagrumab, Bimekizumab, Bivatuzumab mertansine, Blinatumomab, Blosozumab, Bococizumab, Brentuximab vedotin, Briakinumab, Brodalumab,

Brolucizumab, Brontictuzumab, Canakinumab, Cantuzumab mertansine, Cantuzumab ravtansine, Caplacizumab, Capromab pendetide, Carlumab, Catumaxomab, cBR96-doxorubicin immunoconjugate, CC49, Cedelizumab, Certolizumab pegol, Cetuximab, Ch.14.18, Citatuzumab bogatox, Cixutumumab, Clazakizumab, Clenoliximab, Clivatuzumab tetraxetan, Codrituzumab, Coltuximab ravtansine, Conatumumab, Concizumab, Crenezumab, CR6261, Dacetuzumab, Daclizumab, Dalotuzumab, Dapirolizumab pegol, Daratumumab, Dectrekumab, Demcizumab, Denintuzumab mafodotin, Denosumab, Derlotuximab biotin, Detumomab, Dinutuximab, Diridavumab, Dorlimomab aritox, Drozitumab, Duligotumab, Dupilumab, Durvalumab, Dusigitumab, Ecromeximab, Eculizumab, Edobacomab, Edrecolomab, Efalizumab, Efungumab, Eldelumab, Elgemtumab, Elotuzumab, Elsilimomab, Emactuzumab, Emibetuzumab, Enavatuzumab, Enfortumab vedotin, Enlimomab pegol, Enoblituzumab, Enokizumab, Enoticumab, Ensituximab, Epitumomab cituxetan, Epratuzumab, Erlizumab, Ertumaxomab, Etaracizumab, Etrolizumab, Evinacumab, Evolocumab, Exbivirumab, Fanolesomab, Faralimomab, Farletuzumab, Fasinumab, FBTA05, Felvizumab, Fezakinumab, Ficlatuzumab, Figitumumab, Firivumab, Flanvotumab, Fletikumab, Fontolizumab, Foralumab, Foravirumab, Fresolimumab, Fulranumab, Futuximab, Galiximab, Ganitumab, Gantenerumab, Gavilimomab, Gemtuzumab ozogamicin, Gevokizumab, Girentuximab, Glembatumumab vedotin, Golimumab, Gomiliximab, Guselkumab, Ibalizumab, Ibritumomab tiuxetan, Icrucumab, Idarucizumab, Igovomab, IMAB362, Imalumab, Imciromab, Imgatuzumab, Inclacumab, Indatuximab ravtansine, Indusatumab vedotin, Infliximab, Intetumumab, Inolimomab, Inotuzumab ozogamicin, Ipilimumab, Iratumumab, Isatuximab, Itolizumab, Ixekizumab, Keliximab, Labetuzumab, Lambrolizumab, Lampalizumab, Lebrikizumab, Lemalesomab, Lenzilumab, Lerdelimumab, Lexatumumab, Libivirumab, Lifastuzumab vedotin, Ligelizumab, Lilotomab satetraxetan, Lintuzumab, Lirilumab, Lodelcizumab, Lokivetmab, Lorvotuzumab mertansine, Lucatumumab, Lulizumab pegol, Lumiliximab, Lumretuzumab, Mapatumumab, Margetuximab, Maslimomab, Mavrilimumab, Matuzumab, Mepolizumab, Metelimumab, Milatuzumab, Minretumomab, Mitumomab, Mogamulizumab, Morolimumab, Motavizumab, Moxetumomab pasudotox, Muromonab-CD3, Nacolomab tafenatox, Namilumab, Naptumomab estafenatox, Narnatumab, Natalizumab, Nebacumab, Necitumumab, Nemolizumab, Nerelimomab, Nesvacumab, Nimotuzumab, Nivolumab, Nofetumomab merpentan, Obiltoxaximab, Ocaratuzumab, Ocrelizumab, Odulimomab, Ofatumumab, Olaratumab, Olokizumab, Omalizumab, Onartuzumab, Ontuxizumab, Opicinumab, Oportuzumab monatox, Oregovomab, Orticumab, Otelixizumab, Otlertuzumab, Oxelumab, Ozanezumab, Ozoralizumab, Pagibaximab, Palivizumab, Panitumumab, Pankomab, Panobacumab, Parsatuzumab, Pascolizumab, Pasotuxizumab, Pateclizumab, Patritumab,

Pembrolizumab, Pemtumomab, Perakizumab, Pertuzumab, Pexelizumab, Pidilizumab, Pinatuzumab vedotin, Pintumomab, Placulumab, Polatuzumab vedotin, Ponezumab, Priliximab, Pritoxaximab, Pritumumab, PRO 140, Quilizumab, Racotumomab, Radretumab, Rafivirumab, Ralpancizumab, Ramucirumab, Ranibizumab, Raxibacumab, Refanezumab, Regavirumab, Reslizumab, Rilotumumab, Rinucumab, Rituximab, Robatumumab, Roledumab, Romosozumab, Rontalizumab, Rovelizumab, Ruplizumab, Samalizumab, Sarilumab, Satumomab pendetide, Secukinumab, Seribantumab, Setoxaximab, Sevirumab, Sibrotuzumab, SGN-CD19A, SGN-CD33A, Sifalimumab, Siltuximab, Simtuzumab, Siplizumab, Sirukumab, Sofituzumab vedotin, Solanezumab, Solitomab, Sonepcizumab, Sontuzumab, Stamulumab, Sulesomab, Suvizumab, Tabalumab, Tacatuzumab tetraxetan, Tadocizumab, Talizumab, Tanezumab, Taplitumomab paptox, Tarextumab, Tefibazumab, Telimomab aritox, Tenatumomab, Teneliximab, Teplizumab, Teprotumumab, Tesidolumab, TGN1412, Ticilimumab (tremelimumab), Tildrakizumab, Tigatuzumab, TNX-650, Tocilizumab (atlizumab), Toralizumab, Tosatoxumab, Tositumomab, Tovetumab, Tralokinumab, Trastuzumab, TRBS07, Tregalizumab, Tremelimumab, Tucotuzumab celmoleukin, Tuvirumab, Ublituximab, Ulocuplumab, Urelumab, Urtoxazumab, Ustekinumab, Vandortuzumab vedotin, Vantictumab, Vanucizumab, Vapaliximab, Varlilumab, Vatelizumab, Vedolizumab, Veltuzumab, Vepalimomab, Vesencumab, Visilizumab, Volociximab, Vorsetuzumab mafodotin, Votumumab, Zalutumumab, Zanolimumab, Zatuximab, Ziralimumab, or Zolimomab aritox.

[0072] In additional embodiments, the methods of treatment described herein i.e., decoy polypeptide formulations described herein are administered in combination with a monoclonal antibody targeting one or more of VWF, vimentin, VEGFR2, VEGFR-1, VEGF-A, TYRP1(glycoprotein 75), TWEAK receptor, tumor specific glycosylation of MUC1, tumor antigen CTAA16.88, TRAIL-R2, TRAIL-R1, TNF-α, TGF-β, TGF beta 2, TGF beta 1, TFPI, tenascin C, TEM1, TAG-72, T-cell receptor, STEAP1, sphingosine-1-phosphate, SOST, SLAMF7, selectin P, SDC1, sclerostin, RTN4, RON, Rhesus factor, RHD, respiratory syncytial virus, RANKL, rabies virus glycoprotein, platelet-derived growth factor receptor beta, phosphatidylserine, phosphate-sodium co-transporter, PDGF-R α, PDCD1, PD-1, PCSK9, oxLDL, OX-40, NRP1, Notch receptor 4, Notch receptor 3, Notch receptor 2, Notch receptor 1, NOGO-A, NGF, neural apoptosis-regulated proteinase 1, NCA-90 (granulocyte antigen), NARP-1, N-glycolylneuraminic acid, myostatin, myelin-associated glycoprotein, mucin CanAg, MUC1, MSLN, MS4A1, MIF, mesothelin, MCP-1, LTA, LOXL2, lipoteichoic acid, LINGO-1, LFA-1 (CD11a), Lewis-Y antigen, L-selectin (CD62L), KIR2D, ITGB2 (CD18), ITGA2, interferon α/β receptor, interferon receptor, interferon gamma-induced protein, integrin αvβ3, integrin αIIbβ3, integrin α7β7, integrin α5β1, integrin α4β7, integrin α4, insulin-like growth

factor I receptor, Influenza A hemagglutinin, ILGF2, IL9, IL6, IL4, IL31RA, IL23, IL17A, IL-6 receptor, IL-6, IL-5, IL-4, IL-23, IL-22, IL-1β, IL-17A, IL-17, IL-13, IL-12, IL-1, IL 20, IGHE, IgG4, IGF-I, IGF-1 receptor, IgE Fc region, IFN-γ, IFN-α, ICAM-1 (CD54), human TNF, human scatter factor receptor kinase, Hsp90, HNGF, HLA-DR, HIV-1, histone complex, HHGFR, HGF, HER3, HER2/neu, HER1, hepatitis B surface antigen, hemagglutinin, GUCY2C, GPNMB, GMCSF receptor α-chain, glypican 3, GD3 ganglioside, GD2, ganglioside GD2, Frizzled receptor, folate receptor 1, folate hydrolase, fibronectin extra domain-B, fibrin II, beta chain, FAP, F protein of respiratory syncytial virus, ERBB3, episialin, EpCAM, endotoxin, EGFR, EGFL7, E. coli shiga toxin type-2, E. coli shiga toxin type-1, DR5, DPP4, DLL4, dabigatran, cytomegalovirus glycoprotein B, CTLA-4, CSF2, CSF1R, clumping factor A, CLDN18.2, ch4D5, CFD, CEA-related antigen, CEA, CD80, CD79B, CD74, CD70, CD6, CD56, CD52, CD51, CD5, CD44 v6, CD41, CD40 ligand, CD40, CD4, CD38, CD37, CD33, CD30 (TNFRSF8), CD3 epsilon, CD3, CD28, CD274, CD27, CD25 (α chain of IL-2 receptor), CD23 (IgE receptor), CD221, CD22, CD200, CD20, CD2, CD19, CD154, CD152, CD15, CD147 (basigin), CD140a, CD125, CD11, CD-18, CCR5, CCR4, CCL11 (eotaxin-1), cardiac myosin, carbonic anhydrase 9 (CA-IX), Canis lupus familiaris IL31, CA-125, C5, C242 antigen, C-X-C chemokine receptor type 4, beta-amyloid, BAFF, B7-H3, B-lymphoma cell, AOC3 (VAP-1), anthrax toxin, protective antigen, angiopoietin 3, angiopoietin 2, alpha-fetoprotein, AGS-22M6, adenocarcinoma antigen, ACVR2B, activin receptor-like kinase 1, 5T4, 5AC, 4-1BB or 1-40-β-amyloid.

[0073] In additional embodiments, the methods of treatment described herein i.e., decoy polypeptide formulations described herein are administered in combination with radiotherapy (e.g., γ-rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells, microwaves, UV radiation and the like. In additional embodiments, the methods of treatment described herein i.e., decoy polypeptides described herein are administered in combination with gene therapy. Therapeutic genes include an antisense version of an inducer of cellular proliferation (oncogene), an inhibitor of cellular proliferation (tumor suppressor), or an inducer of programmed cell death (pro-apoptotic gene). In some embodiments, the combination therapies described herein are administered with a surgery (e.g., resection).

[0074] In additional embodiments, the methods of treatment described herein i.e., decoy polypeptides described herein are administered in combination with anti-diarrheal agents, anti-emetic agents, analgesics, opioids and/or non-steroidal anti-inflammatory agents.

[0075] In further embodiments, decoy polypeptide described herein (e.g., a composition comprising a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide) is administered to an individual who has been pre-treated with cyclophosphamide, or imitanib, or daclizumab and/or

any other anti-cancer agent. In other embodiments, a decoy polypeptide described herein is administered to an individual who has not been pre-treated with cyclophosphamide and/or any other anti-cancer agent.

[0076] In some of the above embodiments, treatment with a decoy polypeptide described herein (e.g., a composition comprising a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide) prolongs lifespan and/or increases survival rates for individuals suffering from cancer. In some of the above embodiments, treatment with a decoy polypeptide described herein (e.g., a composition comprising a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide) improves quality of life for an individual suffering from cancer (e.g., an individual needs a lower dose of an anti-cancer drug that causes side-effects when the individual is treated with a decoy polypeptide described herein).

[0077] In some of the above embodiments, treatment with a decoy polypeptide described herein (e.g., a composition comprising a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide) induces and/or sustains phagocytosis or ADCC in an individual. Phagocytosis includes phagocytosis by professional phagocytes (e.g. monocytes, macrophages, neutrophils, dendritic cells or mast cells), non-professional phagocytes (e.g. epithelial cells, endothelial cells, fibroblasts or mesenchymal cells) or both. ADCC includes antibody dependence cell-mediated cytotoxicity by myeloid cells including neutrophils, monocytes, and natural killer cells. Measurement of phagocytosis and ADCC is accomplished by any known method including, for example, fluorescence microscopy or flow cytometry.

[0078] In further embodiments, treatment with a decoy polypeptide described herein (e.g., a composition comprising a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide) induces and/or enhances antibody-dependent cell-mediated phagocytosis (ADCP) or ADCC of IgE producing B and plasma cells by combining the SIRP-beta or SIRP-gamma agents with antibodies against M1prime or CD38 in an individual with asthma or allergy.

[0079] Also provided herein are methods for treating a viral infection, disorder or condition in an individual comprising administering to an individual having a viral infection, disorder or condition a decoy polypeptide described herein. In particular aspects, the viral infection, disorder or condition is chronic. In further aspects, the viral infection, disorder or condition is acute. In further aspects, the viral infection, disorder or condition is an Adenoviridae such as, Adenovirus; a Herpesviridae such as Herpes simplex, type 1, Herpes simplex, type 2, Varicella-zoster virus, Epstein-barr virus, Human cytomegalovirus, Human herpesvirus, type 8; a Papillomaviridae such as Human papillomavirus; a Polyomaviridae such as BK virus or JC virus; a Poxviridae such as Smallpox; a Hepadnaviridae such as Hepatitis B virus; a Parvoviridae such as Human bocavirus or Parvovirus; a Astroviridae such as Human astrovirus;

a Caliciviridae such as Norwalk virus; a Picornaviridae such as coxsackievirus, hepatitis A virus, poliovirus, rhinovirus; a Coronaviridae such as Severe acute respiratory syndrome virus; a Flaviviridae such as Hepatitis C virus, yellow fever virus, dengue virus, West Nile virus; a Togaviridae such as Rubella virus; a Hepeviridae such as Hepatitis E virus; a Retroviridae such as Human immunodeficiency virus (HIV); a Orthomyxoviridae such as Influenza virus; a Arenaviridae such as Guanarito virus, Junin virus, Lassa virus, Machupo virus, Sabiá virus; a Bunyaviridae such as Crimean-Congo hemorrhagic fever virus ; a Filoviridae such as Ebola virus, Marburg virus; a Paramyxoviridae such as Measles virus, Mumps virus, Parainfluenza virus, Respiratory syncytial virus, Human metapneumovirus, Hendra virus, Nipah virus; a Rhabdoviridae such as Rabies virus; Hepatitis D virus; or a Reoviridae such as Rotavirus, Orbivirus, Coltivirus, Banna virus. In particular aspects, the viral infection, disorder or condition is Human immunodeficiency virus (HIV), Human cytomegalovirus, Epstein-barr virus, Hepatitis C virus, or Hepatitis B virus.

[0080] Also provided herein are methods for treating a bacterial infection, disorder or condition in an individual comprising administering to an individual having a bacterial infection, disorder or condition a decoy polypeptide described herein. In particular aspects, the bacterial infection, disorder or condition is chronic. In other aspects, the bacterial infection, disorder or condition is acute. In further aspects the bacterial infection is a Bacillus such as Bacillus anthracis or Bacillus cereus; a Bartonella such as Bartonella henselae or Bartonella quintana; a Bordetella such as Bordetella pertussis; a Borrelia such as Borrelia burgdorferi, Borrelia garinii, Borrelia afzelii, Borrelia recurrentis; a Brucella such as Brucella abortus, a Brucella canis, Brucella melitensis or Brucella suis; a Campylobacter such as Campylobacter jejuni; a Chlamydia or Chlamydophila such as Chlamydia pneumoniae, Chlamydia trachomatis, Chlamydophila psittaci; a Clostridium such as Clostridium botulinum, a Clostridium difficile, Clostridium perfringens, Clostridium tetani; a Corynebacterium such as Corynebacterium diphtheriae; an Enterococcus such as Enterococcus faecalis or Enterococcus faecium; a Escherichia such as Escherichia coli; a Francisella such as Francisella tularensis; a Haemophilus such as Haemophilus influenzae; a Helicobacter such as Helicobacter pylori; a Legionella such as Legionella pneumophila; a Leptospira such as Leptospira interrogans, Leptospira santarosai, Leptospira weilii or Leptospira noguchii; a Listeria such as Listeria monocytogenes; a Mycobacterium such as Mycobacterium leprae, Mycobacterium tuberculosis or Mycobacterium ulcerans; a Mycoplasma such as Mycoplasma pneumoniae; a Neisseria such as Neisseria gonorrhoeae or Neisseria meningitidis; a Pseudomonas such as Pseudomonas aeruginosa; a Rickettsia such as Rickettsia rickettsii; a Salmonella such as Salmonella typhi or Salmonella typhimurium; a Shigella such as Shigella sonnei; a Staphylococcus such as Staphylococcus

aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus; a Streptococcus such as Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes; a Treponema such as Treponema pallidum; a Vibrio such as Vibrio cholerae; a Yersinia such as Yersinia pestis, Yersinia enterocolitica or Yersinia pseudotuberculosis.

[0081] Also provided herein are methods for treating anemia in an individual comprising administering to an individual having anemia a decoy polypeptide described herein. In particular embodiments the anemia is a thalassemia, an aplastic anemia, a haemolytic anemia, a sickle cell anemia, a pernicious anemia or a fanconi anemia.

[0082] Also provided herein are methods for treating a person undergoing a transplant comprising administering to an individual undergoing an organ transplant a decoy polypeptide described herein. In particular aspects, the transplant is a heart, a lung, a heart and lung, a kidney, a liver, a pancreas, an intestine, a stomach, a testis, a hand, a cornea, skin, islets of Langerhans, bone marrow, stem cells, blood, a blood vessel, a heart valve , or a bone.

[0083] Also provided herein are methods for treating a person with autoimmune disease comprising administering to an individual with autoimmune disease a decoy polypeptide described herein. In particular aspects, the autoimmune disease is Acute Disseminated Encephalomyelitis (ADEM), Acute necrotizing hemorrhagic leukoencephalitis, Addison's disease, Agammaglobulinemia, Alopecia areata, Amyloidosis, Ankylosing spondylitis, Anti-GBM/Anti-TBM nephritis, Antiphospholipid syndrome (APS), Autoimmune angioedema, Autoimmune aplastic anemia, Autoimmune dysautonomia, Autoimmune hepatitis, Autoimmune hyperlipidemia, Autoimmune immunodeficiency, Autoimmune inner ear disease (AIED), Autoimmune myocarditis, Autoimmune oophoritis, Autoimmune pancreatitis, Autoimmune retinopathy, Autoimmune thrombocytopenic purpura (ATP), Autoimmune thyroid disease, Autoimmune urticaria, Axonal & neuronal neuropathies, Balo disease, Behcet's disease, Bullous pemphigoid, Cardiomyopathy, Castleman disease, Celiac disease, Chagas disease, Chronic fatigue syndrome, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic recurrent multifocal ostomyelitis (CRMO), Churg-Strauss syndrome, Cicatricial pemphigoid/benign mucosal pemphigoid, Crohn's disease, Cogans syndrome, Cold agglutinin disease, Congenital heart block, Coxsackie myocarditis, CREST disease, Essential mixed cryoglobulinemia, Demyelinating neuropathies, Dermatitis herpetiformis, Dermatomyositis, Devic's disease (neuromyelitis optica), Discoid lupus, Dressler's syndrome, Endometriosis, Eosinophilic, esophagitis, Eosinophilic fasciitis, Erythema nodosum, Experimental allergic encephalomyelitis, Evans syndrome, Fibromyalgia, Fibrosing alveolitis, Giant cell arteritis (temporal arteritis), Giant cell myocarditis, Glomerulonephritis, Goodpasture's syndrome, Granulomatosis with Polyangiitis (GPA) (formerly called Wegener's Granulomatosis), Graves'

disease, Guillain-Barre syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, Hemolytic anemia, Henoch-Schonlein purpura, Herpes gestationis, Hypogammaglobulinemia, Idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, IgG4-related sclerosing disease, Immunoregulatory lipoproteins, Inclusion body myositis, Interstitial cystitis, Juvenile arthritis, Juvenile diabetes (Type 1 diabetes), Juvenile myositis, Kawasaki syndrome, Lambert-Eaton syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous conjunctivitis, Linear IgA disease (LAD), Lupus (SLE), Lyme disease, chronic, Meniere's disease, Microscopic polyangiitis, Mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, Multiple sclerosis, Myasthenia gravis, Myositis, Narcolepsy, Neuromyelitis optica (Devic's), Neutropenia, Ocular cicatricial pemphigoid, Optic neuritis, Palindromic rheumatism, PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus), Paraneoplastic cerebellar degeneration, Paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Parsonnage-Turner syndrome, Pars planitis (peripheral uveitis), Pemphigus, Peripheral neuropathy, Perivenous encephalomyelitis, Pernicious anemia, POEMS syndrome, Polyarteritis nodosa, Type I, II, & III autoimmune polyglandular syndromes, Polymyalgia rheumatica, Polymyositis, Postmyocardial infarction syndrome, Postpericardiotomy syndrome, Progesterone dermatitis, Primary biliary cirrhosis, Primary sclerosing cholangitis, Psoriasis, Psoriatic arthritis, Idiopathic pulmonary fibrosis, Pyoderma gangrenosum, Pure red cell aplasia, Raynauds phenomenon, Reactive Arthritis, Reflex sympathetic dystrophy, Reiter's syndrome, Relapsing polychondritis, Restless legs syndrome, Retroperitoneal fibrosis, Rheumatic fever, Rheumatoid arthritis, Sarcoidosis, Schmidt syndrome, Scleritis, Scleroderma, Sjogren's syndrome, Sperm & testicular autoimmunity, Stiff person syndrome, Subacute bacterial endocarditis (SBE), Susac's syndrome, Sympathetic ophthalmia, Takayasu's arteritis, Temporal arteritis/Giant cell arteritis, Thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome, Transverse myelitis, Type 1 diabetes, Ulcerative colitis, Undifferentiated connective tissue disease (UCTD), Uveitis, Vasculitis, Vesiculobullous dermatosis, Vitiligo or Wegener's granulomatosis (now termed Granulomatosis with Polyangiitis (GPA)). The diseases described above fall into this category; depletion of autoreactive T or B cells may both be part of a regimen to treat the listed autoimmune diseases.

Methods of visualization

[0084] Also provided herein are methods for visualizing a cell expressing CD47 comprising contacting a population of cells with a decoy polypeptide described herein and a detectable label. In some embodiments of the methods described herein the methods include visualization of a tumor cell, a virally infected cell, a bacterially infected cell, an autoreactive T cell, damaged red blood cells, arterial plaques, or fibrotic tissue. In additional embodiments, the cell is a

healthy normal cell such as hematopoietic stem cell, a healthy myeloid or lymphoid precursor cell, or a healthy differentiated hematopoietic cell type such as T, B, plasma, or NK cells. In additional embodiments of the methods described herein the methods include visualization of a cell in vivo, visualization of a cell ex vivo or visualization of a cell in vitro. In particular aspects, the method is microscopy, fluorescent microscopy, fluorescence activated cell sorting or positron emission tomography (PET) imaging. In particular aspects, the detectable label comprises an enzymatic label such as horseradish peroxidase (HRP), alkaline phosphatase (AP) or glucose oxidase. In additional aspects, the detectable label comprises a fluorescent label such as Alexa Fluor® 350, Alexa Fluor® 405, Alexa Fluor® 488, Alexa Fluor® 532, Alexa Fluor® 546, Alexa Fluor® 555, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 647, Alexa Fluor® 680, Alexa Fluor® 750, BODIPY® FL, Coumarin, Cy®3, Cy®5, Fluorescein (FITC), Oregon Green®, Pacific Blue™, Pacific Green™, Pacific Orange™, Tetramethylrhodamine (TRITC), Texas Red® or other fluorescent label. In further aspects, the detectable label comprises a radioactive isotope such as 32P, 33P, 3H, 14C, 125I or other radioactive isotope. In further aspects, the method is a diagnostic.

Dosages

[0085] When a SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide described herein, (e.g., a variant SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide), is being given to an individual, one of skill in the art understands that the dosage depends on several factors, including, but not limited to, the individual's weight and disease state. Generally, as used herein, an individual that receives a SIRP-gamma, SIRP-beta or SIRP-beta2 decoy polypeptide described herein (e.g., a composition comprising a variant SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide), is a single organism. In certain embodiments, an individual will be a mammal. Specifically, an individual is a human, including being a male or a female. In many embodiments, the individual will be a patient, or an individual awaiting or under medical care and treatment.

[0086] In some embodiments, an individual is administered a dose normalized to the body weight of the individual. In some embodiments, an individual is administered a dose of about 10μg/kg, about 50 μg/kg, about 100 μg/kg, about 200 μg/kg, about 300 μg/kg, about 400 μg/kg, about 500 μg/kg, about 600 μg/kg, about 700 μg/kg, about 800 μg/kg, about 900 μg/kg, about 1,000 μg/kg, about 1,100 μg/kg, 1,200 μg/kg, 1,300 μg/kg, 1,400 μg/kg, 1,500 μg/kg, 1,600 μg/kg, 1,700 μg/kg, 1,800 μg/kg, 1,900 μg/kg, about 2,000 μg/kg, about 3000 μg/kg, about 4000 μg/kg, about 5000 μg/kg, about 6000 μg/kg, about 7000 μg/kg, about 8000 μg/kg, about 9000 μg/kg, about 10 mg/kg, about 20 mg/kg, about 30 mg/kg, about 40 mg/kg, about 50 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg about 90 mg/kg, about 100 mg/kg, about 200

mg/kg, about 300 mg/kg, about 400 mg/kg, about 500 mg/kg, about 600 mg/kg, about 700 mg/kg, about 800 mg/kg, about 900 mg/kg, or about 1000 mg/kg of a decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide described herein (e.g., composition comprising a variant SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide), in either single or cumulative applications. In specific embodiments, the dose given to the individual is about 7000 mg/kg of decoy polypeptide per week. In specific embodiments, the dose given to the individual is about 70 mg/kg of decoy polypeptide per week. In specific embodiments, the dose given to the individual is about 7 mg/kg of decoy polypeptide per week. In specific embodiments, the dose given to the individual is about 1,000 μg of decoy polypeptide per week. In specific embodiments, the dose given to the individual is 500 μg of decoy polypeptide per week. In specific embodiments, the dose given to the individual is 250 μg of decoy polypeptide per week. In specific embodiments, the dose given to the individual is 100 μg of decoy polypeptide per week. In specific embodiments, the dose given to the individual is 50 μg of decoy polypeptide per week.

[0087] In some embodiments, an individual will receive a dose of the decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide described herein (e.g., a composition comprising a variant SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide), for example, multiple times daily, every day, every other day, once a week, once every other week, once every three weeks, once per month or any other suitable dosing regimen. In one embodiment, an individual will receive a dose of the decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide as a continuous infusion. In one embodiment, routinely administering encompasses administering a dose of a decoy polypeptide described herein once a week for a period of time. Of course, the dosing regimen optionally comprises other permutations of decoy polypeptide delivery. That is, the decoy polypeptide is administered once, twice, three times, four times, five times, six times, or more times a week at a physician's discretion. In some embodiments, individuals will be given at least 5 doses over a period of time. In other embodiments, individuals will be given greater than or fewer than 5 doses. Thus, in one embodiment, an individual will receive a dose of about 10 mg/kg of the decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide every week. Alternatively, the individual will receive two doses of 5 mg/kg twice a week, or a daily 2 mg/kg dose over five days.

[0088] These dosage examples are not limiting and only used to exemplify particular dosing regimens for administering about 10 mg/kg of the decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide. For instance, if the appropriate dose for a given situation is 10 mg/kg per week, the doses is optionally broken down into any number of permutations, e.g., four injections of 2.5 mg/kg per week. This also holds true if the appropriate dose for a particular situation is greater than or less than 10 mg/kg.

[0089] In some embodiments, the period of time that a decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide (e.g., a composition comprising a variant SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide), is administered to the individual is any suitable period as determined by the stage of the disease, the patient's medical history and the attending physician's discretion. Examples of such suitable periods include, but are not limited to, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 13 months, at least about 14 months, at least about 15 months, at least about 16 months, at least about 17 months, at least about 18 months, at least about 19 months, at least about 20 months, at least about 21 months, at least about 22 months, at least about 23 months, or at least about 24 months or longer. In particular aspects, the treatment period is continued for longer than 24 months, if desired, such as for 30 months, 31 months, 32 months, 33 months, 34 months, 35 months, 36 months, or longer than 36 months. In some embodiments, the period is 6 months, 1 year or 2 years.

[0090] In another embodiment, the period of time of dosing for any of the methods described herein is for at least about 2 weeks, at least about 4 weeks, at least about 8 weeks, at least about 16 weeks, at least about 17 weeks, at least about 18 weeks, at least about 19 weeks, at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 60 weeks, at least about 68 weeks, at least about 72 weeks, at least about 80 weeks, at least about 88 weeks, at least about 96 weeks, or at least about 104 weeks.

[0091] In some embodiments, any decoy polypeptide described herein (e.g., a composition comprising a variant SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide), is administered in different phases of treatment. For example, the decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide is administered in both a treatment phase and a maintenance phase. In some embodiments, the treatment phase will comprise administration of the decoy polypeptide formulation in weekly dosages, whereas the maintenance phase is for longer time periods, such as about every 6 weeks, about every 7 weeks, about every 8 weeks, about every 9 weeks, about every 10 weeks, about every 11 weeks, about every 12 weeks, or longer. In some cases, the dosage given in the treatment phase will be greater than the dosage given in the maintenance phase. However, treatment and maintenance phases are designed to a particular individual so the time and dosages between the treatment and maintenance phases vary from the above examples. Generally, the maintenance phase begins at any time deemed appropriate. For example, in some embodiments, the treatment phase will be eight weeks and the maintenance phase will continue

throughout the individual's lifetime. In other embodiments, only a treatment or a maintenance phase will be undertaken.

[0092] In yet further embodiments, decoy polypeptide described herein (e.g., a composition comprising a variant SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide), is given prophylactically. In these embodiments, the administration of decoy polypeptide prevents onset of disease in an individual (e.g., an individual genetically pre-disposed to developing cancer, such as breast cancer; an individual predisposed to developing a bacterial or viral infection; an individual about to undergo an organ transplant; or an individual predisposed to developing anemia or autoimmune disease.)

[0093] The amount of time that an individual should remain on a decoy polypeptide described herein is determined by the attending physician. In some cases, it is advantageous to administer the decoy polypeptide for the rest of an individual's lifetime. In some of such embodiments, a decoy polypeptide is administered in four quadrants of the body, e.g., near lymph nodes, (e.g., in each armpit), in each buttock (e.g., subcutaneously) and the like. In some of such embodiments, a decoy polypeptide is administered via a pump. In some embodiments, a pump and/or delivery device is implanted in an individual to allow chronic dosing. Examples of implantable pumps include and are not limited to Alzet® osmotic pumps.

Kits

[0094] Provided herein are kits for dispensing the decoy polypeptides described herein. Such kits comprise a first drug product vial containing a decoy polypeptide comprising a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide, and a second vial containing a suitable sterile liquid as described herein for reconstitution. In some embodiments

[0095] For example, in one embodiment, such kits comprise a first vial, i.e., a drug product vial containing 300μg of a decoy polypeptide comprising a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide, which represents a 120% fill. This excess is intended to facilitate the withdrawal and administration of the specified dose. The kit further comprises a second vial containing up to 1 mL of 0.9% sodium chloride solution for injection. After reconstitution of the drug product with 0.6mL of sodium chloride solution for injection (0.9% w/v), a drug product vial yields 0.5mL for delivery corresponding to 250μg of a decoy polypeptide comprising a SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide. By way of example, if the dose is 1mg total, 4 vials are required per dose.

Certain definitions

[0096] The terms “decoy ,“ “inhibitor” and “blocking agent” as used herein refer to molecules that prevent binding of CD47 to its binding partner. For development purposes the binding may be performed under experimental conditions, e.g. using isolated proteins as binding partners,

using portions of proteins as binding partners, using yeast display of proteins or portions of proteins as binding partners, and the like. For physiologically relevant purposes the binding of CD47 to its binding partner is often an event between two cells, where each cell expresses one of the binding partners. Of particular interest is the expression of SIRP polypeptides on phagocytotic cells, such as macrophages; and the expression of CD47 on cells that could be targets for phagocytosis, e.g. tumor cells, circulating hematopoietic cells, and the like. Inhibitors may be identified using in vitro and in vivo assays for receptor or ligand binding or signaling.

[0097] The terms “polypeptide,” “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms also apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers.

[0098] The term "amino acid" as used herein refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine. The term “amino acid analogs” as used herein refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an .alpha. carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. The term “amino acid mimetics” as used herein refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0099] The terms “recipient”, “individual”, “subject”, “host”, and “patient”, are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. "Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and laboratory, zoo, sports, or pet animals, such as dogs, horses, cats, cows, sheep, goats, pigs, mice, rats, rabbits, guinea pigs, monkeys etc. In some embodiments, the mammal is human.

[00100] As used herein “cancer” includes any form of cancer, including but not limited to solid tumor cancers (e.g., lung, prostate, breast, bladder, colon, ovarian, pancreas, kidney, liver, glioblastoma, medulloblastoma, leiomyosarcoma, head & neck squamous cell carcinomas, melanomas, neuroendocrine; etc.) and liquid cancers (e.g., hematological cancers); carcinomas;

soft tissue tumors; sarcomas; teratomas; melanomas; leukemias; lymphomas; and brain cancers, including minimal residual disease, and including both primary and metastatic tumors. Any cancer is a suitable cancer to be treated by the subject methods and compositions.

[00101] The term “binding partner” as used herein refers to a member of a specific binding pair (i.e., two molecules, usually two different molecules, where one of the molecules, e.g., a first binding partner, through non-covalent means specifically binds to the other molecule, e.g., a second binding partner).

[00102] As used herein, the terms "treatment," "treating," and the like, refer to administering an agent, or carrying out a procedure, for the purposes of obtaining an effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of effecting a partial or complete cure for a disease and/or symptoms of the disease. "Treatment," as used herein, may include treatment of a tumor in a mammal, particularly in a human, and includes: (a) preventing the disease or a symptom of a disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it (e.g., including diseases that may be associated with or caused by a primary disease; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease. Treating may refer to any indicia of success in the treatment or amelioration or prevention of an cancer, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the disease condition more tolerable to the patient; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of an examination by a physician. Accordingly, the term "treating" includes the administration of the compounds or agents of the present invention to prevent or delay, to alleviate, or to arrest or inhibit development of the symptoms or conditions associated with cancer or other diseases. The term "therapeutic effect" refers to the reduction, elimination, or prevention of the disease, symptoms of the disease, or side effects of the disease in the subject.

[00103] "In combination with", "combination therapy" and "combination products" refer, in certain embodiments, to the concurrent administration to a patient of a first therapeutic and the compounds as used herein. When administered in combination, each component can be administered at the same time or sequentially in any order at different points in time. Thus, each component can be administered separately but sufficiently closely in time so as to provide the desired therapeutic effect.

[00104] "Dosage unit" refers to physically discrete units suited as unitary dosages for the particular individual to be treated. Each unit can contain a predetermined quantity of active

compound(s) calculated to produce the desired therapeutic effect(s) in association with the required pharmaceutical carrier. The specification for the dosage unit forms can be dictated by (a) the unique characteristics of the active compound(s) and the particular therapeutic effect(s) to be achieved, and (b) the limitations inherent in the art of compounding such active compound(s).

[00105] "Pharmaceutically acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients can be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous.

[00106] The terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a human without the production of undesirable physiological effects to a degree that would prohibit administration of the composition.

[00107] A "therapeutically effective amount" means the amount that, when administered to a subject for treating a disease, is sufficient to effect treatment for that disease.

[00108] The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), peptibodies, human antibodies, humanized antibodies, camelid antibodies (including camelid single domain antibodies), alternative scaffold antibodies (e.g., affibodies, avimers, Fn3 domains, DARPins, Kunitz domains, SMIPs, Domain antibodies, BiTEs, Adnectins, Nanobodies, Stable scFvs, Anticalins) and antibody fragments so long as they exhibit the desired biological activity. "Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity.

EXAMPLES

Example 1: Methods

[00109] *Protein expression and purification*. The human CD47 lgSF domain (residues 1-117), with a C15G mutation and C-terminal 8· histidine tag (SEQ ID NO: 40), were secreted from Trichoplusia in (Hi-5) cells using baculovirus and purified by Ni-NTA. Monomeric SIRP-gamma, SIRP-beta and SIRP-beta 2 variants were expressed as MBP-fusions in the periplasm of BL-21 (DE3) E. coli using a modified pMal-p2X expression vector (New England Biolabs) containing a rhinovirus 3C protease cleavage site after the MBP tag and a C-terminal 8xhistidine tag (SEQ ID NO: 40). Cells were induced at an OD-600 of 0.8 with 1 mM IPTG and incubated

with shaking at 22° C for 24 hours. Periplasmic protein was obtained by osmotic shock and the MBP-fusion proteins purified using nickel-nitrilotriacetic acid (Ni-NTA) chromatography. Eluted proteins were digested with 3C protease at 4°C for 12 hours to remove MBP and further purified by an additional Ni-NTA chromatography step. Endotoxin was removed using Triton X-114 as previously described and endotoxin removal confirmed using the ToxinSensor Chromogenic LAL Endotoxin Assay Kit (Genscript). SIRP-gamma, SIRP-beta and SIRP-beta2-Fc fusions were produced by cloning SIRP-gamma, SIRP-beta and SIRP-beta2 variants into a modified pFUSE-hlgG4-Fc vector (Invivogen) with an IL-2 signal sequence and engineered Ser228 Pro mutation. Proteins were expressed by transient transfection in Freestyle 293-F cells (Invitrogen) and purified over HiTrap Protein A columns (GE Healthcare).

[00110] Biotinylated proteins were obtained by expression with a carboxy-terminal biotin acceptor peptide tag (GLNDIFEAQKIEWHE (SEQ ID NO: 41)) and purified as described above. The purified proteins were biotinylated in vitro with BirA ligase and then repurified from the reaction mixture by Ni-NTA chromatography.

[00111] *Yeast display and library generation of SIRP-gamma, SIRP-beta or SIRP-beta2 variants*. The N-terminal V-set domains of SIRP-gamma, SIRP-beta or SIRP-beta2 were displayed on the surface of S. cerevisiae strain BJ5465 in the pYDS649HM vector as described. The libraries were generated by assembly PCR reactions that randomized the CD47-contact residues and the hydrophobic 'core' residues of SIRP-gamma, SIRP-beta or SIRP-beta2 using the primer sets with degenerate codons. The PCRs were further amplified with primers containing homology to the pYDS649HM vector, combined with linearized pYDS649HM vector DNA, and co-electroporated into BJ5465 yeast. The resulting libraries contained 4.0-8.0x10^8 transformants.

[00112] *Selection of library.* Transformed yeast were expanded in SD-W liquid media at 30 °C and induced in SG-W liquid medium at 20 °C. All media were supplemented with 100 mM maltose to prevent flocculation. All selection steps were carried out at 4 °C. For the first round of selection, 8 x 10^9 induced yeast, representing ten-fold coverage of the number of library transformants, were resuspended in 5 ml PBE (phosphate buffered saline supplemented with 0.5 % bovine serum albumin and 0.5 mM EDTA). Yeast were mixed with 500µl paramagnetic streptavidin microbeads (Miltenyi) that are pre-coated with biotinylated CD47 and the mixture was incubated with rotation for one hour. The yeast were pelleted by centrifugation at 5,000 x g for five minutes and washed twice with 1 ml PBE. Magnetically-labeled yeast were resuspended in 5 ml PBE and separated with an LS MAGS column according to the manufacturer's instructions (Miltenyi). Eluted yeast were pelleted, resuspended in SD-W medium, and expanded for the next round of selection. Additional rounds of selection were performed similarly to the first round with the following modifications: 1 x10^8 yeast were resuspended in

500 µl PBE containing Alexa Fluor 488-labeled anti-HA antibody (Cell Signaling) or successively decreasing concentrations of biotinylated CD47 protein, from 100 nM to 1 nM. After incubation for one hour, yeast were washed with PBE and for selections with CD47, labeled with streptavidin-PE (Invitrogen) or streptavidin-Alexa Fluor 647 (produced in house) for 15 minutes. Yeast were washed twice more with PBE and magnetically labeled with 50 µl of the appropriate anti-fluorophore microbeads (anti-FITC, anti-PE, or anti-Alexa Fluor 647; Miltenyi) for 15 minutes. Yeast were washed once, resuspended in 3 ml PBE, and separated with an LS column as in the first round.

[00113] For the final rounds of selection, kinetic selection was performed. Briefly, yeast were stained with 10 nM biotinylated CD47 for one hour, washed with PBE, and then resuspended in 500 µl PBE containing 1 µM nonbiotinylated CD47. The cells were incubated at 25 °C for 300 minutes, after which they were washed with ice-cold PBE and stained with fluorescently labeled streptavidin. Yeast were then co-labeled with Alexa Fluor 488 labeled anti-HA and streptavidin-Alexa Fluor 647 and selected with FACS cell sorter.

[00114] *Surface plasmon resonance (SPR).* Experiments were conducted with a Biacore T100 at 25°C. Protein concentrations were quantified by 280 nm absorbance with a Nanodrop2000 spectrometer (Thermo Scientific). A Biacore SA sensor chip (GE Healthcare) was used to capture biotinylated CD47 (R_{max} ~150 RU). An unrelated biotinylated protein was immobilized with an RU value matching that of the reference surface to control for nonspecific binding. Measurements were made with serial dilutions of the SIRP-gamma, SIRP-beta or SIRP-beta2 variants in HBS-P+ buffer (GE Healthcare). The CD47 surface was regenerated by three 60 second injections of 2 M $MgCI_2$. All data were analyzed with the Biacore T100 evaluation software version 2.0 with a 1:1 Langmuir binding model.

[00115] *Cell lines and GFP-luciferase+ transduction.* DLD-1 cells (ATCC), HT-29 cells (ATCC), Raji cells (ATCC), Jurkat cells (ATCC), and 639-V cells (DSMZ) were cultured in RPMI+GlutaMax (Invitrogen) supplemented with 10% fetal bovine serum (Omega Scientific), 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen). GFP-luciferase+ lines were generated by transduction using a pCDH-CMV-MCS-EF1 puro HIV-based lentiviral vector (Systems Biosciences) engineered to express an eGFP-luciferase2 (pgl4) fusion protein. Stable lines were created by sorting for GFP expression on a FACS cell sorter.

[00116] *Cell-based CD47 binding assays.* Varying concentrations of biotinylated SIRP-gamma, SIRP-beta or SIRP-beta2 monomers, SIRP-gamma, SIRP-beta or SIRP-beta2-hIgG4 fusion proteins were incubated with cancer cells. Binding of biotinylated monomers was detected using 100 nM Alexa Fluor 647-conjugated streptavidin as a secondary staining reagent and is analyzed on an Accuri C6 flow cytometer (BD Biosciences). Binding of SIRP-gamma, SIRP-beta or

SIRP-beta2 -hlgG4 fusion proteins or antiCD47 antibodies is detected with goat anti-human lgG antibody (Invitrogen) and is analyzed on an Accuri C6 flow cytometer. Data represent the mean fluorescence intensity normalized to maximal binding for each class of reagents, and points are fit to sigmoidal dose-response curves using Prism 5 (Graphpad).

[00117] *Cell-based CD47 blocking assays.* Biotinylated SIRP-gamma, SIRP-beta or SIRP-beta2 variants were incubated with Alexa Fluor 647-conjugated streptavidin to form SIRP-gamma, SIRP-beta or SIRP-beta2 variant tetramers. 100 nM SIRP-gamma, SIRP-beta or SIRP-beta2 variant tetramers were combined with titrating concentrations of CD47 antagonists and simultaneously added to 50,000 GFP-luciferase+ Raji cells. Cells were incubated for 30 min at 4 °C then washed to remove unbound tetramer. Samples were stained with DAPI (Sigma) to exclude dead cells, and fluorescence was assayed using an Accuri C6 flow cytometer. Data represent the geometric mean fluorescence intensity normalized to maximal tetramer binding, and were fit to sigmoidal dose response curves using Prism 5 (Graphpad).

[00118] *Macrophage derivation and phagocytosis assays.* Leukocyte reduction system (LRS) chambers were obtained from anonymous donors, and peripheral blood mononuclear cells were enriched by density gradient centrifugation over Ficoll-Paque Premium (GE Healthcare). Monocytes were purified on an AutoMACS (Miltenyi) using anti-CD14 microbeads (Miltenyi) and differentiated to macrophages by culture for 7-10 days in IMDM+GlutaMax (Invitrogen) supplemented with 10% AB-Human Serum (Invitrogen) and 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen). Phagocytosis assays were performed by co-culture of 50,000 macrophages with 100,000 GFP +tumor cells for 2 hours, then analyzed using an LSRFortessa cell analyzer with high throughput sampler (BD Biosciences). Antibodies used for treatment include: mouse lgG1 isotype control (eBioscience), anti-CD47 clone 203 (eBioscience), anti-EpCam (Biolegend), cetuximab (Bristol-Myers Squibb), and rituximab (Genentech). Macrophages were identified by flow cytometry using anti-CD14, anti-CD45, or anti-CD206 antibodies (Biolegend). Dead cells were excluded from the analysis by staining with DAPI (Sigma). Phagocytosis was evaluated as the percentage of GFP+ macrophages and was normalized to the maximal response by each independent donor against each cell line. Statistical significance was determined by 2-way ANOVA with Bonferroni post-tests, and, when indicated, data were fit to sigmoidal dose response curves using Prism 5 (Graphpad).

[00119] *FACS-based phagocytosis assay.* Assessment of phagocytosis was performed by co-culture of 100,000 target cells and 50,000 macrophages for two hours in ultra-low attachment 96 well U-bottom plates (Corning) in IMDM+GlutaMax (Life Technologies) without antibiotics or serum added. Macrophages were generated by magnetic bead-based purification of primary human monocytes using the CD14+ Whole Blood isolation kit (Miltenyi) followed by 7 days of

culture with human serum (Gemini); on day 7, these cells were harvested from plates using TrypLE Express (Life Technologies). Macrophages were marked with Calcein AM red/orange cell stain (Life Technologies) according to manufacturer indications. Target cells were engineered to stably express green fluorescent protein. Protein treatments were added to co-culture of target cells and macrophages, and the mix was incubated at 37°C for 2 hours. Reactions were run on an LSRFortessa Analyzer outfitted with a high-throughput auto-sampler (BD Biosciences). Phagocytosis was evaluated as GFP+ macrophages expressed as a percentage of the total macrophages, as analyzed using FlowJo v.9.4.10 (Tree Star) and was normalized as indicated in the figure legends.

[00120] *Mice*. Nod.Cg-Prkdc^{scid} IL2rg$_{tm1Wjl}$/SzJ (NSG) mice were used for all in vivo experiments. Mice are engrafted with tumors at approximately 6-10 weeks of age, and experiments are performed with age and sex-matched cohorts of 8-15 mice.

[00121] *Tumor models.* To model human colon cancer, 1 x 10^5 GFP-luciferase+ DLD-1 cells were injected into the peritoneal cavities of NSG mice. Bladder cancer is modeled by engraftment of 1.25 x 10^5 GFP-luciferase+ 639-V cells into the dorsal subcutaneous tissue of NSG mice in 25% Matrigel (BD Biosciences). 1 x 10^6 GFP-luciferase+ Raji cells are engrafted subcutaneously on the lower flank for a localized model of human lymphoma. In all models, treatment is initiated upon confirmation of engraftment and continued as indicated. For all treatments, 200 µg SIRP-gamma, SIRP-beta or SIRP-beta2 variant or antibody is administered by intraperitoneal injection on a daily schedule. Tumor growth is monitored by bioluminescence imaging, and tumor dimensions are measured to calculate volumes according to the ellipsoid formula (π/6 x length x width^2). Statistical significance is determined by Mann-Whitney test or Kruskai-Wallis with Dunn's post-tests as appropriate. Survival is analyzed by Mantel-Cox test.

[00122] *Bioluminescence imaging.* Anesthetized mice are injected with 200 µl D-luciferin (firefly) potassium salt (Biosynth) reconstituted at 16.67 mg/ml in sterile PBS. Bioluminescence imaging is performed using an IVIS Spectrum (Caliper Life Sciences) over 20 minutes to record maximal radiance. Peak total flux values are assessed from the anatomical region of interest using Living Image 4.0 (Caliper Life Sciences) and used for analysis.

[00123] *Protein sequences.* Among the proteins used in the examples described herein, the following are included:

Wild-type SIRPgamma:

EEELQMIQPEKLLLVTVGKTATLHCTVTSLLPVGPVLWFRGVGPGRELIYNQKEGHFPR VTTVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 1)

GV1 (high affinity SIRPgamma):

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPVLWFRGVGPGRVLIYNQRQGPFPRV

TTVSDTTKRNNMDFSIRISSITPADVGTYYCIKFRKGSPENVEFKSGPGTEMALGAKPS

(SEQ ID NO: 3)

GV1.2:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQREGPFPRVT

TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS

(SEQ ID NO: 13)

HGV1:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQRDGPFPRV

TTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS

(SEQ ID NO: 4)

HGV2:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGPFPRV

TTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS

(SEQ ID NO: 5)

HGV3:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRV

TTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS

(SEQ ID NO: 6)

MGV1:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRV

TTVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGTPEDVEFKSGPGTEMALGAKPS

(SEQ ID NO: 7)

SIRP-gamma library residues:

M6	MILF
V27	FILV
L30	LIVHND
L31	FILV
V33	VILPTA
V36	VI
L37	LQ
V42	VA
E47	EV
Q52	QPLVAE

K53	KR
E54	EDKNQH
H56	HPR
L66	LIVPTARSG
T67	TINFSYVAD
V92	VI
S98	SRNKTIM
N101	NKDEHQ

Wild-type SIRPbeta:

EDELQVIQPEKSVSVAAGESATLRCAMTSLIPVGPIMWFRGAGAGRELIYNQKEGHFPR VTTVSELTKRNNLDFSISISNITPADAGTYYCVKFRKGSPDDVEFKSGAGTELSVRAKPS (SEQ ID NO: 43)

BV1 (high affinity SIRPbeta):

EDELQ**I**IQPEKSVSVAAGESATLRCA**I**TSL**F**PVGPI**Q**WFRGAGAGR**V**LIYNQ**RQ**G**P**FPRV TTVSE**T**TKRNNLDFSISISNITPADAGTYYC**I**KFRKGSPDDVEFKSGAGTELSVRAKPS (SEQ ID NO: 26)

Wild-type SIRPbeta2:

EEELQVIQPDKSISVAAGESATLHCTVTSLIPVGPIQWFRGAGPGRELIYNQKEGHFPRVT TVSDLTKRNNMDFSIRISNITPADAGTYYCVKFRKGSPDHVEFKSGAGTELSVRAKPS (SEQ ID NO: 44)

B2V1 (high affinity SIRPbeta2):

EEELQ**I**IQPDKSISVAAGESATLHCT**I**TSL**F**PVGPIQWFRGAGPGR**V**LIYNQ**RQ**G**P**FPRVT TVSD**T**TKRNNMDFSIRISNITPADAGTYYC**I**KFRKGSPD**D**VEFKSGAGTELSVRAKPS (SEQ ID NO: 28)

[00124] Additional protein sequences for SIRP-gamma polypeptide include:

HLib1:

EEELQIIQPEKLLLVTVGKTATLHCTITSHFPVGPIQWFRGVGPGRVLIYNQKDGHFPRV TTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 8);

HLib2:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 9);

HLib3:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPVLWFRGVGPGRVLIYNQRQGPFPRVT TVSDTTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 10);

HLib4:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRELIYNAREGRFPRVT TVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 11);

HMLib1:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRQGPFPRVT TVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFRKGSPDDVEFKSGAGTELSVRAKPS (SEQ ID NO: 12);

HMLib2:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPVLWFRGVGPGRVLIYNQRQGPFPRVT TVSDTTKRNNMDFSIRISSITPADVGTYYCIKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 3);

HMLib3:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQREGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 13);

HMLib4:

EEELQMIQPEKLLLVTVGKTATLHCTVTSLLPVGPVLWFRGVGPGRELIYNQKEGHFPR VTTVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 42);

HMLib5:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 14);

HMLib6:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTXWH (SEQ ID NO: 15), wherein X is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, or V;

HMLib7:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQKDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGTPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 16);

MLib1:

EEELQIIQPEKLLLVTVGKTATLHCTITSLLPVGPIQWFRGVGPGRELIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 17);

MLib2:

EEELQIIQPEKLLLVTVGKTATLHCTLTSLLPVGPILWFRGVGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGNPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 18);

MLib3:

EEELQLIQPEKLLLVTVGKTATLHCTITSLFPPGPIQWFRGVGPGRVLIYNQKDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGIPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 19);

MLib4:

EEELQIIQPEKLLLVTVGKTATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 20);

MLib5:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPIGPILWFRGVGPGRVLIYNQKDGPFPRVTT VSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 21);

MLib6:

EEELQMIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRV TTVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 22);

MLib7:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 23); and

MLib8:

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQKDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGTPEDVEFKSGPGTEMALXAKPS (SEQ ID NO: 24).

[00125] In additional embodiments, the decoy polypeptide is a fusion or chimeric polypeptide. In additional embodiments, the decoy polypeptide comprises a SIRP-gamma polypeptide sequence fused to a polypeptide sequence comprising an immune checkpoint inhibitor, a co-stimulatory molecule, or a cytokine or an attenuated cytokine, wherein the sequences are connected by a Gly-Ser linker of varying length and composition. In further embodiments, the decoy polypeptide comprises one of the following sequences:

1) Fusion to immune checkpoint inhibitors

    a. PD-1/PD-L1 antagonist

    Example: HAC-GV3 (high-affinity PD-1 decoy fused to GV3)
    DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFHVVWHRESPSGQTDTLA AFPEDRSQPGQDARFRVTQLPNGRDFHMSVVRARRNDSGTYVCGVISLAPKIQI KESLRAELRVTERGGGGSGGGGSEEELQIIQPEKLLLVTVGKTATLHCTITSLFPV GPIQWFRGVGPGRVLIYNQKDGHFPRVTTVSDGTKRNNMDFSIRISSITPADVGT YYCVKFRKGSPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 30);

    b. BTLA/CD160 antagonist

    Example: GV3-BTLA decoy
    EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSWNIHGKESCDVQLYIKRQSEHSILAGDPFELECPVK YCANRPHVTWCKLNGTTCVKLEDRQTSWKEEKNISFFILHFEPVLPNDNGSYRC SANFQSNLIESHSTTLYVTDVK (SEQ ID NO: 31);

    c. Phosphatidylserine antagonist

    Example: GV3-MFGE8 decoy
    EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSELNGCANPLGLKNNSIPDKQITASSSYKTWGLHLFS WNPSYARLDKQGNFNAWVAGSYGNDQWLQVDLGSSKEVTGIITQGARNFGSV QFVASYKVAYSNDSANWTEYQDPRTGSSKIFPGNWDNHSHKKNLFETPILARYV RILPVAWHNRIALRLELLGC (SEQ ID NO: 32);

    Example: GV3-Tim1 decoy

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSVAGSVKVGGEAGPSVTLPCHYSGAVTSMCWNRGS CSLFTCQNGIVWTNGTHVTYRKDTRYKLLGDLSRRDVSLTIENTAVSDSGVYCC RVEHRGWFNDMKITVSLEIVPPKVTT (SEQ ID NO: 33);

Example: GV3-Tim3 decoy

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSSEVEYRAEVGQNAYLPCFYTPAAPGNLVPVCWGK GACPVFECGNVVLRTDERDVNYWTSRYWLNGDFRKGDVSLTIENVTLADSGIY CCRIQIPGIMNDEKFNLKLVIKPAKVTPA (SEQ ID NO: 34);

Example: GV3-Tim4 decoy

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSTSETVVTEVLGHRVTLPCLYSSWSHNSNSMCWGK DQCPYSGCKEALIRTDGMRVTSRKSAKYRLQGTIPRGDVSLTILNPSESDSGVYC CRIEVPGWFNDVKINVRLNLQRASTTTDEKFNLKLVIKPAKVTPA (SEQ ID NO: 35);

2) Fusion to co-stimulatory molecules

   a. CD40 agonist

Example: GV3-CD40L

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSGDQNPQIAAHVISEASSKTTSVLQWAEKGYYTMSN NLVTLENGKQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILL RAANTHSSAKPCGQQSIHLGGVFELQPGASVFVNVTDPSQVSHGTGFTSFGLLKL (SEQ ID NO: 36);

   b. 41BB (CD137) agonist

Example: GV3-41BBL

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDP GLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQMELRRVVAGEGSGSVSLALH LMPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEA RARHAWQLTQGATVLGLFRVTPEIPA (SEQ ID NO: 37);

3) Fusion to cytokines or attenuated cytokines

Example: GV3-IL2

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINV IVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT (SEQ ID NO: 38);

Example: GV3-IL2 (an "attenuated" cytokine with mutations F42A/D20T)

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGH FPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTE MALGAKPSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLTLQMILNGINNYKNPKL TRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINV IVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT (SEQ ID NO: 39).

Example 2: Surface plasmon resonance-based measurement of binding affinity and kinetics of the SIRP-gamma variant GV3 for human CD47

[00126] Using the surface plasmon resonance method described above, one could measure the binding affinity and kinetics of the SIRP-gamma variant GV3 for human CD47. For example, as shown in **FIG. 1A**, varying concentrations of the SIRP-gamma variant GV3 was used, including 100 pM, 316 pM, 1 nM, 3.16 nM, 10 nM, and 100 nM, in binding reactions with a known concentration of biotinylated human CD47. Using a 1:1 Langmuir binding model, the calculated dissociation constant K_d was 92 pM. The calculated dissociation half-life $T_{1/2}$ was about 44 minutes. **FIG. 1B** shows a graphic representation of a biotinylated human CD47 bound to the SIRP-gamma variant GV3.

Example 3: Surface plasmon resonance-based measurement of binding affinity and kinetics of HAC-GV3, a fusion protein of the SIRP-gamma variant GV3 and a high-affinity PD-1 variant HAC, for human CD47

[00127] Using the surface plasmon resonance method described above, one could measure the binding affinity and kinetics of HAC-GV3, a fusion protein of the SIRP-gamma variant GV3 and a high-affinity PD-1 variant HAC, for human CD47. For example, as shown in **FIG. 2A**, varying concentrations of the HAC-GV3 fusion protein was used, including 100 pM, 316 pM, 1 nM, 3.16 nM, 10 nM, and 100 nM, in binding reactions with a known concentration of biotinylated human CD47. Using a 1:1 Langmuir binding model, the calculated dissociation constant K_d was 160 pM. The calculated dissociation half-life $T_{1/2}$ was about 40 minutes. **FIG. 2B** shows a graphic representation of a biotinylated human CD47 bound to the fusion protein comprising SIRP-gamma variant GV3 and HAC.

Example 4: Surface plasmon resonance-based measurement of binding affinity and kinetics of a PD-1 variant HAC for human PD-L1

[00128] In another example, surface plasmon resonance was used to measure the binding affinity and kinetics of PD-1 variant HAC for human PD-L1. Varying concentrations of HAC, including 100 pM, 316 pM, 1 nM, 3.16 nM, 10 nM, and 100 nM, were used in binding reactions with a known concentration of biotinylated human PD-L1. Using a 1:1 Langmuir binding model, the calculated dissociation constant K_d was 110 pM. The calculated dissociation half-life $T_{1/2}$ was about 42 minutes.

Example 5: Surface plasmon resonance-based measurement of binding affinity and kinetics of HAC-GV3, a fusion of the SIRP-gamma variant GV3 with a PD-1 variant HAC, for human PD-L1

[00129] Using the surface plasmon resonance method described above, one could measure the binding affinity and kinetics of HAC-GV3, a fusion of the SIRP-gamma variant GV3 with a PD-1 variant HAC, for human PD-L1. For example, as shown in **FIG. 3A**, varying concentrations of the HAC-GV3 fusion protein, including 100 pM, 316 pM, 1 nM, 3.16 nM, 10 nM, and 100 nM, were added to binding reactions with a known concentration of biotinylated human PD-L1. Using a 1:1 Langmuir binding model, the calculated dissociation constant K_d was 134 pM. The calculated dissociation half-life $T_{1/2}$ was about 38 minutes. **FIG. 3B** shows a graphic representation of a biotinylated human PD-L1 bound to HAC-GV3.

Example 6: Surface plasmon resonance-based measurement of the simultaneous binding of both human CD47 and human PD-L1 by HAC-GV3

[00130] In another example of surface plasmon resonance-based measurement, the binding affinity and kinetics of simultaneous binding of both human CD47 and human PD-L1 by HAC-GV3 were measured by adding varying concentrations of either HGV3 or HAC-GV3 to a known concentration of biotinylated human CD47. As shown in **FIG. 4A**, in comparison to human GV3 alone, the binding curve for fusion protein HAC-GV3 showed two distinct peaks – a first peak for binding to biotinylated CD47 and a second peak for binding to PD-L1. **FIG. 4B** shows a graphic representation of a biotinylated human CD47 bound to the GV3 portion of the HAC-GV3 fusion protein, wherein the HAC portion is bound to PD-L1.

Example 7: Flow cytometry-based measurement of blockade of CD47/SIRP-alpha interactions on the surface of CD47+ cells by GV3 and HAC-GV3

[00131] Using the cell-based CD47 blocking assay described above, 50 nM of biotinylated SIRP-alpha tetramers were combined with titrating concentrations of either GV3 or HAC-GV3 in the presence of the CD47+ cells. **FIG. 5** shows the flow cytometry-based measurement of blockade of CD47/SIRP-alpha interactions on the surface of CD47+ GFP-luciferase+ DLD1-Tg

cells by GV3 and HAC-GV3. Percent median fluorescence intensity (% MFT) as a readout of SIRP-alpha binding is plotted against log concentration in M.

Example 8: Flow cytometry-based measurement of blockade of CD47/SIRP-alpha interactions on the surface of CD47+ PD-L1+ cells by GV3 and HAC-GV3

[00132] In another example of the cell-based CD47 blocking assay described above, flow cytometry was used to measure blockade of CD47/SIRP-alpha interactions on the surface of CD47+ PD-L1+ GFP-luciferase+ DLD1-Tg cells by GV3 and HAC-GV3. As **FIG. 6** shows, 50 nM of biotinylated SIRP-alpha tetramers were combined with titrating concentrations of either GV3 or HAC-GV3 in the presence of the CD47+ PD-L1+ cells. Percent median fluorescence intensity (% MFT) as a readout of SIRP-alpha binding is plotted against log concentration in M.

Example 9: Flow cytometry-based measurement of blockade of PD-1/PD-L1 interactions on the surface of PD-L1+ cells by HAC and HAC-GV3

[00133] In another example of cell-based CD47 blocking assay, flow cytometry was used to measure blockade of PD-1/PD-L1 interactions on the surface of human PD-L1+ yeast cells by HAC and HAC-GV3. As **FIG. 7** shows, 100 nM of biotinylated PD-1 tetramers were combined with titrating concentrations of either HAC or HAC-GV3 in the presence of hPD-L1+ yeast cells. Percent median fluorescence intensity (% MFT) as a readout of PD-1 binding is plotted against log concentration in M.

Example 10: Flow cytometry-based measurement of blockade of PD-1/PD-L1 interactions on the surface of CD47+ PD-L1+ cells by HAC and HAC-GV3

[00134] In another example of cell-based CD47 blocking assay, flow cytometry was used to measure of blockade of PD-1/PD-L1 interactions on the surface of CD47+ PD-L1+ GFP-luciferase+ DLD1-Tg cells by HAC and HAC-GV3. As shown in **FIG. 8**, 100 nM of biotinylated PD-1 tetramers were combined with titrating concentrations of either HAC or HAC-GV3 in the presence of the CD47+ PD-L1+ cells. Percent median fluorescence intensity (% MFT) as a readout of PD-1 binding is plotted against log concentration in M.

Example 11: FACS-based measurement of phagocytosis shows GV3 microbody and HAC-GV3 potentiate phagocytosis across a wide range of opsonizing antibody concentrations

[00135] **FIG. 9** shows the FACS-based measurement of phagocytosis by donor-derived human macrophages against the human colon cancer cell line DLD1, indicating that GV3 microbody and HAC-GV3 potentiate phagocytosis across a wide range of opsonizing antibody concentrations. Assessment of phagocytosis was performed by co-culture of 100,000 target cells and 50,000 macrophages for two hours in ultra-low attachment 96 well U-bottom plates (Corning) in IMDM+GlutaMax (Life Technologies) without antibiotics or serum added. Macrophages were generated by magnetic bead-based purification of primary human monocytes

using the CD14+ Whole Blood isolation kit (Miltenyi) followed by 7 days of culture with human serum (Gemini); on day 7, these cells were harvested from plates using TrypLE Express (Life Technologies). Macrophages were marked with Calcein AM red/orange cell stain (Life Technologies) according to manufacturer indications. Target cells were engineered to stably express green fluorescent protein. A dimerized microbody form of HGV3 (GV3mb) or HAC-GV3 fusion protein was added to reaction wells at a saturating concentration of 10nM, while the opsonizing antibody Cetuximab was titrated across three logs of concentration. Error bars represent the standard deviation of duplicate experiments. Reactions were run on an LSRFortessa Analyzer outfitted with a high-throughput auto-sampler (BD Biosciences). Phagocytosis was evaluated as GFP+ macrophages expressed as a percentage of the total macrophages, as analyzed using FlowJo v.9.4.10 (Tree Star) and was normalized as indicated in the figure legends.

Example 12: Occupancy and Persistence of HAC-GV3 and GV3

[00136] The experiments to determine occupancy and persistence of HAC-GV3 and GV3 are summarized in FIG. 10. To test occupancy and persistence of HAC-GV3 and GV3 NSG mice were injected on day 0 with B16-F0 (subcutaneously), as well as a mixture of unmodified HL60 and HL60-Tg (hPDL1, GFP) (intravenously). On days 11, 12, and 13, mice were injected with PBS, GV3, or HAC-GV3. One hour or 24 hours after the last injection, spleens or tumors were harvested from sacrificed mice and cells were dissociated into single cell suspensions on ice without the use of enzymatic digestion. FACS was used to analyze SIRPA-tetramer binding to either mouse splenocytes, mouse solid tumor cells (B16F0), or Human tumor cells (HL60-Tg (hPDL1, GFP)).

[00137] Occupancy and persistence of HAC-GV3 and GV3 are shown in FIGS. 11A-11D. HAC-GV3 was shown to have both increased occupancy and persistence compared to GV3 and PBS control in total splenic cells at 1 hour and 24 hours (FIG. 11A). HAC-GV3 was demonstrated to have both increased occupancy and persistence compared to GV3 and PBS control in mouse splenocytes at 1 hour and 24 hours (FIG. 11B). HAC-GV3 was observed to have both increased occupancy and persistence compared to GV3 and PBS control in human cells isolated from the spleen at 1 hour and 24 hours (FIG. 11C). HAC-GV3 was demonstrated to have increased occupancy compared to GV3 and PBS control at 1 hour and 24 hours in B16-F0 melanoma cells isolated from a tumor (FIG. 11D).

Example 13: Clinical trial to test effect of a decoy polypeptide for treatment of B cell lymphoma

[00138] This is a prospective open label, controlled, randomized study to test the safety and efficacy of a decoy polypeptide for the treatment of patients with stage IIIB or stage IV B cell lymphoma. To be eligible, patients entering the trial will have demonstrated either stable disease

or a clinical response after first-line treatment (chemotherapy alone, or chemotherapy and radiotherapy) and have an ECOG performance status of 0, 1 or 2. Following a 3 week washout period, patients will be stratified by disease status, and randomized to either anti-CD20 antibody (e.g. rituximab) alone, or anti-CD20 antibody plus decoy polypeptide.

[00139] Eight weekly subcutaneous treatments with 10 mg/kg of a decoy polypeptide will be administered at weeks 0, 1, 2, 3, 4, 5, 6 and 7. All patients will be additionally treated with 375 mg/m^2 anti-CD20 at week 0, and 500 mg/m^2 anti-CD20 at week 1, 2, 3, 4, 5, 6, and 7.

[00140] Primary outcome measures will be documentation of safety profile of the decoy polypeptide, and comparison of survival rate of patients in the two arms of the trial. Secondary outcome measures will be to measure activation of phagocytosis elicited by the decoy polypeptide, and to evaluate quality of life for patients undergoing immunotherapy.

Example 14: Clinical trial to test maximum tolerated dose and/or recommended dosage of decoy polypeptide in patients with advanced solid tumors

[00141] This is an open label, Phase I dose escalation study to evaluate the safety and immunogenicity of repeat dose vaccination with a decoy polypeptide in patients with previously treated Stage 3 or 4 solid tumors, including but not limited to: breast, non-small cell lung, ovarian, colorectal, gastric, prostate, pancreatic, and renal cell cancers.

[00142] Part 1 evaluates escalating dose levels of the decoy polypeptide administered subcutaneously once every other week (Q2W) over 8 weeks (for a total of 4 doses) or once every week (QW) over 8 weeks (for a total of 8 doses), and uses a 3+3 dose escalation design to identify the maximum tolerated dose (MTD) and/or recommended dose (RD) for each dosing schedule, for further evaluation in Part 2 of the study. Part 2 evaluates the safety, immunogenicity, and potential anti-tumor activity of the decoy polypeptide of Example 1 administered over 8 weeks at the Q2W and QW MTD/RD in cohorts of 15 patients each. After the 8 week administration period, patients are evaluated for safety, immune response and tumor response to Week 20.

[00143] The study population includes patients with previously treated Stage 3 or 4 solid tumors.

[00144] Inclusion criteria:

- 18-70 years of age at time of consent
- Life expectancy of at least 6 months, according to investigator's opinion
- Have histologically confirmed breast, non-small cell lung, ovarian, colorectal, gastric, prostate, pancreatic, or renal cell cancer, or other tumor type

- Have evidence of persistent, recurrent, or progressive disease after at least one course of systemic therapy for locally advanced or metastatic disease, including chemotherapy, targeted therapy, or immunotherapy
- Clinical stage 3 or 4 disease
- ECOG 0 or 1
- Adequate hematological, renal and hepatic function parameters

[00145] Exclusion criteria:

- Has received treatment with any systemic chemotherapy, radiation, or experimental agent within 4 weeks of study drug dosing
- Has any preexisting medical condition requiring chronic steroid or immunosuppressive therapy
- HIV, hepatitis B or hepatitis C positive

[00146] Mode of Administration: The decoy polypeptide is administered subcutaneously, starting on Day 1 per cohort assignment. All patients receive doses of the decoy polypeptide administered as four separate injections at four separate injection sites (one injection each in the right upper arm or thigh, left upper arm or thigh, and right and left lower abdomen). The starting dose is 0.01 mg/kg of the decoy polypeptide. The dose levels are selected using a dose-doubling design. An intermediate dose level, 750 mg/kg, is considered when the MTD is less than 1000 mg/kg.

[00147] In the Part 2 evaluation and assessment of the eight week administration, measures include measurement of activation of phagocytosis. Tumor response is assessed as defined by RECIST or Immune RECIST 1.1.

[00148] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A decoy polypeptide comprising a SIRP-gamma, a SIRP-beta or a SIRP-beta2 polypeptide, wherein the polypeptide comprises at least one amino acid modification to increase affinity of the decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide binding to CD47 as compared to the affinity for CD47 of the corresponding wild type SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide.

2. The decoy polypeptide of claim 1, wherein the polypeptide comprises a SIRP-gamma polypeptide with the sequence EEELQMIQPEKLLLVTVGKTATLHCTVTSLLPVGPVLWFRGVGPGRELIYNQKEGHFPR VTTVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 1).

3. The decoy polypeptide of claim 1 or claim 2, wherein the SIRP-gamma polypeptide is 90% identical to a wildtype SIRP-gamma polypeptide.

4. The decoy polypeptide of any one of claims 1 to 3, wherein the SIRP-gamma polypeptide has an amino acid substitution at M6, V27, L30, L31, V33, V36, L37, V42, E47, Q52, K53, E54, H56, L66, T67, V92, S98 or N101.

5. The decoy polypeptide of any one of claims 1 to 4, wherein the SIRP-gamma polypeptide has a substitution at M6 wherein the substitution is I, L or F.

6. The decoy polypeptide of any one of claims 1 to 5, wherein the SIRP-gamma polypeptide has a substitution at V27 wherein the substitution is F, I or L.

7. The decoy polypeptide of any one of claims 1 to 6, wherein the SIRP-gamma polypeptide has a substitution at L30 wherein the substitution is I, V, H, N or D.

8. The decoy polypeptide of any one of claims 1 to 7, wherein the SIRP-gamma polypeptide has a substitution at L31 wherein the substitution is F, I or V.

9. The decoy polypeptide of any one of claims 1 to 8, wherein the SIRP-gamma polypeptide has a substitution at V33 wherein the substitution is I, L, P, T or A.

10. The decoy polypeptide of any one of claims 1 to 9, wherein the SIRP-gamma polypeptide has a substitution at V36 wherein the substitution is I.

11. The decoy polypeptide of any one of claims 1 to 10, wherein the SIRP-gamma polypeptide has a substitution at L37 wherein the substitution is Q.

12. The decoy polypeptide of any one of claims 1 to 11, wherein the SIRP-gamma polypeptide has a substitution at V42 wherein the substitution is A.

13. The decoy polypeptide of any one of claims 1 to 12, wherein the SIRP-gamma polypeptide has a substitution at E47 wherein the substitution is V.

14. The decoy polypeptide of any one of claims 1 to 13, wherein the SIRP-gamma polypeptide has a substitution at Q52 wherein the substitution is P, L, V, A or E.

15. The decoy polypeptide of any one of claims 1 to 14, wherein the SIRP-gamma polypeptide has a substitution at K53 wherein the substitution is R

16. The decoy polypeptide of any one of claims 1 to 15, wherein the SIRP-gamma polypeptide has a substitution at E54 wherein the substitution is D, K, N, Q or H.

17. The decoy polypeptide of any one of claims 1 to 16, wherein the SIRP-gamma polypeptide has a substitution at H56 wherein the substitution is P or R.

18. The decoy polypeptide of any one of claims 1 to 17, wherein the SIRP-gamma polypeptide has a substitution at L66 wherein the substitution is I, V, P, T, A, R, S or G.

19. The decoy polypeptide of any one of claims 1 to 18, wherein the SIRP-gamma polypeptide has a substitution at T67 wherein the substitution is I, N, F, S, Y, V, A or D.

20. The decoy polypeptide of any one of claims 1 to 19, wherein the SIRP-gamma polypeptide has a substitution at V92 wherein the substitution is I.

21. The decoy polypeptide of any one of claims 1 to 20, wherein the SIRP-gamma polypeptide has a substitution at S98 wherein the substitution is R, N, K, T, I or M.

22. The decoy polypeptide of any one of claims 1 to 21, wherein the SIRP-gamma polypeptide is substituted at N101 wherein the substitution is K, D, E, H or Q.

23. The decoy polypeptide of any one of claims 1 to 4, wherein the SIRP-gamma polypeptide has the sequence EEELQX_1IQPEKLLLVTVGKTATLHCTX_2TS$X_3$$X_4PX_5GPX_6$$X_7$WFRG$X_8$GPGR$X_9$LIYN$X_{10}$$X_{11}$$X_{12}GX_{13}$FPRVTTVSD$X_{14}$$X_{15}$KRNNMDFSIRISSITPADVGTYYC$X_{16}$KFRKG$X_{17}PEX_{18}$VEFKSGPGTEMALGAKPS (SEQ ID NO: 2), wherein X_1 is M, I, L or F; X_2 is F, I, L or V; X_3 is L, I, V, H, N or D; X_4 is F, I, L or V; X_5 is V, I, L, P, T or A; X_6 is V or I; X_7 is L or Q; X_8 is V or A; X_9 is E or V; X_{10} is Q, P, L, V, A or E; X_{11} is K or R; X_{12} is E, D, K, N, Q or H; X_{13} is H, P or R; X_{14} is L, I, V, P, T, A, R, S or G; X_{15} is T, I, N, F, S, Y, V, A or D; X_{16} is V or I; X_{17} is S, R, N, K, T, I or M; and X_{18} is N, K, D, E, H or Q.

24. The decoy polypeptide of any one of claims 1 to 4, wherein the SIRP-gamma polypeptide has the sequence EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPVLWFRGVGPGRVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRISSITPADVGTYYCIKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 3).

25. The decoy polypeptide of any one of claims 1 to 4, wherein the SIRP-gamma polypeptide has the sequence

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQRDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 4).

26. The decoy polypeptide of any one of claims 1 to 4, wherein the SIRP-gamma polypeptide has the sequence
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGPFPRV
TTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 5).

27. The decoy polypeptide of any one of claims 1 to 4, wherein the SIRP-gamma polypeptide has the sequence
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRV
TTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 6).

28. The decoy polypeptide of any one of claims 1 to 4, wherein the SIRP-gamma polypeptide has the sequence
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGTPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 7).

29. The decoy polypeptide of claim 1, wherein the polypeptide comprises a SIRP-beta polypeptide with the sequence
EDELQVIQPEKSVSVAAGESATLRCAMTSLIPVGPIMWFRGAGAGRELIYNQKEGHFPR
VTTVSELTKRNNLDFSISISNITPADAGTYYCVKFRKGSPDDVEFKSGAGTELSVRAKPS
(SEQ ID NO: 25).

30. The decoy polypeptide of claim 1 or claim 29, wherein the SIRP-beta polypeptide is at least 90% identical to a wildtype SIRP-beta polypeptide.

31. The decoy polypeptide of any one of claims 1 or 29 to 30, wherein the SIRP-beta polypeptide has an amino acid substitution at I6, I27, F31, Q37, V47, R53, Q54, P56, T66 or I92.

32. The decoy polypeptide of any one of claims 1 or 29 to 31, wherein the SIRP-beta polypeptide has a substitution at V6 wherein the substitution is I.

33. The decoy polypeptide of any one of claims 1 or 29 to 32, wherein the SIRP-beta polypeptide has a substitution at M27 wherein the substitution is I.

34. The decoy polypeptide of any one of claims 1 or 29 to 33, wherein the SIRP-beta polypeptide has a substitution at I31 wherein the substitution is F.

35. The decoy polypeptide of any one of claims 1 or 29 to 34, wherein the SIRP-beta polypeptide has a substitution at M37 wherein the substitution is Q.

36. The decoy polypeptide of any one of claims 1 or 29 to 35, wherein the SIRP-beta polypeptide has a substitution at E47 wherein the substitution is V.

37. The decoy polypeptide of any one of claims 1 or 29 to 36, wherein the SIRP-beta polypeptide has a substitution at K53 wherein the substitution is R.

38. The decoy polypeptide of any one of claims 1 or 29 to 37, wherein the SIRP-beta polypeptide has a substitution at E54 wherein the substitution is Q.

39. The decoy polypeptide of any one of claims 1 or 29 to 38, wherein the SIRP-beta polypeptide has a substitution at H56 wherein the substitution is P.

40. The decoy polypeptide of any one of claims 1 or 29 to 39, wherein the SIRP-beta polypeptide has a substitution at L66 wherein the substitution is T.

41. The decoy polypeptide of any one of claims 1 or 29 to 40, wherein the SIRP-beta polypeptide has a substitution at V92 wherein the substitution is I.

42. The decoy polypeptide of any one of claims 1 or 29 to 41, wherein the SIRP-beta polypeptide has the sequence EDELQIIQPEKSVSVAAGESATLRCAITSLFPVGPIQWFRGAGAGRVLIYNQRQGPFPRVT TVSETTKRNNLDFSISISNITPADAGTYYCIKFRKGSPDDVEFKSGAGTELSVRAKPS (SEQ ID NO: 26).

43. The decoy polypeptide of claim 1, wherein the polypeptide comprises a SIRP-beta2 polypeptide with the sequence EEELQVIQPDKSISVAAGESATLHCTVTSLIPVGPIQWFRGAGPGRELIYNQKEGHFPRVT TVSDLTKRNNMDFSIRISNITPADAGTYYCVKFRKGSPDHVEFKSGAGTELSVRAKPS (SEQ ID NO: 27).

44. The decoy polypeptide of claim 1 or claim 43, wherein the SIRP-beta2 polypeptide is at least 90% identical to a wildtype SIRP-beta2 polypeptide.

45. The decoy polypeptide of any one of claims 1 or 43 to 44, wherein the SIRP-beta2 polypeptide has an amino acid substitution at I6, I27, F31, V47, R53, Q54, P56, T66, I92 or D101.

46. The decoy polypeptide of any one of claims 1 or 43 to 45, the SIRP-beta2 polypeptide is substituted at V6 wherein the substitution is I.

47. The decoy polypeptide of any one of claims 1 or 43 to 46, the SIRP-beta2 polypeptide is substituted at V27 wherein the substitution is I.

48. The decoy polypeptide of any one of claims 1 or 43 to 47, the SIRP-beta2 polypeptide is substituted at I31 wherein the substitution is F.

49. The decoy polypeptide of any one of claims 1 or 43 to 48, the SIRP-beta2 polypeptide is substituted at E47 wherein the substitution is V.

50. The decoy polypeptide of any one of claims 1 or 43 to 49, the SIRP-beta2 polypeptide is substituted at K53 wherein the substitution is R.

51. The decoy polypeptide of any one of claims 1 or 43 to 50, the SIRP-beta2 polypeptide is substituted at E54 wherein the substitution is Q.

52. The decoy polypeptide of any one of claims 1 or 43 to 51, the SIRP-beta2 polypeptide is substituted at H56 wherein the substitution is P.

53. The decoy polypeptide of any one of claims 1 or 43 to 52, the SIRP-beta2 polypeptide is substituted at L66 wherein the substitution is T.

54. The decoy polypeptide of any one of claims 1 or 43 to 53, the SIRP-beta2 polypeptide is substituted at V92 wherein the substitution is I.

55. The decoy polypeptide of any one of claims 1 or 43 to 54, the SIRP-beta2 polypeptide is substituted at H101 wherein the substitution is D.

56. The decoy polypeptide of any one of claims 1 or 43 to 55, wherein the SIRP-beta 2 polypeptide has the sequence EEELQIIQPDKSISVAAGESATLHCTITSLFPVGPIQWFRGAGPGRVLIYNQRQGPFPRVTT VSDTTKRNNMDFSIRISNITPADAGTYYCIKFRKGSPDDVEFKSGAGTELSVRAKPS (SEQ ID NO: 28).

57. The decoy polypeptide of any one of claims 1 to 56, wherein the decoy polypeptide blocks binding of CD47 to a ligand.

58. The decoy polypeptide of claim 57, wherein the ligand is SIRP-alpha, SIRPgamma, or thrombospoindin-1.

59. The decoy polypeptide of any one of claims 1 to 58, wherein the decoy polypeptide binds to a cell.

60. The cell of claim 59, wherein the cell is a tumor cell, virally infected cell, bacterially infected cell, damaged red blood cell, arterial plaque cell, fibrotic tissue cell, a healthy normal cell such as hematopoietic stem cell, a healthy myeloid or lymphoid precursor cell, or a healthy differentiated hematopoietic cell type such as T, B, plasma, or NK cell.

61. The decoy polypeptide of any one of claims 1 to 60, wherein the decoy polypeptide enables phagocytosis or ADCC of a tumor cell, virally infected cell, bacterially infected cell, damaged red blood cell, arterial plaque cell, fibrotic tissue cell, healthy normal cell such as hematopoietic stem cell, healthy myeloid or lymphoid precursor cell or healthy differentiated hematopoietic cell type such as T, B, plasma, or NK cells.

62. The decoy polypeptide of any one of claims 1 to 61, wherein the polypeptide is fused to an immunoglobulin Fc sequence.

63. The decoy polypeptide of any one of claims 1 to 62, wherein the SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide is multimeric.

64. The decoy polypeptide of any one of claims 1 to 62, wherein the SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide is monomeric.

65. The decoy polypeptide of any one of claims 1 to 64, wherein the polypeptide further comprises a detectable label.

66. The decoy polypeptide of any one of claims 1 to 64, wherein the polypeptide has increased occupancy.

67. The decoy polypeptide of any one of claims 1 to 66, wherein the polypeptide has increased persistence.

68. A composition comprising the decoy polypeptide of any one of claims 1 to 67 and a pharmaceutically acceptable carrier or excipient.

69. The composition of claim 68, further comprising an additional drug.

70. The composition of claim 69, wherein the drug comprises one or more of a chemotherapeutic agent, kinase inhibitor, proteasome inhibitor, or inhibitor of viral DNA or RNA polymerase.

71. The composition of any one of claims 68 to 70, further comprising a monoclonal antibody.

72. The composition of claim 71, wherein the monoclonal antibody binds an antigen on a cancer cell, an immune cell, a pathogen-infected cell, or a hematopoietic stem cell.

73. The composition of claim 72, wherein the antigen on a cancer cell comprises EGFR, Her2/neu, CD19, CD20, CD22, CD25, CD30, CD33, CD38, CD45, CD47, CD56, CD70, CD117, or EpCAM.

74. The composition of claim 72, wherein the antigen on the immune cell comprises M1prime, CD2, CD3, CD4, CD5, CD8, CD19, CD20, CD22, CD25, CD38, CD56, PD-1, PD-L1, CTLA4, BTLA, TIM3, LAG3, OX40, GITR or CD137 (4-1BB).

75. The composition of claim 72, wherein the antigen on a pathogen-infected cell comprises CMV proteins, including UL18, UL11, pp65, gB, and pp150, HIV envelope proteins, including Gp41, Gp120, V1V2 glycan, and V3 glycan, and influenza hemagglutinin.

76. The composition of claim 72, wherein the antigen on the hematopoietic stem cell, comprises CD11, CD45, CD117 or Sca1.

77. An isolated nucleic acid encoding the decoy polypeptide of any one of claims 1 to 67.

78. A cell expressing the decoy polypeptide of any one of claims 1 to 67.

79. A method of modulating phagocytosis or ADCC of a cell expressing CD47, the method comprising contacting the cell with the decoy polypeptide of claims 1 to 67 or the composition of claims 68 to 76.

80. A method of treating a subject in need thereof comprising administering an effective amount of the decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide of any one of claims 1 to 67 or the composition of claims 68 to 76.

81. The method of claim 80, wherein the subject has cancer, anemia, viral infection, bacterial infection, auto-immune disease, asthma, allergy, transplant rejection, atherosclerosis, or fibrosis.

82. The decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide of any one of claims 1 to 67 or the composition of any one of claims 68 to 76 for use in treating cancer, viral infection, bacterial infection, auto-immune disease, asthma, allergy, transplant rejection, atherosclerosis, or fibrosis.

83. The decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide of any one of claims 1 to 67 or the composition of any one of claims 68 to 76 for use in preconditioning for a hematopoietic stem cell transplant.

84. A method of visualizing a cell expressing CD47, the method comprising contacting a population of cells with the decoy polypeptide of any one of claims 1 to 67 and a detectable label.

85. The method of claim 84, wherein the cell is a tumor cell, virally infected cell, bacterially infected cell, autoreactive T or B cell, damaged red blood cell, arterial plaque cell, fibrotic tissue cell, healthy normal cell such as hematopoietic stem cell, a healthy myeloid or lymphoid precursor cell or healthy differentiated hematopoietic cell type such as T, B, plasma, or NK cell.

86. The method of claim 84 or claim 85, wherein the contact is in vivo.

87. The method of claim 84 or claim 85, wherein the contact is in vitro.

88. A method of purifying a cell expressing CD47, the method comprising contacting a population of cells with the decoy polypeptide of any one of claims 1 to 67 and a detectable label and purifying cells bound to the detectable label.

89. The decoy polypeptide of any one of claims 1 to 4, wherein the SIRP-gamma polypeptide has the sequence EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQREGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 13).

90. The decoy polypeptide of any one of claims 1 to 4, wherein the SIRP-gamma polypeptide has one of the following sequences:

EEELQIIQPEKLLLVTVGKTATLHCTITSHFPVGPIQWFRGVGPGRVLIYNQKDGHFPRV TTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 8);

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 9);

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPVLWFRGVGPGRVLIYNQRQGPFPRVT TVSDTTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 10);

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRELIYNAREGRFPRVT TVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 11);

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRQGPFPRVT TVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFRKGSPDDVEFKSGAGTELSVRAKPS (SEQ ID NO: 12);

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPVLWFRGVGPGRVLIYNQRQGPFPRVT TVSDTTKRNNMDFSIRISSITPADVGTYYCIKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 3);

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQREGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 13);

EEELQMIQPEKLLLVTVGKTATLHCTVTSLLPVGPVLWFRGVGPGRELIYNQKEGHFPR VTTVSDLTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPENVEFKSGPGTEMALGAKPS (SEQ ID NO: 42);

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 14);

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTXWH (SEQ ID NO: 15), wherein X is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, or V;

EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQKDGPFPRVT TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGTPEDVEFKSGPGTEMALGAKPS (SEQ ID NO: 16);

EEELQIIQPEKLLLVTVGKTATLHCTITSLLPVGPIQWFRGVGPGRELIYNQRDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGTPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 17);
EEELQIIQPEKLLLVTVGKTATLHCTLTSLLPVGPILWFRGVGPGRVLIYNQRDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGNPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 18);
EEELQLIQPEKLLLVTVGKTATLHCTITSLFPPGPIQWFRGVGPGRVLIYNQKDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGIPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 19);
EEELQIIQPEKLLLVTVGKTATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 20);
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPIGPILWFRGVGPGRVLIYNQKDGPFPRVTT
VSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 21);
EEELQMIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGAGPGRVLIYNQRDGPFPRV
TTVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 22);
EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGIPEDVEFKSGPGTEMALGAKPS
(SEQ ID NO: 23); and
EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIYNQKDGPFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCIKFRKGTPEDVEFKSGPGTEMALXAKPS
(SEQ ID NO: 24).

91. The decoy polypeptide of any one of claims 1 to 67 , wherein the SIRP-gamma polypeptide is a fusion or chimeric polypeptide, and wherein the SIRP-gamma polypeptide is fused to a polypeptide sequence comprising an immune checkpoint inhibitor, a co-stimulatory molecule, or a cytokine through a linker sequence.

92. The decoy polypeptide of claim 91, wherein the linker sequence comprises Gly and Ser.

93. The decoy polypeptide of claim 91, wherein the linker sequence comprises GGGGSGGGGS (SEQ ID NO: 29).

94. The decoy polypeptide of claim 91, wherein the SIRP-gamma polypeptide is located either N-terminal or C-terminal of the polypeptide sequence comprising an immune checkpoint inhibitor, co-stimulatory molecule, cytokine, or attenuated cytokine.

95. The decoy polypeptide of claim 91, wherein the immune checkpoint inhibitor polypeptide has a sequence of a PD-1 or PD-L1 antagonist, a BTLA or CD160 antagonist, or a phosphatidylserine antagonist, e.g., MFGE8, TIM1, TIM3 or TIM4.

96. The decoy polypeptide of claim 91, wherein the co-stimulatory molecule polypeptide has a sequence of a CD40 agonist, a 41BBL or CD137 agonist.

97. The decoy polypeptide of claim 91, wherein the cytokine polypeptide has a sequence of an IL2.

98. The decoy polypeptide of claim 91, wherein the cytokine is attenuated.

99. The decoy polypeptide of 98, wherein the IL2 polypeptide sequence contains mutations D20T and F42A.

100. The decoy polypeptide of claims 91 to 95, wherein the fusion or chimeric polypeptide has a sequence of

DSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFHVVWHRESPSGQTDTLAAFPED
RSQPGQDARFRVTQLPNGRDFHMSVVRARRNDSGTYVCGVISLAPKIQIKESLRAELRV
TERGGGGSGGGGSEEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRV
LIYNQKDGHFPRVTTVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSG
PGTEMALGAKPS (SEQ ID NO: 30).

101. The decoy polypeptide of claims 91 to 95, wherein the fusion or chimeric polypeptide has a sequence of

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPSG
GGGSGGGGSWNIHGKESCDVQLYIKRQSEHSILAGDPFELECPVKYCANRPHVTWCKL
NGTTCVKLEDRQTSWKEEKNISFFILHFEPVLPNDNGSYRCSANFQSNLIESHSTTLYVT
DVK (SEQ ID NO: 31).

102. The decoy polypeptide of claims 91 to 95, wherein the fusion or chimeric polypeptide has one of the following sequences:

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPSG
GGGSGGGGSELNGCANPLGLKNNSIPDKQITASSSYKTWGLHLFSWNPSYARLDKQGN
FNAWVAGSYGNDQWLQVDLGSSKEVTGIITQGARNFGSVQFVASYKVAYSNDSANWT
EYQDPRTGSSKIFPGNWDNHSHKKNLFETPILARYVRILPVAWHNRIALRLELLGC (SEQ
ID NO: 32);

EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPSG
GGGSGGGGSVAGSVKVGGEAGPSVTLPCHYSGAVTSMCWNRGSCSLFTCQNGIVWTN

GTHVTYRKDTRYKLLGDLSRRDVSLTIENTAVSDSGVYCCRVEHRGWFNDMKITVSLE
IVPPKVTT (SEQ ID NO: 33);
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPSG
GGGSGGGGSSEVEYRAEVGQNAYLPCFYTPAAPGNLVPVCWGKGACPVFECGNVVLR
TDERDVNYWTSRYWLNGDFRKGDVSLTIENVTLADSGIYCCRIQIPGIMNDEKFNLKLV
IKPAKVTPA (SEQ ID NO: 34); or
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPSG
GGGSGGGGSTSETVVTEVLGHRVTLPCLYSSWSHNSNSMCWGKDQCPYSGCKEALIRT
DGMRVTSRKSAKYRLQGTIPRGDVSLTILNPSESDSGVYCCRIEVPGWFNDVKINVRLN
LQRASTTTDEKFNLKLVIKPAKVTPA (SEQ ID NO: 35).

103. The decoy polypeptide of claims 91 to 95 or 96, wherein the fusion or chimeric polypeptide has one of the following sequences:
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPSG
GGGSGGGGSGDQNPQIAAHVISEASSKTTSVLQWAEKGYYTMSNNLVTLENGKQLTV
KRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIH
LGGVFELQPGASVFVNVTDPSQVSHGTGFTSFGLLKL (SEQ ID NO: 36); or
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPSG
GGGSGGGGSDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYK
EDTKELVVAKAGVYYVFFQMELRRVVAGEGSGSVSLALHLMPLRSAAGAAALALTVD
LPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTP
EIPA (SEQ ID NO: 37).

104. The decoy polypeptide of claims 91 to 95 or 97 to 99, wherein the fusion or chimeric polypeptide has one of the following sequences:
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPSG
GGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKK
ATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD
ETATIVEFLNRWITFCQSIISTLT (SEQ ID NO: 38); or
EEELQIIQPEKLLLVTVGKTATLHCTITSLFPVGPIQWFRGVGPGRVLIYNQKDGHFPRVT
TVSDGTKRNNMDFSIRISSITPADVGTYYCVKFRKGSPEDVEFKSGPGTEMALGAKPSG
GGGSGGGGSAPTSSSTKKTQLQLEHLLLTLQMILNGINNYKNPKLTRMLTAKFYMPKK

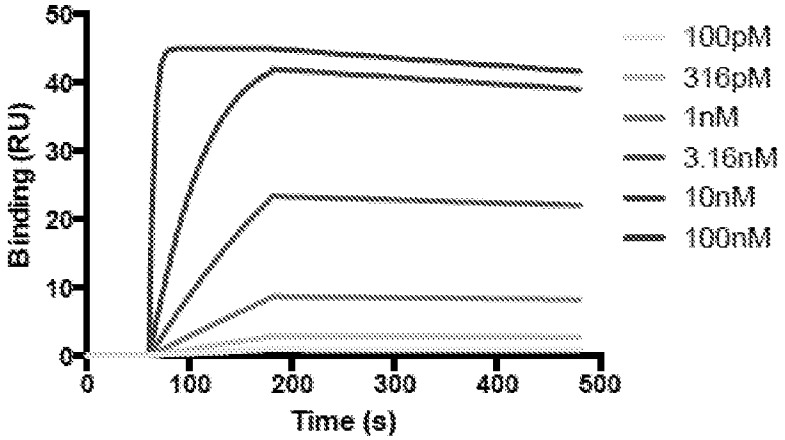
ATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD

ETATIVEFLNRWITFCQSIISTLT (SEQ ID NO: 39).

FIG. 1

Surface plasmon resonance-based measurement of binding affinity and kinetics of the SIRP-gamma variant GV3 for human CD47.

FIG. 1A



KD = 92 pM

$T_{1/2}$ = ~44 min

FIG. 1B

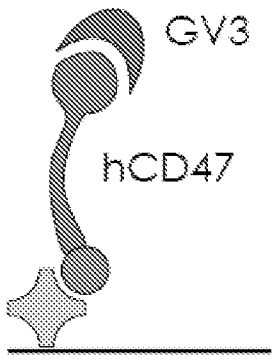
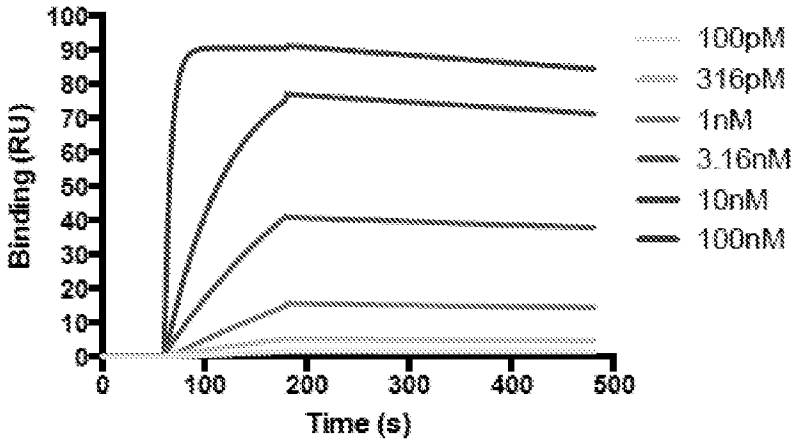


FIG. 2

Surface plasmon resonance-based measurement of binding affinity and kinetics of HAC-GV3, a fusion of the SIRP-gamma variant GV3 with a PD-1 variant HAC, for human CD47.

FIG. 2A



KD = 160 pM

$T_{1/2}$ = ~40 min

FIG. 2B

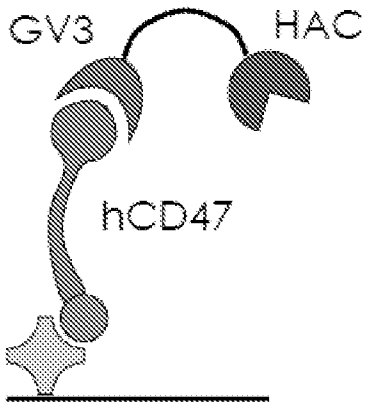
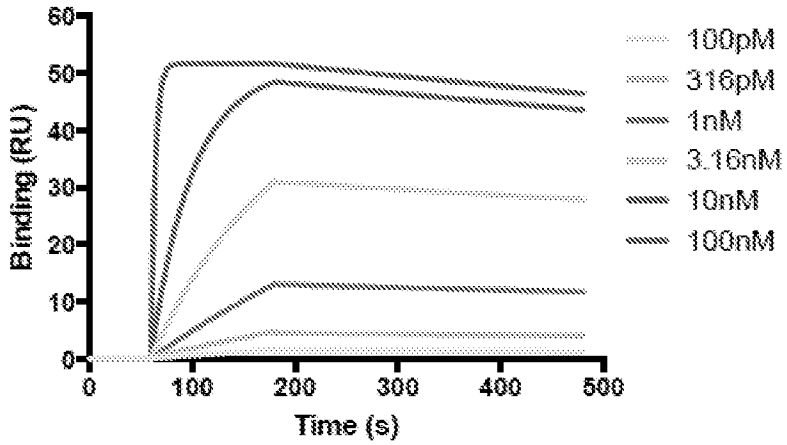


FIG. 3

Surface plasmon resonance-based measurement of binding affinity and kinetics of HAC-GV3, a fusion of the SIRP-gamma variant GV3 with a PD-1 variant HAC, for human PD-L1.

FIG. 3A



KD = 134 pM

$T_{1/2}$ = ~38 min

FIG. 3B

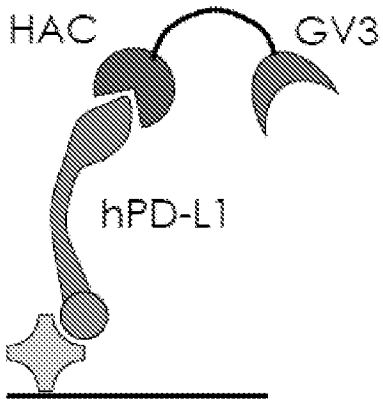


FIG. 4

Surface plasmon resonance-based measurement of the simultaneous binding of both human CD47 and human PD-L1 by HAC-GV3.

FIG. 4A

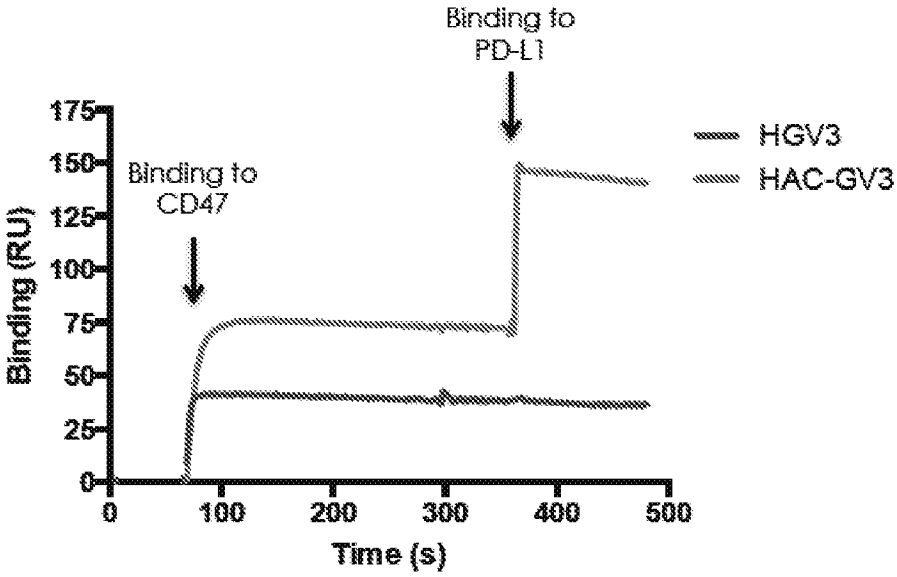


FIG. 4B

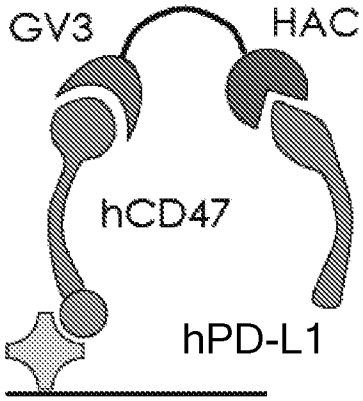


FIG. 5

Flow cytometry-based measurement of blockade of CD47/SIRP-alpha interactions on the surface of CD47+ cells by GV3 and HAC-GV3.

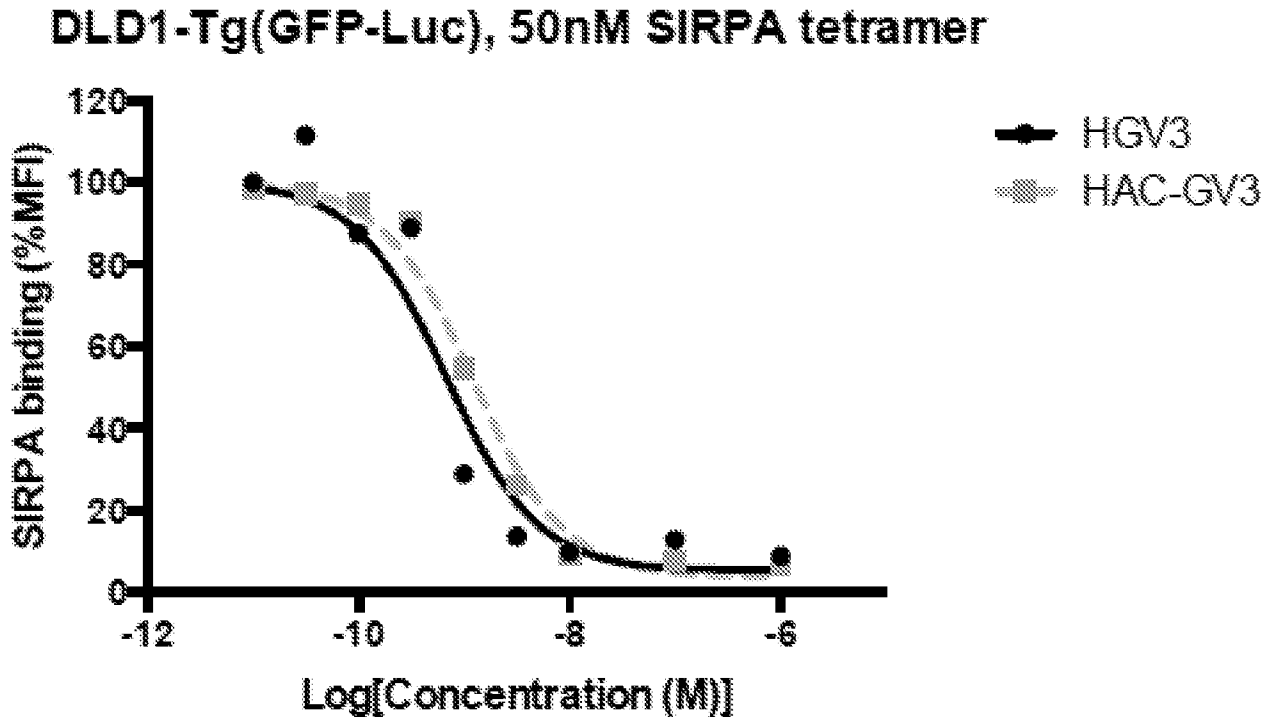


FIG. 6

Flow cytometry-based measurement of blockade of CD47/SIRP-alpha interactions on the surface of CD47+ PD-L1+ cells by GV3 and HAC-GV3.

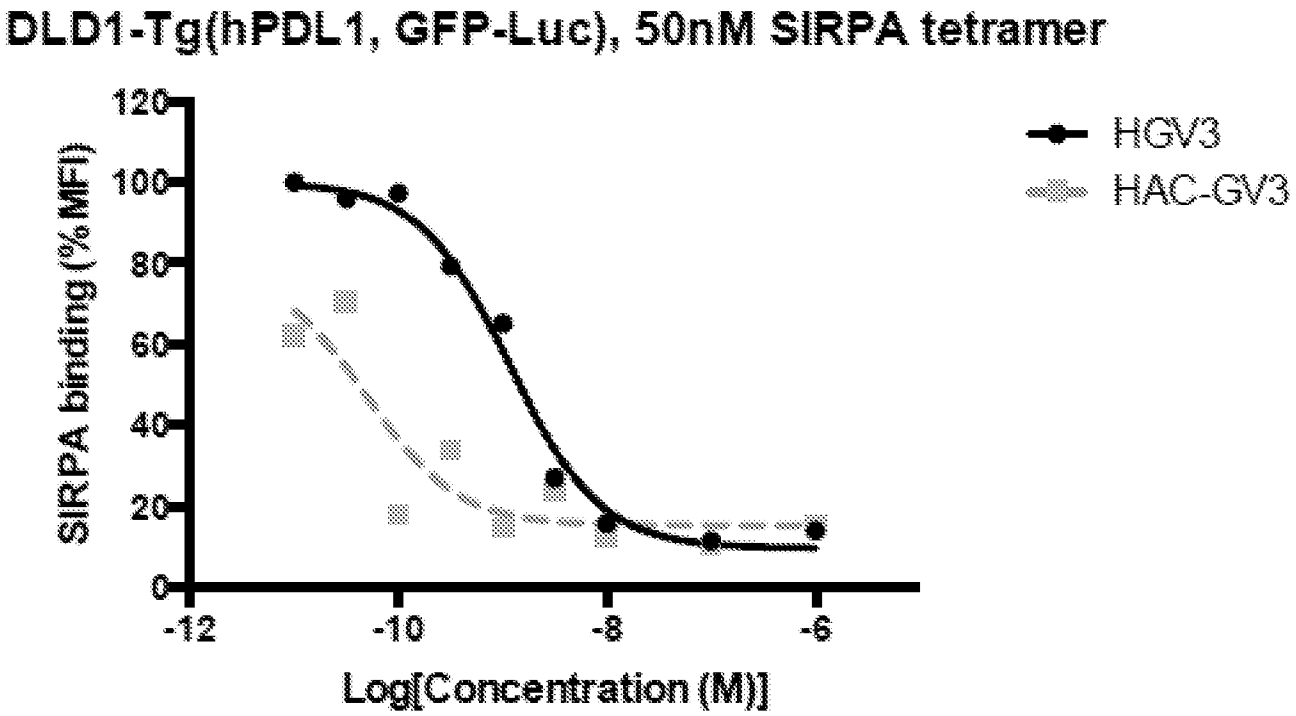


FIG. 7

Flow cytometry-based measurement of blockade of PD-1/PD-L1 interactions on the surface of PD-L1+ cells by HAC and HAC-GV3.

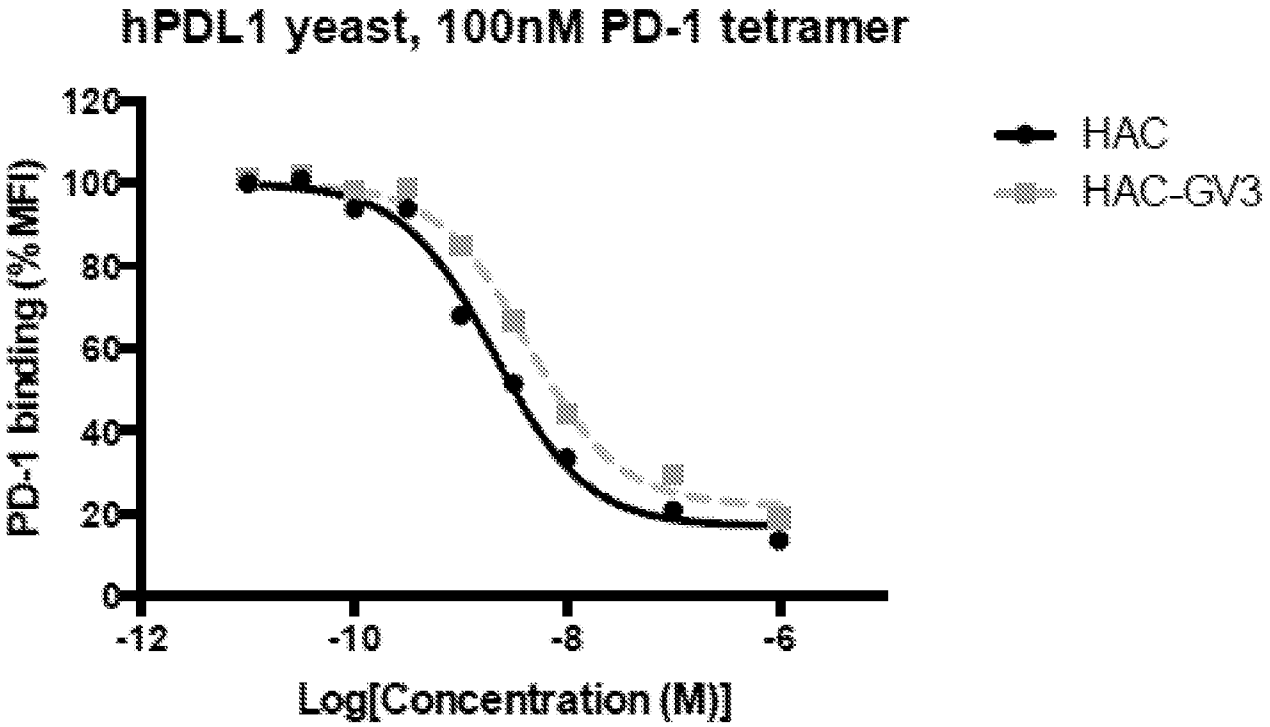


FIG. 8

Flow cytometry-based measurement of blockade of PD-1/PD-L1 interactions on the surface of CD47+ PD-L1+ cells by HAC and HAC-GV3.

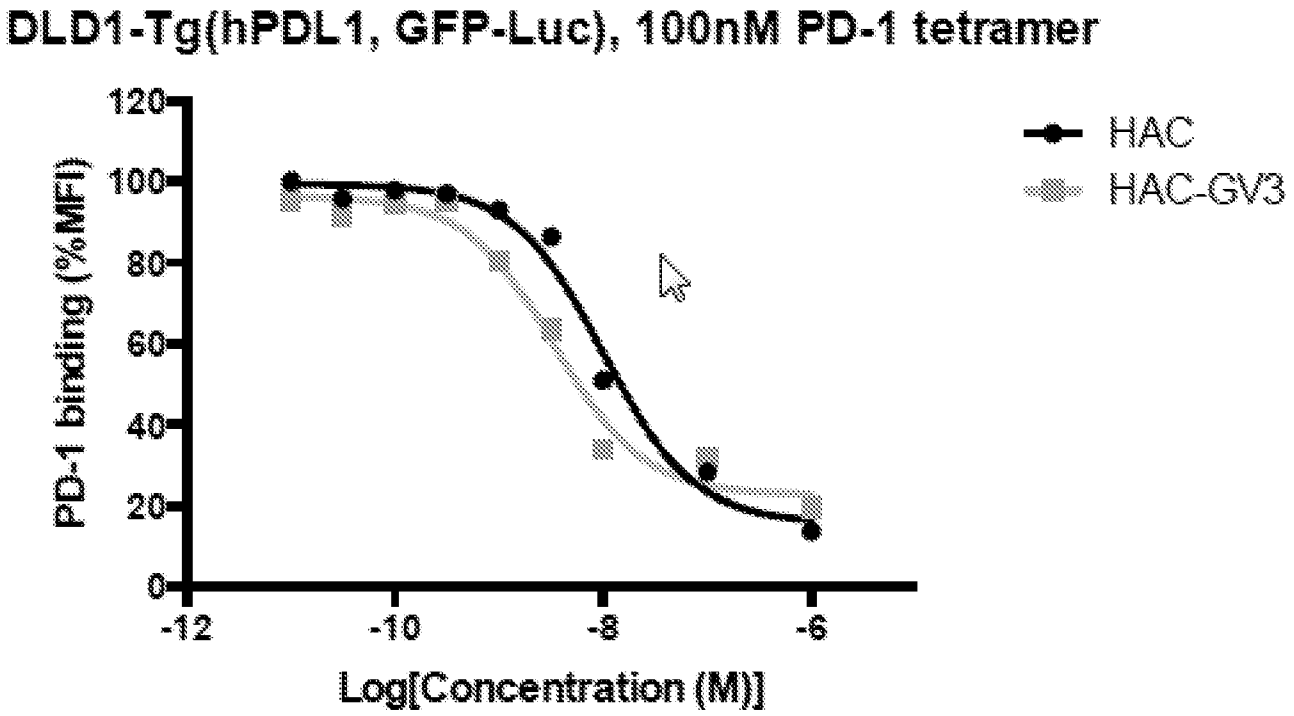


FIG. 9

GV3 microbody and HAC-GV3 potentiate phagocytosis across a wide range of opsonizing antibody concentrations.

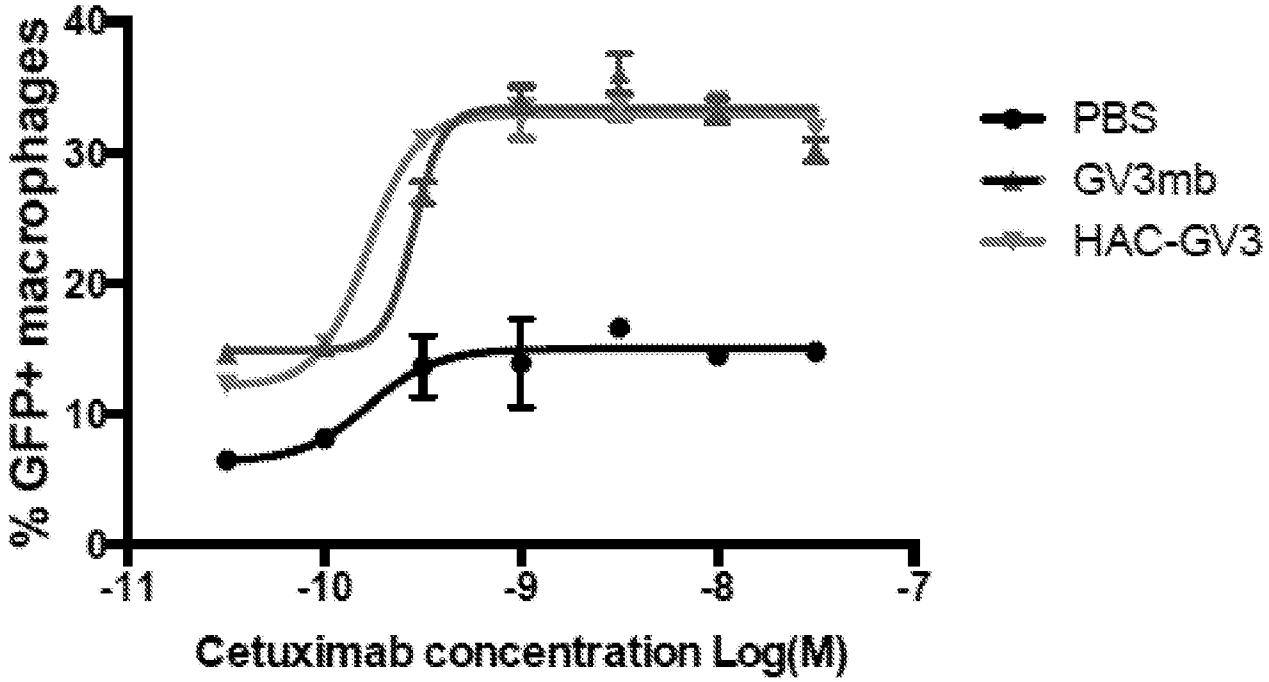


FIG. 10

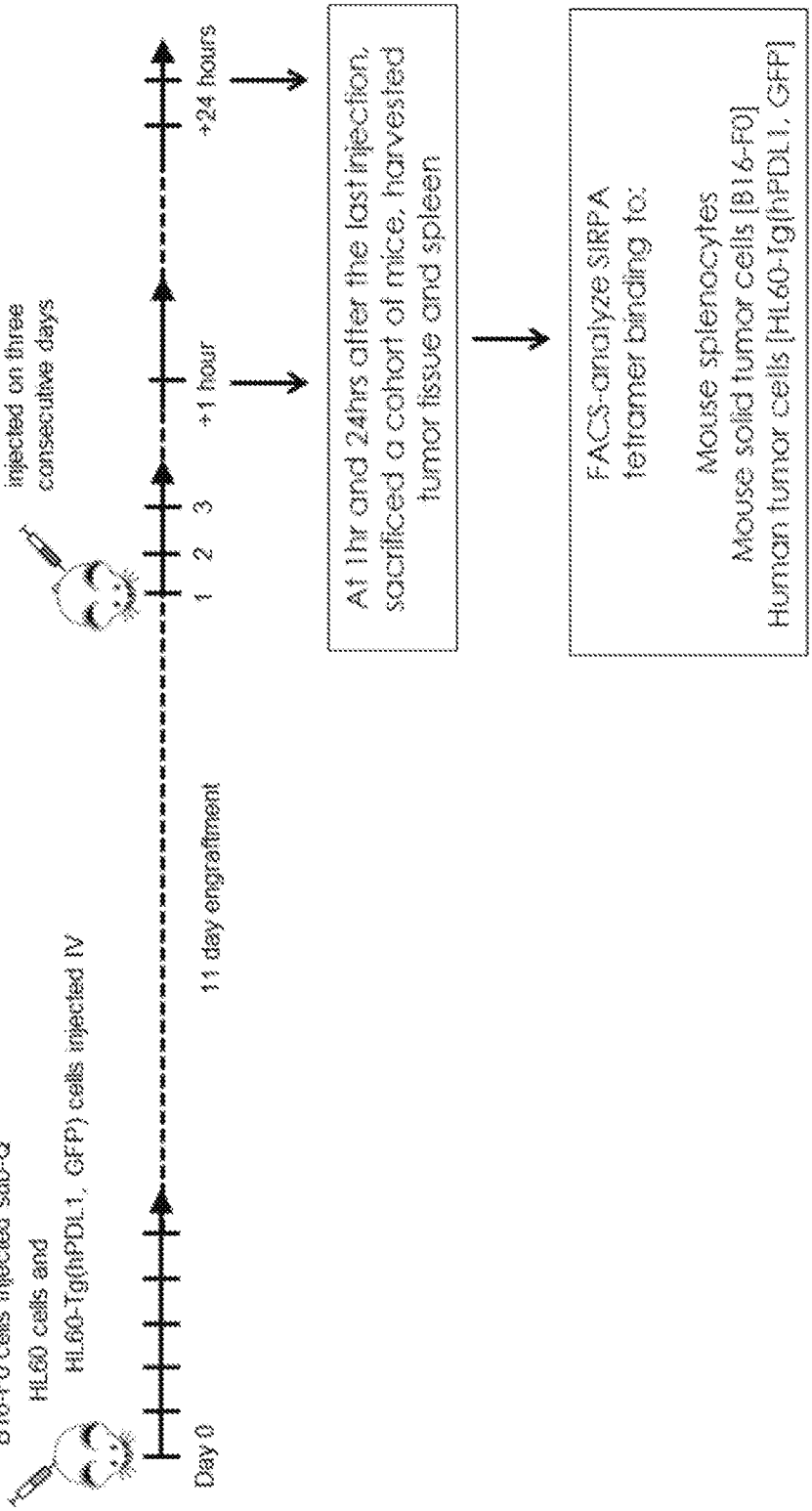

B16-F0 cells injected sub-Q
HL60 cells and
HL60-Tg(hPDL1, GFP) cells injected IV
Day 0
11 day engraftment
PBS, GV3, or HAC-GV3
injected on three
consecutive days
1 2 3
+1 hour
+24 hours
At 1hr and 24hrs after the last injection,
sacrificed a cohort of mice, harvested
tumor tissue and spleen
FACS-analyze SIRPA
tetramer binding to:
Mouse splenocytes
Mouse solid tumor cells [B16-F0]
Human tumor cells [HL60-Tg(hPDL1, GFP)]

FIG. 11A

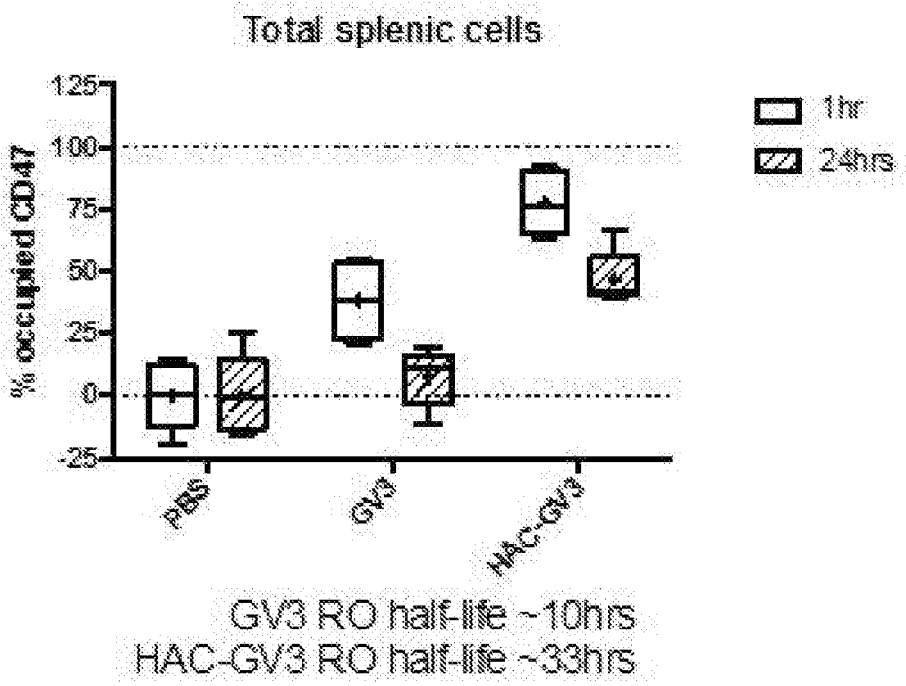


FIG. 11B

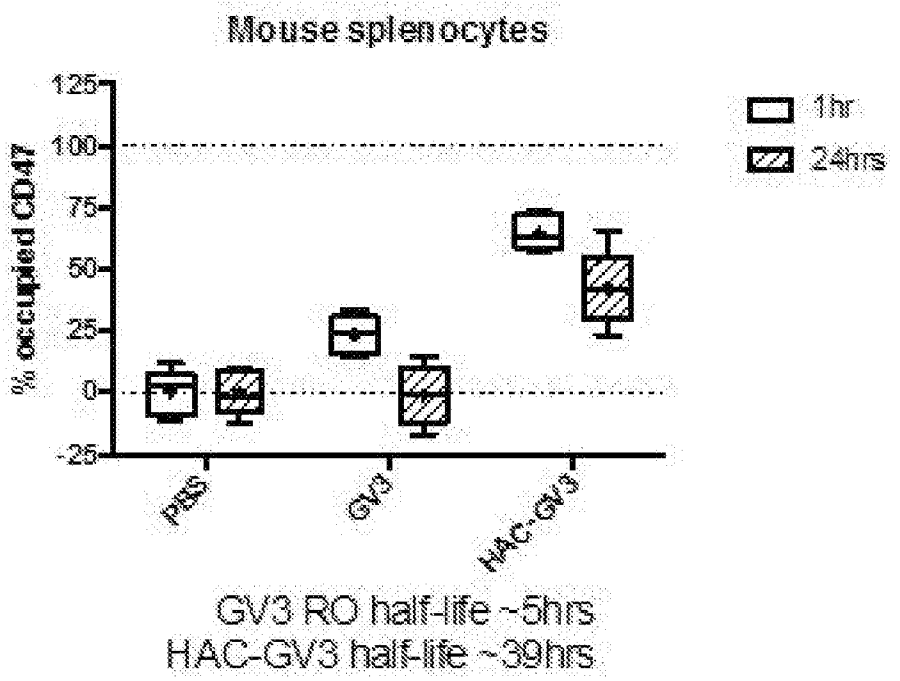


FIG. 11C

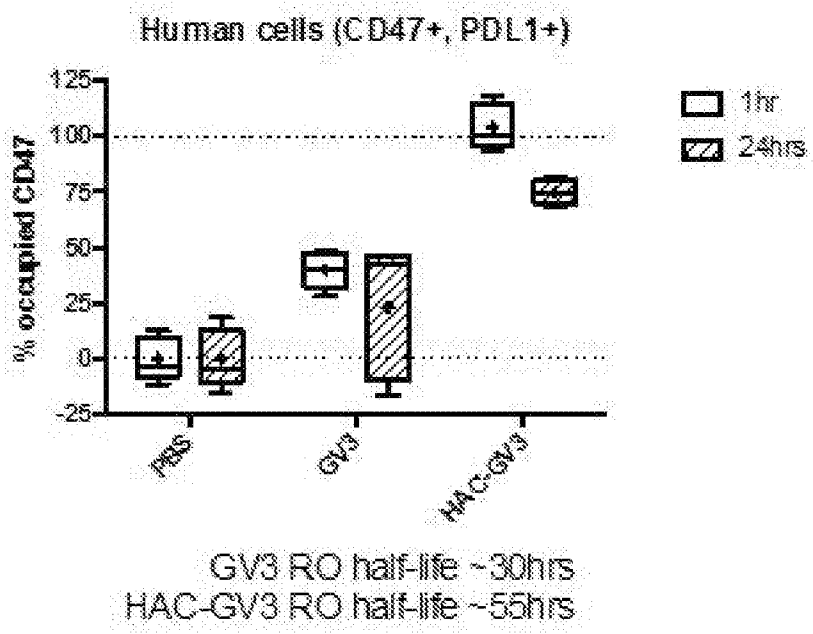
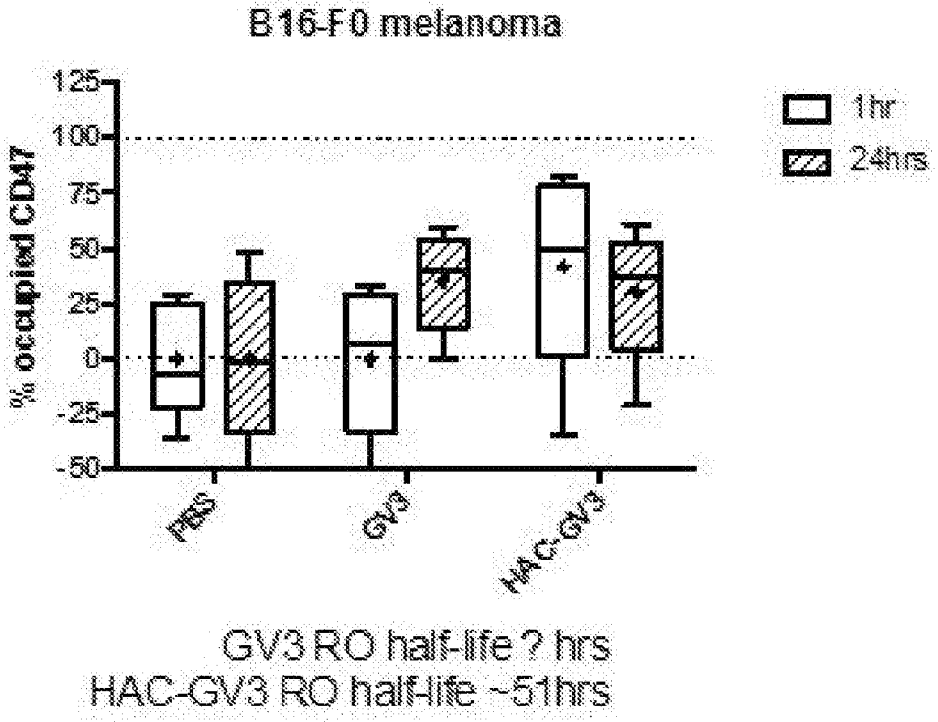


FIG. 11D



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/32921

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 16/28, 14/705, 14/435; A61K 47/42, 38/16 (2016.01)
CPC - C07K 16/2896, 14/70596, 14/435; A61K 47/42, 38/16, 38/1709
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C07K 16/28, 14/705, 14/435, 14/43; A61K 47/42, 38/16, 39/00, 37/00; G01N 33/15, 33/53; C12N 15/63, 5/10; C07H 21/04
CPC: C07K 16/2896, 14/70596, 14/435, 2319/32, 2319/30; A61K 47/42, 38/16, 38/1709, 38/00, 38/177

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); LENS sequence search database; NCBI database; Google Scholar; Pubmed; EBSCO; SIRP, signal, regulat*, protein, beta, gamma, CD, affinity, variant, modif*, amino acid, increas*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	(HATHERLEY, D et al.) Paired Receptor Specificity Explained by Structures of Signal Regulatory Proteins Alone and Complexed with CD47. Molecular Cell. 25 July, 2008; Vol. 31, No. 2; pages 266-277; page 272, column 2, paragraph 2 – page 273, column 1, paragraph 1; figures 5B-5D; Genbank Supplement pages.	1-2, 3/1-2
A	US 2010/0239579 A1 (SMITH, CA et al.) 23 September, 2010; whole document	1-2, 3/1-2
A	(LEE, WY et al.) Novel Structural Determinants on SIRP alpha that Mediate Binding to CD47. The Journal of Immunology. 01 December, 2007; Vol. 179, No. 11; pages 7741-7750; whole document	1-2, 3/1-2
A	(STEFANIDAKIS, M et al.) Endothelial CD47 Interaction with SIRP gamma is Required for Human T-cell Transendothelial Migration Under Shear Flow Conditions In Vitro. Blood. 04 June, 2008; Vol. 112, No. 4; pages 1280-1289; whole document; DOI: 10.1182/blood-2008-01-134429	1-2, 3/1-2

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 2016 (21.09.2016)	14 OCT 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/32921

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 13*ter*.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 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/32921

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-28, 31-42, 45-104
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-Please See Supplemental Page-*-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Group 1+: Claims 1-3 SEQ ID NO: 1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US16/32921

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+: Groups I+: Claims 1-3, 29, 30/1, 30/29, 43, 44/1 and 44/43 are directed toward a decoy polypeptide comprising a SIRP-gamma, a SIRP-beta or a SIRP-beta2 polypeptide.

The decoy polypeptide comprising a SIRP-gamma, a SIRP-beta or a SIRP-beta2 polypeptide will be searched to the extent that they encompass SEQ ID NO: 1 (SIRP-gamma wild-type polypeptide amino acid sequence). It is believed that Claims 1-3 encompass SEQ ID NO: 1 (SIRP-gamma wild-type polypeptide amino acid sequence). Applicant is invited to elect additional SIRP sequence(s), with specified SEQ ID NO(s): for each, to be searched. Additional SIRP sequence(s) will be searched upon the payment of additional fees. Applicants must specificy the claims that encompass any additionally elected SIRP sequence(s). Applicants must further indnicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An Exemplary Election would be: SEQ ID NO: 29 (SIRP-beta polypeptide synthetic peptide amino acid sequence).

No technical features are shared between the sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including a decoy polypeptide comprising a SIRP-gamma, a SIRP-beta or a SIRP-beta2 polypeptide, wherein the polypeptide comprises at least one amino acid modification to increase affinity of the decoy SIRP-gamm, SIRP-beta or SIRP-beta2 polypeptide binding to CD47 as compared to the affinity for CD47 of the corresponding wild type SIRP-gamm, SIRP-beta or SIRP-beta2 polypeptide.

However, these shared features are previously disclosed by the publication entitled 'Endothelial CD47 Interaction with SIRP-gamma Is Required for Human T-Cell Transendothelial Migration Under Shear Flow Conditions In Vitro' by Stefanidakis, et al. (hereinafter 'Stefanidakis') in view of the publication entitled 'Novel Structural Determinants on SIRP-alpha that Mediate Binding to CD47' by Lee, et al. (hereinafter 'Lee').

Stefanidakis discloses a decoy polypeptide comprising a SIRP-gamma (abstract), a SIRP-beta (page 1280, right column) or a SIRP-beta2 polypeptide (page 1280, right column), including modulatiation of the affinity of the decoy SIRP-gamma (page 1287, right column, first paragraph), SIRP-beta or SIRP-beta2 polypeptide binding to CD47 as compared to the affinity for CD47 of the corresponding wild type SIRP-gamma (page 1287, right column, first paragraph), SIRP-beta or SIRP-beta 2 polypeptide (data suggests that CD47 engagement by SIRP-gamma activates CD47-induced unidirectional signaling patways in the endothelial cell; page 1287, right column, first paragraph). Stefanidakis does not disclose wherein the polypeptide comprises at least one amino acid modification to increase affinity of the decoy SIRP-gamma, SIRP-beta or SIRP-beta2 polypeptide binding to CD47. Lee discloses at least one amino acid modification (page 7744, right column, second paragraph) to increase affinity of the SIRP-gamma, SIRP-beta (page 7744, right column, second paragraph) or SIRP-beta2 polypeptide binding to CD47 (in order for SIRP-Beta to gain the ability to bind to CD47, it would be necessary to replace cumulatively all residues that correspond to the cirticla residues in SIRP-alpha ... when these mutations were introduced singly and in combinations, no binding to CD47 was observed except for the recombinant protein carrying all the critical mutations; page 7744, right column, second paragraph). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the decopy polypeptide comprising a SIRP-beta polypeptide, as previously disclosed by Stefanidakis, with the mutations in SIRP-beta polypeptide, as previously disclosed by Lee, as the Lee reference indicates that such mutations enhances the binding affinity of SIRP-beta with CD47 (page 7744, right column, second paragraph), for achieving enhanced applications in immunotherapy.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Stefanidakis and Lee references, unity of invention is lacking.