SIRP-ALPHA VARIANT CONSTRUCTS AND USES THEREOF

BACKGROUND OF THE INVENTION

Signal-regulatory protein α (SIRP-α) is a protein widely expressed on the membrane of myeloid cells. SIRP-α interacts with CD47, a protein broadly expressed on many cell types in the body. The interaction of SIRP-α with CD47 prevents engulfment of "self" cells, which could otherwise be recognized by the immune system. SIRP-α was first discovered as a binder of SHP-2 (an SH-2 domain containing tyrosine phosphatase). CD47 was early characterized as an overexpressed antigen on ovarian carcinoma cells.

In 2000, Oldenborg et al. showed that administration of CD47-deficient red blood cells (RBCs) in a mouse model resulted in rapid clearance of the RBCs from the system, demonstrating CD47 to be a "protective" signal on some subset of "self" cells. Subsequently, the potential link between the SIRP-α and cancer was further explored. It was found that high CD47 expression on tumor cells acted, in acute myeloid leukemia (AML) and several solid tumor cancers, as a negative prognostic factor for survival. Strategies focused on disrupting the interaction between CD47 and SIRP-α, such as administration of agents that mask either CD47 or SIRP-α, have been explored as potential anticancer therapies.

However, in considering these therapeutic strategies, it is a concerning issue that SIRP-α could bind to CD47 on many different cell types in the human body. Thus, there exists a need to engineer SIRP-α to preferentially bind to CD47 only on diseased cells or on cells at a diseased site.

SUMMARY

The disclosure features signal-regulatory protein α (SIRP-α) variant constructs. The SIRP-α variant constructs include SIRP-α variants. In some examples, the SIRP-α variant constructs have preferential activity at a diseased site (e.g., at the site of a tumor than at a non-diseased site). In certain examples, the SIRP-α variant constructs have higher binding affinity to CD47 on diseased cells (e.g., tumor cells). In some examples, the SIRP-α variants bind with higher affinity to CD47 under acidic pH (e.g., less than around pH 7) and/or under hypoxic condition than under physiological conditions. In some examples, the SIRP-α variants contain one or more substitutions of amino acids with histidine residues or with other amino acids that allow preferential binding of SIRP-α variant constructs at a diseased site. In some examples, the SIRP-α variant constructs are prevented from binding to CD47 in a non-diseased site by a blocking peptide. In some examples, the SIRP-α variant constructs are targeted to the diseased site (e.g., the tumor) by a targeting moiety (e.g., an antibody directed to a tumor-associated antigen or an antibody-binding peptide). The disclosure also features methods and pharmaceutical compositions containing SIRP-α variant constructs to treat various diseases, such as cancer, preferably solid tumor cancer and hematological cancer.

In one example, the disclosure features a signal-regulatory protein α (SIRP-α) variant construct, wherein the SIRP-α variant construct preferentially binds CD47 on diseased cells or at a diseased site than on non-diseased cells. In some examples, the SIRP-α variant construct binds to CD47 on diseased cells or at a diseased site with higher affinity than it binds CD47 on non-diseased cells.

In some examples, the SIRP-α variant construct includes a SIRP-α variant attached to a blocking peptide. In some examples, the blocking peptide binds with higher affinity to a wild-type

SIRP-α than to the SIRP-α variant. In some examples, the SIRP-α variant binds with higher affinity to a wild-type CD47 than to the blocking peptide.

In some examples, the blocking peptide is a CD47-based blocking peptide. In some examples, the CD47-based blocking peptide includes a portion that has at least 80% amino acid sequence identity to the sequence of the wild-type, IgSF domain of CD47 (SEQ ID NO: 35) or a fragment thereof. In some examples, the CD47-based blocking peptide has the sequence of SEQ ID NO: 38 or 40.

Provided herein are SIRP-α variant constructs comprising a SIRP-α variant described herein, wherein said SIRP-α variant is attached to a blocking peptide described herein by use of at least one linker (e.g, a cleavable linker). In some examples, the SIRP-α variant may comprise the same CD47 binding site as a wild type SIRP-α. In some examples, the SIRP-α variant may comprise one or more mutations, or insertions as compared to a wild type SIRP-α. In some examples, the SIRP-α variant may be a truncated form of the wild type SIRP-α. In some examples, the blocking peptide may be a CD47 mimic, variant, or fragment described herein. In some examples, the blocking peptide may exhibit a higher affinity for a wild-type SIRP-α, as compared to the SIRP-α variant in the SIRP-α variant construct. In some examples, the blocking peptide may be a CD47 variant polypeptide that demonstrates a lower affinity for a SIRP-α variant as compared to the wild-type CD47. In some examples, the linker between the SIRP-α variant and the blocking peptide may be at least one linker that is optionally cleavable by one or more proteases. In some examples, the linker optionally also comprises one or more spacers.

In some examples, the SIRP-α variant is attached to a blocking peptide by way of a cleavable linker and optionally one or more spacers. In some examples, the cleavable linker is cleaved under acidic pH and/or hypoxic condition. In some examples, the cleavable linker is cleaved by a tumor-associated enzyme. In some examples, the tumor-associated enzyme is a protease. In some examples, the protease is selected from the group consisting of matriptase (MTSP1), urinary-type plasminogen activator (uPA), legumain, PSA (also called KLK3, kallikrein-related peptidase-3), matrix metalloproteinase-2 (MMP-2), MMP9, human neutrophil elastase (HNE), and proteinase 3 (Pr3). In some examples, the protease is matriptase. In some examples, the cleavable linker has the sequence of LSGRSDNH (SEQ ID NO: 47) or any one of the sequences listed in Table 7. In some examples, the cleavable linker includes one or a combination of the following sequences (see, e.g., Table 7): PRFKIIGG, PRFRIIGG, SSRHRRALD, RKSSIIIRMRDVVL, SSSFDKGKYKKGDDA, SSSFDKGKYKRGDDA, IEGR, IDGR, GGSIDGR, PLGLWA, GPLGIAGI, GPEGLRVG, YGAGLGVV, AGLGVVER, AGLGISST, DVAQFVLT, VAQFVLTE, AQFVLTEG, PVQPIGPQ, L/S/G-/R--/S-/D/N/H, -/s/gs/Rk-/rv/-/-/-, SGR-SA, L/S/G-/R--/S-/D/N/H, r/-/-/Rk-/v-/-/g/-, RQAR-VV, r/-/-/Rk/v/-/g, /Kr/RKQ/gAS/RK/A, L/S/G-/R-/S-/D/N/H, -/-/-/N/-/-/-, AAN-L, ATN-L, si/sq/-/yqrs/s/-/-, S/S/K/L/Q, -/p/-/- /li/-/-/-, g/pa/-/gl/-/g/-, G/P/L/G/I/A/G/Q, P/V/G/L/I/G, H/P/V/G/L/L/A/R, -/-/-/viat-/-/-/-, and -/y/y/vta -/-/-/-, where "–" means any amino acid (i.e., any naturally occurring amino acid), capital case indicates an strong preference for that amino acid, lower case indicates a minor preference for that amino acid, and "/" separates amino acid positions in cases where more than one amino acid at a position adjacent to the "/"is possible.

In some examples, the SIRP-α variant is attached to an antibody-binding peptide. In some examples, the antibody-binding peptide binds to a constant region of an antibody reversibly or

irreversibly. In some examples, the antibody-binding peptide binds to the fragment antigen-binding (Fab) region of an antibody reversibly or irreversibly. In some examples, the antibody-binding peptide binds to a variable region of an antibody reversibly or irreversibly. In some examples, the antibody is Cetuximab. In some examples, the antibody-binding peptide has at least 75% amino acid sequence identity to the sequence of a disease localization peptide (DLP) (CQFDLSTRRLKC (SEQ ID NO: 64) or CQYNLSSRALKC (SEQ ID NO: 65)) or a fragment thereof. In some examples, the antibody-binding peptide has the sequence of SEQ ID NO: 64.

In some examples, the SIRP-α variant is attached to an Fc domain monomer. In some examples, the SIRP-α variant is attached to a human serum albumin (HSA). In some examples, the HSA includes amino acid substitution C34S and/or K573P, relative to SEQ ID NO: 67. In some examples, the HSA has the sequence of

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLF
GDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYL
YEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGER
AFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECC
EKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRL
AKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVS
TPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCF
SALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCC
KADDKETCFAEEGKKLVAASQAALGL (SEQ ID NO: 68).

In some examples, the SIRP-α variant is attached to an albumin-binding peptide. In some examples, the albumin-binding peptide has the sequence of SEQ ID NO: 2. In some examples, the SIRP-α variant is attached to a polymer, wherein the polymer is polyethylene glycol (PEG) chain or polysialic acid chain.

In some examples, the SIRP-α variant is attached to an antibody. In some examples, the antibody is a tumor-specific antibody. In some examples, the antibody (e.g., a tumor-specific antibody) is selected from the group consisting of cetuximab, pembrolizumab, nivolumab, pidilizumab, MEDI0680, MEDI6469, Ipilimumab, tremelimumab, urelumab, vantictumab, varlilumab, mogamalizumab, anti-CD20 antibody, anti-CD19 antibody, anti-CS1 antibody, herceptin, trastuzumab, and pertuzumab. In some examples, the antibody (e.g., a tumor-specific antibody) may bind to one or more of the following: 5T4, AGS-16, ALK1, ANG-2, B7-H3, B7-H4, c-fms, c-Met, CA6, CD123, CD19, CD20, CD22, EpCAM, CD30, CD32b, CD33, CD37, CD38, CD40, CD52, CD70, CD74, CD79b, CD98, CEA, CEACAM5, CLDN18.2, CLDN6, CS1, CXCR4, DLL-4, EGFR, EGP-1, ENPP3, EphA3, ETBR, FGFR2, fibronectin, FR-alpha, GCC, GD2, glypican-3, GPNMB, HER-2, HER3, HLA-DR, ICAM-1, IGF-1R, IL-3R, LIV-1, mesothelin, MUC16, MUC1, NaPi2b, Nectin-4, Notch 2, Notch 1, PD-L1, PD-L2, PDGFR-α, PS, PSMA, SLTRK6, STEAP1, TEM1, VEGFR, CD25, CD27L, DKK-1, and/or CSF-1R.

In some examples, the SIRP-α variant in the SIRP-α variant construct has at least 80% (e.g., at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to a sequence of any one of SEQ ID NOs: 3-12 and 24-34.

In some examples, the SIRP-α variant in the SIRP-α variant construct has a sequence of EEEX$_1$QX$_2$IQPDKSVLVAAGETX$_3$TLRCTX$_4$TSLX$_5$PVGPIQWFRGAGPGRX$_6$LIYNQX$_7$X$_8$GX$_9$FPRVTTVSD X$_{10}$TX$_{11}$RNNMDFSIRIGX$_{12}$ITX$_{13}$ADAGTYYCX$_{14}$KX$_{15}$RKGSPDDVEX$_{16}$KSGAGTELSVRAKPS (SEQ ID

NO: 13), wherein X_1 is L, I, or V; X_2 is V, L, or, I; X_3 is A or V; X_4 is A, I, or L; X_5 is I, T, S, or F; X_6 is E, V, or L; X_7 is K or R; X_8 is E or Q; X_9 is H, P, or R; X_{10} is L, T, or G; X_{11} is K or R; X_{12} is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X_{13} is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X_{14} is V or I; X_{15} is F, L, or V; and X_{16} is F or V.

In one aspect, the present disclosure refers to a signal-regulatory protein α (SIRP-α) variant construct, comprising a SIRP-α variant attached to a CD47-based blocking peptide by way of a cleavable linker,

wherein the CD47-based blocking peptide has at least 80% sequence identity to a sequence of wild-type, IgSF domain of CD47 (SEQ ID NO: 35) or a fragment thereof that can bind a SIRP-α variant, and

wherein the SIRP-α variant comprises the amino acid sequence set forth in EEEX$_1$QX$_2$IQPDKSVLVAAGETX$_3$TLRCTX$_4$TSLX$_5$PVGPIQWFRGAGPGRX$_6$LIYNQX$_7$X$_8$GX$_9$FPRVTTVSD X$_{10}$TX$_{11}$RNNMDFSIRIGX$_{12}$ITX$_{13}$ADAGTYYCX$_{14}$KX$_{15}$RKGSPDDVEX$_{16}$KSGAGTELSVRAKPS (SEQ ID NO: 13),

wherein X_1 is L, I, or V; X_2 is V, L, or, I; X_3 is A or V; X_4 is A, I, or L; X_5 is I, T, S, or F; X_6 is E, V, or L; X_7 is K or R; X_8 is E or Q; X_9 is H, P, or R; X_{10} is L, T, or G; X_{11} is K or R; X_{12} is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X_{13} is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X_{14} is V or I; X_{15} is F, L, or V; and X_{16} is F or V.

In some examples, the SIRP-α variant in the SIRP-α variant construct has a sequence of EEGX$_1$QX$_2$IQPDKSVSVAAGESX$_3$ILHCTX$_4$TSLX$_5$PVGPIQWFRGAGPGRX$_6$LIYNQX$_7$X$_8$GX$_9$FPRVTTVSD X$_{10}$TX$_{11}$RNNMDFSIRIGX$_{12}$ITX$_{13}$ADAGTYYCX$_{14}$KX$_{15}$RKGSPDDVEX$_{16}$KSGAGTELSVRAKPS (SEQ ID NO: 16), wherein X_1 is L, I, or V; X_2 is V, L, or, I; X_3 is A or V; X_4 is A, I, or L; X_5 is I, T, S, or F; X_6 is E, V, or L; X_7 is K or R; X_8 is E or Q; X_9 is H, P, or R; X_{10} is L, T, or G; X_{11} is K or R; X_{12} is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X_{13} is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X_{14} is V or I; X_{15} is F, L, or V; and X_{16} is F or V.

In some examples, the SIRP-α variant in the SIRP-α variant construct has a sequence of EEEX$_1$QX$_2$IQPDKFVLVAAGETX$_3$TLRCTX$_4$TSLX$_5$PVGPIQWFRGAGPGRX$_6$LIYNQX$_7$X$_8$GX$_9$FPRVTTVSD X$_{10}$TX$_{11}$RNNMDFSIRIGX$_{12}$ITX$_{13}$ADAGTYYCX$_{14}$KX$_{15}$RKGSPDDVEX$_{16}$KSGAGTELSVRAKPS (SEQ ID NO: 17), wherein X_1 is L, I, or V; X_2 is V, L, or, I; X_3 is A or V; X_4 is A, I, or L; X_5 is I, T, S, or F; X_6 is E, V, or L; X_7 is K or R; X_8 is E or Q; X_9 is H, P, or R; X_{10} is L, T, or G; X_{11} is K or R; X_{12} is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X_{13} is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X_{14} is V or I; X_{15} is F, L, or V; and X_{16} is F or V.

In some examples, the SIRP-α variant in the SIRP-α variant construct has a sequence of EEEX$_1$QX$_2$IQPDKSVLVAAGETX$_3$TLRCTX$_4$TSLX$_5$PVGPIQWFRGAGPGRX$_6$LIYNQX$_7$X$_8$GX$_9$FPRVTTVSD X$_{10}$TX$_{11}$RNNMDFPIRIGX$_{12}$ITX$_{13}$ADAGTYYCX$_{14}$KX$_{15}$RKGSPDDVEX$_{16}$KSGAGTELSVRAKPS (SEQ ID NO: 18), wherein X_1 is L, I, or V; X_2 is V, L, or, I; X_3 is A or V; X_4 is A, I, or L; X_5 is I, T, S, or F; X_6 is E, V, or L; X_7 is K or R; X_8 is E or Q; X_9 is H, P, or R; X_{10} is L, T, or G; X_{11} is K or R; X_{12} is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X_{13} is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X_{14} is V or I; X_{15} is F, L, or V; and X_{16} is F or V.

In some examples, the SIRP-α variant in the SIRP-α variant construct has a sequence of EEEX$_1$QX$_2$IQPDKSVLVAAGETX$_3$TLRCTX$_4$TSLX$_5$PVGPIQWFRGAGPGRX$_6$LIYNQX$_7$X$_8$GX$_9$FPRVTTVSD X$_{10}$TX$_{11}$RNNMDFSIRISX$_{12}$ITX$_{13}$ADAGTYYCX$_{14}$KX$_{15}$RKGSPDDVEX$_{16}$KSGAGTELSVRAKPS (SEQ ID NO: 21), wherein X_1 is L, I, or V; X_2 is V, L, or, I; X_3 is A or V; X_4 is A, I, or L; X_5 is I, T, S, or F; X_6 is E, V, or L; X_7 is K or R; X_8 is E or Q; X_9 is H, P, or R; X_{10} is L, T, or G; X_{11} is K or R; X_{12} is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X_{13} is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X_{14} is V or I; X_{15} is F, L, or V; and X_{16} is F or V.

In some examples, the SIRP-α variant in the SIRP-α variant construct has a sequence of EEEX$_1$QX$_2$IQPDKSVSVAAGESX$_3$ILHCTX$_4$TSLX$_5$PVGPIQWFRGAGPARX$_6$LIYNQX$_7$X$_8$GX$_9$FPRVTTVSEX $_{10}$TX$_{11}$RENMDFSISISX$_{12}$ITX$_{13}$ADAGTYYCX$_{14}$KX$_{15}$RKGSPDTEX$_{16}$KSGAGTELSVRAKPS (SEQ ID NO: 14), wherein X_1 is L, I, or V; X_2 is V, L, or, I; X_3 is A or V; X_4 is V, I, or L; X_5 is I, T, S, or F; X_6 is E, V, or L; X_7 is K or R; X_8 is E or Q; X_9 is H, P, or R; X_{10} is S, T, or G; X_{11} is K or R; X_{12} is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X_{13} is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X_{14} is V or I; X_{15} is F, L, or V; and X_{16} is F or V;.

In some examples, the SIRP-α variant in the SIRP-α variant construct has a sequence of EEEX$_1$QX$_2$IQPDKSVSVAAGESX$_3$ILLCTX$_4$TSLX$_5$PVGPIQWFRGAGPARX$_6$LIYNQX$_7$X$_8$GX$_9$FPRVTTVSEX $_{10}$TX$_{11}$RENMDFSISISX$_{12}$ITX$_{13}$ADAGTYYCX$_{14}$KX$_{15}$RKGSPDTEX$_{16}$KSGAGTELSVRAKPS (SEQ ID NO:

15), wherein X_1 is L, I, or V; X_2 is V, L, or, I; X_3 is A or V; X_4 is V, I, or L; X_5 is I, T, S, or F; X_6 is E, V, or L; X_7 is K or R; X_8 is E or Q; X_9 is H, P, or R; X_{10} is S, T, or G; X_{11} is K or R; X_{12} is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X_{13} is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X_{14} is V or I; X_{15} is F, L, or V; and X_{16} is F or V.

In some examples, the SIRP-α variant in the SIRP-α variant construct has a sequence of EEEX$_1$QX$_2$IQPDKSVSVAAGESX$_3$ILHCTX$_4$TSLX$_5$PVGPIQWFRGAGPARX$_6$LIYNQX$_7$X$_8$GX$_9$FPRVTTVSEX$_{10}$TX$_{11}$RENMDFSISISX$_{12}$ITX$_{13}$ADAGTYYCX$_{14}$KX$_{15}$RKGSPDTEX$_{16}$KSGAGTELSVRGKPS (SEQ ID NO: 19), wherein X_1 is L, I, or V; X_2 is V, L, or, I; X_3 is A or V; X_4 is V, I, or L; X_5 is I, T, S, or F; X_6 is E, V, or L; X_7 is K or R; X_8 is E or Q; X_9 is H, P, or R; X_{10} is S, T, or G; X_{11} is K or R; X_{12} is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X_{13} is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X_{14} is V or I; X_{15} is F, L, or V; and X_{16} is F or V.

In some examples, the SIRP-α variant in the SIRP-α variant construct has a sequence of EEEX$_1$QX$_2$IQPDKSVSVAAGESX$_3$ILHCTX$_4$TSLX$_5$PVGPIQWFRGAGPARX$_6$LIYNQX$_7$X$_8$GX$_9$FPRVTTVSEX$_{10}$TX$_{11}$RENMDFSISISX$_{12}$ITX$_{13}$ADAGTYYCX$_{14}$KX$_{15}$RKGSPDTEX$_{16}$KSGAGTELSVRAKPS (SEQ ID NO: 22), wherein X_1 is L, I, or V; X_2 is V, L, or, I; X_3 is A or V; X_4 is V, I, or L; X_5 is I, T, S, or F; X_6 is E, V, or L; X_7 is K or R; X_8 is E or Q; X_9 is H, P, or R; X_{10} is S, T, or G; X_{11} is K or R; X_{12} is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X_{13} is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X_{14} is V or I; X_{15} is F, L, or V; and X_{16} is F or V.

In some examples, the SIRP-α variant in the SIRP-α variant construct has a sequence of EEEX$_1$QX$_2$IQPDKSVLVAAGETX$_3$TLRCTX$_4$TSLX$_5$PVGPIQWFRGAGPARX$_6$LIYNQX$_7$X$_8$GX$_9$FPRVTTVSEX$_{10}$TX$_{11}$RENMDFSISISX$_{12}$ITX$_{13}$ADAGTYYCX$_{14}$KX$_{15}$RKGSPDTEX$_{16}$KSGAGTELSVRAKPS (SEQ ID NO: 20), wherein X_1 is L, I, or V; X_2 is V, L, or, I; X_3 is A or V; X_4 is A, I, or L; X_5 is I, T, S, or F; X_6 is E, V, or L; X_7 is K or R; X_8 is E or Q; X_9 is H, P, or R; X_{10} is S, T, or G; X_{11} is K or R; X_{12} is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X_{13} is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X_{14} is V or I; X_{15} is F, L, or V; and X_{16} is F or V.

In some examples, the SIRP-α variant in the SIRP-α variant construct has a sequence of EEX$_1$X$_2$QX$_3$IQPDKX$_4$VX$_5$VAAGEX$_6$X$_7$X$_8$LX$_9$CTX$_{10}$TSLX$_{11}$PVGPIQWFRGAGPX$_{12}$RX$_{13}$LIYNQX$_{14}$X$_{15}$GX$_{16}$FPRVTTVSX$_{17}$X$_{18}$TX$_{19}$RX$_{20}$NMDFX$_{21}$IX$_{22}$IX$_{23}$X$_{24}$ITX$_{25}$ADAGTYYCX$_{26}$KX$_{27}$RKGSPDX$_{28}$X$_{29}$EX$_{30}$KSGAGTELSVRX$_{31}$KPS (SEQ ID NO: 23), wherein X_1 is E or G; X_2 is L, I, or V; X_3 is V, L, or, I; X_4 is S or F; X_5 is L or S; X_6 is S or T; X_7 is A or V; X_8 is I or T; X_9 is H or R; X_{10} is A, V, I, or L; X_{11} is I, T, S, or F; X_{12} is A or G; X_{13} is E, V, or L; X_{14} is K or R; X_{15} is E or Q; X_{16} is H, P, or R; X_{17} is D or E; X_{18} is S, L, T, or G; X_{19} is K or R; X_{20} is E or N; X_{21} is S or P; X_{22} is S or R; X_{23} is S or G; X_{24} is N, A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; X_{25} is P, A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; X_{26} is V or I; X_{27} is F, L, V; X_{28} is D or absent; X_{29} is T or V; X_{30} is F or V; and X_{31} is A or G.

In some examples, the SIRP-α variant in the SIRP-α variant construct has at least 80% (e.g., at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to a sequence of any one of SEQ ID NOs: 13-23.

In some examples, a SIRP-α variant in the SIRP-α variant construct does not include the sequence of any one of SEQ ID NOs: 3-12 and 24-34.

In some examples, the SIRP-α variant in the SIRP-α variant construct includes one or more substitutions of amino acid residues with histidine residues. In some examples, the one or more substitutions of amino acid residues with histidine residues are located at one or more of the following

amino acid positions: 29, 30, 31, 32, 33, 34, 35, 52, 53, 54, 66, 67, 68, 69, 74, 93, 96, 97, 98, 100, 4, 6, 27, 36, 39, 47, 48, 49, 50, 57, 60, 72, 74, 76, 92, 94, 103, relative to a sequence of any one of SEQ ID NOs: 3-12.

In some examples, the SIRP-α variant construct binds with at least two, at least four, or at least six fold higher affinity to CD47 on diseased cells or at a diseased site than on non-diseased cells.

In some examples, the SIRP-α variant construct binds with at least two, at least four, or at least six fold higher affinity to CD47 under acidic pH than under neutral pH.

In some examples, the SIRP-α variant construct binds with at least two, at least four, or at least six fold higher affinity to CD47 under hypoxic condition than under physiological condition.

In some examples, the diseased cell is a cancer cell of a cancer disease.

In some examples, the acidic pH is a pH between about 4 to about 7.

In another example, the disclosure features a nucleic acid molecule encoding a SIRP-α variant construct described herein. In one aspect, the present disclosure refers to a nucleic acid encoding the SIRP-α variant construct described here.

In another example, the disclosure features a vector including the nucleic acid molecule encoding a SIRP-α variant construct described herein. In one aspect, the present disclosure refers to a vector comprising the nucleic acid described herein.

In another example, the disclosure features a host cell that expresses a SIRP-α variant construct described herein, wherein the host cell includes a nucleic acid molecule encoding a SIRP-α variant construct described herein or a vector including the nucleic acid molecule, wherein the nucleic acid molecule or vector is expressed in the host cell. In one aspect, the present disclosure refers to a host cell comprising the nucleic acid described herein or the vector described herein.

In another example, the disclosure features a method of preparing a SIRP-α variant construct described herein, wherein the method includes: a) providing a host cell including a nucleic acid molecule of encoding a SIRP-α variant construct described herein or a vector including the nucleic acid molecule; b) expressing the nucleic acid molecule or vector in the host cell under conditions that allow for the formation of the SIRP-α variant construct; and c) recovering the SIRP-α variant construct. In one aspect, the present disclosure refers to a method of making the SIRP-α variant construct described herein, comprising: (a) culturing the host cell described herein under conditions where the construct is produced, and; (b) recovering the construct produced by the host cell.

In another example, the disclosure features a pharmaceutical composition including a therapeutically effective amount of a SIRP-α variant construct described herein. In some examples, the pharmaceutical composition includes one or more pharmaceutically acceptable carriers or excipients. In one aspect, the present disclosure refers to a pharmaceutical composition, comprising: (a) a therapeutically effective amount of the SIRP-α variant construct described herein, and (b) a pharmaceutically acceptable excipient.

In one aspect, the present disclosure refers to the SIRP-α variant construct described herein or the pharmaceutical composition described herein for use as a medicament.

In another example, the disclosure features a method of increasing phagocytosis of a target cell in a subject including administering to the subject a SIRP-α variant construct described herein or a pharmaceutical composition including a therapeutically effective amount of a SIRP-α variant construct described herein. In some examples, the target cell is a cancer cell.

In another example, the disclosure features a method of eliminating regulatory T-cells in a subject including administering to the subject a SIRP-α variant construct described herein or a pharmaceutical composition including a therapeutically effective amount of a SIRP-α variant construct described herein.

In another example, the disclosure features a method for killing a cancer cell, the method includes contacting the cancer cell with a SIRP-α variant construct described herein or the pharmaceutical composition including a therapeutically effective amount of a SIRP-α variant construct described herein.

In another example, the disclosure features a method for treating a disease associated with SIRP-α and/or CD47 activity in a subject, the method includes administering to the subject a therapeutically effective amount of the SIRP-α variant construct described herein or the pharmaceutical composition including a therapeutically effective amount of a SIRP-α variant construct described herein.

In another example, the disclosure features a method of treating a disease associated with SIRP-α and/or CD47 activity in a subject, the method includes: (a) determining the amino acid sequences of SIRP-α of the subject; and (b) administering to the subject a therapeutically effective amount of a SIRP-α variant construct described herein; wherein the SIRP-α variant in the SIRP-α variant construct has the same amino acid sequence as that of a SIRP-α of the subject.

In another example, the disclosure features a method of treating a disease associated with SIRP-α and/or CD47 activity in a subject, the method includes: (a) determining the amino acid sequences of SIRP-α of the subject; and (b) administering to the subject a therapeutically effective amount of a SIRP-α variant construct described herein; wherein the SIRP-α variant in the SIRP-α variant construct has minimal immunogenicity in the subject.

In another example, the disclosure features a method of treating a disease associated with SIRP-α and/or CD47 activity in a subject, the method includes: administering to the subject a SIRP-α variant construct described herein, wherein the SIRP-α variant construct preferentially binds CD47 on diseased cells or at a diseased site over CD47 on non-diseased cells.

In some examples, the disease is cancer. In one aspect, the present disclosure refers to use of the SIRP-α variant construct described herein or the pharmaceutical composition described herein in the manufacture of a medicament for treating cancer in a subject. In some examples, the cancer is selected from solid tumor cancer, hematological cancer, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, non-Hodgkin lymphoma, Hodgkin lymphoma, multiple myeloma, bladder cancer, pancreatic cancer, cervical cancer, endometrial cancer, lung cancer, bronchus cancer, liver cancer, ovarian cancer, colon and rectal cancer, stomach cancer, gastric cancer, gallbladder cancer, gastrointestinal stromal tumor cancer, thyroid cancer, head and neck cancer, oropharyngeal cancer, esophageal cancer, melanoma, non-melanoma skin cancer, Merkel cell carcinoma, virally induced cancer, neuroblastoma, breast cancer, prostate cancer, renal cancer, renal cell cancer, renal pelvis cancer, leukemia, lymphoma, sarcoma, glioma, brain tumor, and carcinoma. In some examples, the cancer is a solid tumor cancer. In some examples, the cancer is a hematological cancer.

In some examples, the disease is an immunological disease. In some examples, the immunological disease is an autoimmune disease or an inflammatory disease. In one aspect, the present disclosure refers to use of the SIRP-α variant construct described herein or the pharmaceutical composition described herein in the manufacture of a medicament for treating an immunological disease, an autoimmune disease, or an inflammatory disease in a subject. In some examples, the autoimmune or inflammatory disease is multiple sclerosis, rheumatoid arthritis, a spondyloarthropathy, systemic lupus erythematosus, an antibody-mediated inflammatory or autoimmune disease, graft versus host disease, sepsis, diabetes, psoriasis, atherosclerosis, Sjogren's syndrome, progressive systemic sclerosis, scleroderma, acute coronary syndrome, ischemic reperfusion, Crohn's Disease, endometriosis, glomerulonephritis, myasthenia gravis, idiopathic pulmonary fibrosis, asthma, acute respiratory distress syndrome (ARDS), vasculitis, or inflammatory autoimmune myositis.

In another example, the disclosure features a method of increasing hematopoietic stem cell engraftment in a subject including modulating the interaction between SIRP-α and CD47 in the subject by administering to the subject a SIRP-α variant described herein or a pharmaceutical composition including a therapeutically effective amount of a SIRP-α variant described herein.

In another example, the disclosure features a method of altering an immune response in a subject including administering the subject a SIRP-α variant construct described herein or a pharmaceutical composition including a therapeutically effective amount of a SIRP-α variant construct described herein, thereby altering the immune response in the subject. In some examples, the immune response includes suppressing the immune response.

In some examples, the subject is a mammal, preferably, the mammal is a human.

Definitions

As used herein, the term “diseased cells” and “diseased tissue” refer to, for example, cancer cells and tissue. In particular, the cancer may be a solid tumor cancer or a hematological cancer. For example, if the cancer is a solid tumor cancer, the diseased cells are the cells of the solid tumor. Diseased cells are often living under conditions characteristic of a diseased site, such as acidic pH and hypoxia. “Diseased cells” and “diseased tissue” can also be associated with other diseases including, but not limited to, cancer. “Diseased cells” and “diseased tissue” can also be associated with an immunological disease or disorder, a cardiovascular disease or disorder, a metabolic disease or disorder, or a proliferative disease or disorder. An immunological disorder includes an inflammatory disease or disorder and an autoimmune disease or disorder.

As used herein, the term “non-diseased cells” refers to normal, healthy cells of the body. Non-diseased cells often live under physiological conditions, such as neutral pH and adequate oxygen concentration that maintain normal metabolism and regulatory functions of the cells.

As used herein, the term “diseased site” refers to the location or area proximal to the location of the disease in the body. For example, if the disease is solid tumor cancer located in the liver, then diseased site is the site of the tumor in the liver and areas close to the tumor in the liver. Cells at a diseased site may include diseased cells as well as cells that support the disease at the diseased site. For example, if the diseased site is the site of a tumor, cells at the site of the tumor include both diseased cells (e.g., tumor cells) and cells supporting tumor growth at the site of the tumor. Similarly, the term “cancer site” refers to the location of the cancer in the body.

As used herein, the term “SIRP-α D1 domain” or “D1 domain” refers to the membrane distal, extracellular domain of SIRP-α. The SIRP-α D1 domain is located at the N-terminus of a full-length, wild-type SIRP-α and mediates binding to CD47. Amino acid sequences of D1 domains are shown in Table 1.

As used herein, the term “SIRP-α D2 domain” or “D2 domain” refers to the second extracellular domain of SIRP-α. The SIRP-α D2 domain includes approximately amino acids 119 to 220 of a full-length, wild-type SIRP-α.

As used herein, the term “SIRP-α D3 domain” or “D3 domain” refers to the third extracellular domain of SIRP-α. The SIRP-α D3 domain includes approximately amino acids 221 to 320 of a full-length, wild-type SIRP-α.

As used herein, the term “SIRP-α polypeptide” refers to a wild-type SIRP-α as well as a SIRP-α variant, as each term is defined and described herein.

As used herein, the term “SIRP-α variant” refers to a polypeptide containing a SIRP-α D1 domain, or a CD47-binding portion of a full-length SIRP-α. In some examples, the SIRP-α variant has at least 80% (e.g., at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.) sequence identity to a sequence of any one of SEQ ID NOs: 3-12 and 24-34. In some examples, a SIRP-α variant has higher affinity to CD47 than a wild-type SIRP-α. In some examples, a SIRP-α variant contains a portion of wild-type human SIRP-α (preferably a CD47-binding portion of the wild-type SIRP-α) and/or has one or more amino acid substitutions. For example, a SIRP-α variant may contain substitutions of one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, etc, with a maximum of 20) amino acid residues relative to a wild-type SIRP-α. For example, a SIRP-α variant may

contain substitutions of one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, etc, with a maximum of 20) amino acid residues with histidine residues. In some examples, SIRP-α variants have a portion that has at least 80% (e.g., at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) amino acid sequence identity to a sequence of wild-type human SIRP-α or to any of the SIRP-α variants described herein (e.g., to a sequence of a CD47-binding portion of wild-type human SIRP-α). A CD47-binding portion of wild-type SIRP-α includes the D1 domain of the wild-type SIRP-α (a sequence of any one of SEQ ID NOs: 3-12).

As used herein, the term "SIRP-α variant construct" refers to a polypeptide containing a SIRP-α variant attached to, e.g., a blocking peptide, an Fc domain monomer, an HSA, an albumin-binding peptide, a polymer, an antibody-binding peptide, an antibody. In some examples, a SIRP-α variant construct has preferential activity at a diseased site. In some examples, SIRP-α variant constructs have preferential activity at a diseased site and include a SIRP-α variant having a portion that has at least 80% (e.g., at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) amino acid sequence identity to a sequence of wild-type human SIRP-α or to any of the SIRP-α variants described herein (e.g., to a sequence of a CD47-binding portion of wild-type human SIRP-α).

As used herein, the term "percent (%) identity" refers to the percentage of amino acid (or nucleic acid) residues of a candidate sequence, e.g., a SIRP-α variant, that are identical to the amino acid (or nucleic acid) residues of a reference sequence, e.g., a wild-type human SIRP-α or a CD47-binding portion thereof, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent identity (i.e., gaps can be introduced in one or both of the candidate and reference sequences for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). Alignment for purposes of determining percent identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, ALIGN, or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. In some examples, the percent amino acid (or nucleic acid) sequence identity of a given candidate sequence to, with, or against a given reference sequence (which can alternatively be phrased as a given candidate sequence that has or includes a certain percent amino acid (or nucleic acid) sequence identity to, with, or against a given reference sequence) is calculated as follows:

$$100 \times (\text{fraction of } A/B)$$

where A is the number of amino acid (or nucleic acid) residues scored as identical in the alignment of the candidate sequence and the reference sequence, and where B is the total number of amino acid (or nucleic acid) residues in the reference sequence. In some examples where the length of the candidate sequence does not equal to the length of the reference sequence, the percent amino acid (or nucleic acid) sequence identity of the candidate sequence to the reference sequence would not equal to the percent amino acid (or nucleic acid) sequence identity of the reference sequence to the candidate sequence.

In particular examples, a reference sequence aligned for comparison with a candidate sequence may show that the candidate sequence exhibits from 50% to 100% identity across the full length of the candidate sequence or a selected portion of contiguous amino acid (or nucleic acid) residues of the candidate sequence. The length of the candidate sequence aligned for comparison

purpose is at least 30%, e.g., at least 40%, e.g., at least 50%, 60%, 70%, 80%, 90%, or 100% of the length of the reference sequence. When a position in the candidate sequence is occupied by the same amino acid (or nucleic acid) residue as the corresponding position in the reference sequence, then the molecules are identical at that position.

As used herein, the term “tumor-associated protease” or “tumor enzyme” refers to an enzyme, e.g., a protease, that is present at an increased level in a cancer, e.g., a solid tumor cancer. In some examples, the tumor-associated protease may cleave a cleavable linker.

As used herein, the term “blocking peptide” refers to a peptide that can bind to a SIRP-α variant and block or “mask” the CD47-binding portion of the SIRP-α variant. In a SIRP-α variant construct, the blocking peptide may be attached to a SIRP-α variant by way of a linker that is optionally cleavable, and optionally one or more spacers. The blocking peptide may be coupled via non-covalent bonds to the SIRP-α variant and cleaved at a diseased site or diseased cell. In some examples, the blocking peptide may bind to a wild-type SIRP-α at the diseased site or diseased cell. A blocking peptide can be used to reduce or minimize binding of the SIRP-α variant with wild-type CD47 under normal physiological conditions or at a non-diseased site. In some examples, the blocking peptide has higher binding affinity to a wild-type SIRP-α than a SIRP-α variant. The blocking peptide may dissociate from the SIRP-α variant to bind to a wild-type SIRP-α at, for e.g., a diseased site or under non-physiological conditions. An example of a blocking peptide is a CD47-based blocking peptide, which is a peptide derived from CD47 or a fragment thereof. In some examples, a CD47-based blocking peptide is the extracellular, SIRP-α binding portion of CD47 (i.e., the IgSF domain of CD47). In some examples, a CD47-based blocking peptide includes one or more amino acid substitutions, additions, and/or deletions relative to the wild-type CD47.

As used herein, the term “cleavable linker” refers to a linker between two portions of a SIRP-α variant construct. In some examples, a cleavable linker may covalently attach a blocking peptide to a SIRP-α variant to block binding of the SIRP-α variant to CD47 under physiological conditions. In some examples, a cleavable linker may be installed within a blocking peptide, which may be non-covalently associated with the SIRP-α variant to block binding of the SIRP-α variant to CD47 under physiological conditions. A cleavable linker may be cleaved under certain conditions. If the cleavable linker is within a blocking peptide, cleavage of the linker would inactivate the blocking peptide. The cleavable linker contains a moiety that acts to cleave or induce cleavage of the linker under conditions characteristic of a diseased site, such as a cancer site, e.g., inside a solid tumor. The cleavable linker is stable under healthy physiological conditions (e.g., neutral pH and adequate oxygen concentration). The moiety may be a pH-sensitive chemical functional group (e.g., acetals, ketals, thiomaleamates, hydrazones, disulfide bonds) capable of being hydrolyzed under acidic pH. The moiety may also be a hypoxia-sensitive chemical functional group (e.g., quinones, N-oxides, and heteroaromatic nitro groups) or amino acid capable of being reduced under hypoxic condition. The moiety in the cleavable linker may also be a protein substrate capable of being recognized and cleaved by a tumor-associated protease, enzyme, or peptidase.

As used herein, the term “spacer” refers to a covalent or non-covalent linkage between two portions of a SIRP-α variant construct, such as the linker (e.g., cleavable linker) and the SIRP-α variant, or the antibody-binding peptide and the SIRP-α variant. Preferably, the spacer is a covalent linkage. A spacer can be a simple chemical bond, e.g., an amide bond, or an amino acid sequence (e.g., a 3-200

amino acid sequence). An amino acid spacer is part of the primary sequence of a polypeptide (e.g., joined to the spaced polypeptides or polypeptide domains via the polypeptide backbone). A spacer provides space and/or flexibility between the two portions. A spacer is stable under physiological conditions (e.g., neutral pH and adequate oxygen concentration) as well as under conditions characteristic of a diseased site, e.g., acidic pH and hypoxia. A spacer is stable at a diseased site, such as a cancer site, e.g., inside a tumor. Descriptions of spacers are provided in detail further herein.

As used herein, the term "antibody" refers to intact antibodies, antibody fragments, provided that they exhibit the desired biological activity, monoclonal antibodies, polyclonal antibodies, monospecific antibodies, and multispecific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies. Preferably, the antibody is specific to a diseased cell, e.g., a tumor cell. For example, the antibody may specifically bind to a cell surface protein on a diseased cell, e.g., a tumor cell.

As used herein, the term "albumin-binding peptide" refers to an amino acid sequence of 12 to 16 amino acids that has affinity for and functions to bind serum albumin. An albumin-binding peptide can be of different origins, e.g., human, mouse, or rat. In some examples of the present disclosure, a SIRP-α variant construct may include an albumin-binding peptide that is fused to the C-terminus of the SIRP-α variant to increase the serum half-life of the SIRP-α variant. An albumin-binding peptide can be fused, either directly or through a spacer, to the SIRP-α variant.

As used herein, the term "human serum albumin (HSA)" refers to the albumin protein present in human blood plasma. Human serum albumin is the most abundant protein in the blood. It constitutes about half of the blood serum protein. In some examples, a human serum albumin has the sequence of amino acids 25-609 (SEQ ID NO: 67) of UniProt ID NO: P02768. In some examples, a human serum albumin further contains C34S relative to the sequence of SEQ ID NO: 67.

As used herein, the term "Fc domain monomer" refers to a polypeptide chain that includes second and third antibody constant domains (C_H2 and C_H3). In some example, the Fc domain monomer also includes a hinge domain. The Fc domain monomer can be any immunoglobulin antibody isotype, including IgG, IgE, IgM, IgA, or IgD. Additionally, the Fc domain monomer can be an IgG subtype (e.g., IgG1, IgG2a, IgG2b, IgG3, or IgG4). An Fc domain monomer does not include any portion of an immunoglobulin that is capable of acting as an antigen-recognition region, e.g., a variable domain or a complementarity determining region (CDR). Fc domain monomers can include as many as ten changes from a wild-type Fc domain monomer sequence (e.g., 1-10, 1-8, 1-6, 1-4 amino acid substitutions, additions, or deletions) that alter the interaction between an Fc domain and an Fc receptor. Examples of suitable changes are known in the art.

As used herein, the term "Fc domain" refers to a dimer of two Fc domain monomers. In the wild-type Fc domain, the two Fc domain monomers dimerize by the interaction between the two C_H3 antibody constant domains, as well as one or more disulfide bonds that form between the hinge domains of the two dimerizing Fc domain monomers. In some examples, an Fc domain may be mutated to lack effector functions, typical of a "dead Fc domain." In certain examples, each of the Fc domain monomers in an Fc domain includes amino acid substitutions in the C_H2 antibody constant domain to reduce the interaction or binding between the Fc domain and an Fcγ receptor.

As used herein, the term "affinity" or "binding affinity" refers to the strength of the binding interaction between two molecules. Generally, binding affinity refers to the strength of the sum total of non-covalent interactions between a molecule and its binding partner, such as a SIRP-α variant and

CD47. Unless indicated otherwise, binding affinity refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of a binding pair. The binding affinity between two molecules is commonly described by the dissociation constant (K_D) or the affinity constant (K_A). Two molecules that have low binding affinity for each other generally bind slowly, tend to dissociate easily, and exhibit a large K_D. Two molecules that have high affinity for each other generally bind readily, tend to remain bound longer, and exhibit a small K_D. The K_D of two interacting molecules may be determined using methods and techniques well known in the art, e.g., surface plasmon resonance. K_D is calculated as the ratio of k_{off}/k_{on}.

As used herein, the term "host cell" refers to a vehicle that includes the necessary cellular components, e.g., organelles, needed to express proteins from their corresponding nucleic acids. The nucleic acids are typically included in nucleic acid vectors that can be introduced into the host cell by conventional techniques known in the art (e.g., transformation, transfection, electroporation, calcium phosphate precipitation, direct microinjection, etc.). A host cell may be a prokaryotic cell, e.g., a bacterial cell, or a eukaryotic cell, e.g., a mammalian cell (e.g., a CHO cell). As described herein, a host cell is used to express one or more SIRP-α variant constructs.

As used herein, the term "pharmaceutical composition" refers to a medicinal or pharmaceutical formulation that contains an active ingredient as well as excipients and diluents to enable the active ingredient suitable for the method of administration. The pharmaceutical composition of the present disclosure includes pharmaceutically acceptable components that are compatible with the SIRP-α variant construct. The pharmaceutical composition may be in tablet or capsule form for oral administration or in aqueous form for intravenous or subcutaneous administration.

As used herein, the term "disease associated with SIRP-α and/or CD47 activity" refers to any disease or disorder that is caused by and/or related to SIRP-α and/or CD47 activity. For example, any disease or disorder that is caused by and/or related to the increase and/or decrease of SIRP-α and/or CD47 activity. Examples of diseases associated with SIRP-α and/or CD47 activity include, but are not limited to, cancers and immunological diseases (e.g., autoimmune diseases and inflammatory diseases).

As used herein, the term "therapeutically effective amount" refers an amount of a SIRP-α variant construct of the disclosure or a pharmaceutical composition containing a SIRP-α variant construct of the disclosure effective in achieving the desired therapeutic effect in treating a patient having a disease, such as a cancer, e.g., solid tumor or hematological cancer. In particular, the therapeutic effective amount of the SIRP-α variant construct avoids adverse side effects.

As used herein, the term "optimized affinity" or "optimized binding affinity" refers to an optimized strength of the binding interaction between a SIRP-α variant and CD47. In some examples, the SIRP-α variant construct binds primarily or with higher affinity to CD47 on cells at a diseased site (i.e., cancer cells) and does not substantially bind or binds with lower affinity to CD47 on cells at a non-diseased site (i.e., non-cancer cells). The binding affinity between the SIRP-α variant and CD47 is optimized such that the interaction does not cause clinically relevant toxicity. In some examples, in order to achieve an optimized binding affinity between the SIRP-α variant and CD47, the SIRP-α variant may be developed to have a lower binding affinity to CD47 than which is maximally achievable.

As used herein, the term "immunogenicity" refers to the property of a protein (e.g., a therapeutic protein) which causes an immune response in the host as though it is a foreign antigen. The immunogenicity of a protein can be assayed in vitro in a variety of different ways, in particular through in

vitro T-cell proliferation assays (see, e.g., Jawa et al., *Clinical Immunology* 149:534-555, 2013), some of which are commercially available (see, e.g., immunogenicity assay services offered by Proimmune).

As used herein, the term "minimal immunogenicity" refers to an immunogenicity of a protein (e.g., a therapeutic protein) that has been modified, i.e., through amino acid substitutions, to be lower (e.g., at least 10%, 25%, 50%, or 100% lower) than what could have been before the amino acid substitutions are introduced. A protein (e.g., a therapeutic protein) is modified to have minimal immunogenicity means it causes no or very little host immune response even though it is a foreign antigen.

As used herein, the term "optimized pharmacokinetics" refers to that the parameters that are generally associated with the pharmacokinetics of a protein are improved and modified to produce an optimized protein for in vitro and/or in vivo use. Parameters that are associated with the pharmacokinetics of a protein are well-known to a skilled artisan, including, for examples, K_D, valency, and half-life. In the present disclosure, the pharmacokinetics of a SIRP-α variant construct of the disclosure are optimized for its interaction with CD47 for use in a therapeutic context.

DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a portion of the co-crystalized structure of CD47:SIRP-α (PDB: 4KJY, 4CMM), the N-terminus of CD47 exists as a pyro-glutamate and makes hydrogen bonding interactions with Thr66 of a SIRP-α variant or Leu66 of a wild-type SIRP-α.

FIG. 2 shows computation models of the interaction site between CD47 having T102Q and a wild-type SIRP-α having A27 (left) and the interaction site between CD47 having T102Q and a SIRP-α variant having I27.

FIG. 3 shows an SDS-PAGE of SIRP-α variant constructs (SEQ ID NOs: 48-56).

FIG. 4A shows an SDS-PAGE of SIRP-α variant construct (SEQ ID NO: 54) after in vitro cleavage with uPA and matriptase.

FIG. 4B shows an SDS-PAGE of SIRP-α variant construct (SEQ ID NO: 54) after in vitro cleavage with different amounts of matriptase.

FIG. 4C shows an SDS-PAGE of various SIRP-α variant constructs (SEQ ID NOs: 57-63) after in vitro cleavage with matriptase.

FIG. 5A shows a bar graph illustrating the different binding affinities of various SIRP-α variant constructs (SEQ ID NOs: 48-55) to CD47 before and after in vitro cleavage with matriptase.

FIG. 5B shows a bar graph illustrating the different binding affinities of various SIRP-α variant constructs (SEQ ID NOs: 52-63) and the SIRP-α variant (SEQ ID NO: 31) to CD47 before and after in vitro cleavage with matriptase.

FIG. 6 shows a sensorgram demonstrating that a SIRP-α variant construct (SEQ ID NO: 66) can bind Cetuximab and CD47 simultaneously.

FIG. 7A shows a scheme of the quaternary complex containing EGFR, Cetuximab, a SIRP-α variant construct (SEQ ID NO: 66), and CD47.

FIG. 7B shows a sensorgram demonstrating the formation of the quaternary complex shown in FIG. 7A.

FIG. 7C is an image of the sensorgram shown in FIG. 7B.

FIG. 8 is a scatter plot showing phagocytosis induced by the SIRP-α variant construct (SEQ ID NO: 66) and the SIRP-α variant (SEQ ID NO: 31).