

REVIEW ARTICLE

CD47-signal regulatory protein α signaling system and its application to cancer immunotherapy

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Tumor cells evade immune surveillance through direct or indirect interactions with various types of immune cell, with much recent attention being focused on modifying immune cell responses as the basis for the development of new cancer treatments. Signal regulatory protein α (SIRP α) and CD47 are both transmembrane proteins that interact with each other and constitute a cell-cell communication system. SIRP α is particularly abundant in myeloid cells such as macrophages and dendritic cells, whereas CD47 is expressed ubiquitously and its expression level is elevated in cancer cells. Recent studies have shown that blockade of CD47-SIRP α interaction enhances the phagocytic activity of phagocytes such as macrophages toward tumor cells in vitro as well as resulting in the efficient eradication of tumor cells in a variety of xenograft or syngeneic mouse models of cancer. Moreover, CD47 blockade has been shown to promote the stimulation of tumor-specific cytotoxic T cells by macrophages or dendritic cells. Biological agents, such as Abs and recombinant proteins, that target human CD47 or SIRP α have been developed and are being tested in preclinical models of human cancer or in clinical trials with cancer patients. Preclinical studies have also suggested that CD47 or SIRP α blockade may have a synergistic antitumor effect in combination with immune checkpoint inhibitors that target the adaptive immune system. Targeting of the CD47-SIRP α signaling system is thus a promising strategy for cancer treatment based on modulation of both innate and acquired immune responses to tumor cells.

KEYWORDS

cancer immunotherapy, CD47, macrophage, phagocytosis, signal regulatory protein α (SIRP α)

1 | INTRODUCTION

The tumor microenvironment consists of immune cells and a variety of stromal cell types, including fibroblasts and endothelial cells, as well as soluble and insoluble factors, such as cytokines, chemokines, and

extracellular matrix.^{1,2} This microenvironment plays an important role in the regulation of tumor progression by promoting tumor cell survival, invasion, and metastasis as well as angiogenesis.¹⁻³ Cross-talk between tumor and immune cells in the tumor microenvironment is also thought to contribute to the evasion of tumor cells from immune surveillance. For example, the binding of PD-1 on cytotoxic T lymphocytes to its ligand PD-L1 on tumor cells prevents killing of the latter cells by the former.⁴ Indeed, Abs to PD-1 and to PD-L1 are now in clinical use for the treatment of diverse solid tumors, including advanced melanoma, renal cell carcinoma, and non-small-cell lung cancer.^{5,6}

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; DC, dendritic cell; HER2, human epidermal growth factor receptor 2; NK, natural killer; PD-1, programmed cell death-1; PD-L1, programmed cell death-ligand 1; RBC, red blood cell; SH2, Src homology 2; Shp, SH2 domain-containing phosphatase; SIRP α , signal regulatory protein α .

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In addition, Abs to the T-cell molecule CTLA-4, which is also thought to suppress T-cell responses on interaction with CD80 or CD86 on antigen-presenting cells, are given to treat melanoma as well as prostate and lung cancers.^{5,7} Molecules that participate in the negative regulation of the antitumor response of immune cells are thus promising targets for cancer therapy, with drugs that target such molecules being known as immune checkpoint inhibitors.⁸ We and others have recently shown that blocking Abs to SIRP α , which is highly expressed in macrophages and DCs, also has the potential to function as immune checkpoint inhibitors—that target innate immunity, in particular—for cancer treatment.^{9–11}

2 | SIGNAL REGULATORY PROTEIN α AND ITS BIOLOGICAL FUNCTIONS

Signal regulatory protein α (also known as SHPS-1, p84, BIT, or CD172a) is a transmembrane protein that was originally identified as a highly expressed glycoprotein in the brain and a binding partner or putative substrate for 2 cytoplasmic-type protein tyrosine phosphatases, SH2 domain-containing phosphatase 1 (Shp1, also known as PTPN6) and Shp2 (PTPN11).^{12–15} Indeed, SIRP α contains 3 Ig-like domains in its extracellular region and 4 tyrosine residues that are putative phosphorylation sites in its cytoplasmic region (Figure 1A).^{16,17} The extracellular region of SIRP α interacts with its ligand, CD47, which was originally identified in association with $\alpha_v\beta_3$ integrin and is also a member of the Ig superfamily of proteins, with an Ig-V-like extracellular domain, 5 putative membrane-spanning segments, and a short cytoplasmic tail (Figure 1A).¹⁸ In their phosphorylated state, the tyrosine phosphorylation sites (in particular, the 2 COOH-terminal sites) in the cytoplasmic region of SIRP α bind to the SH2 domains of Shp1 and Shp2 and thereby activate these phosphatases (Figure 1A). Tyrosine phosphorylation of the cytoplasmic region of SIRP α is triggered by various growth factors and cytokines as well as by integrin-mediated cell adhesion to extracellular matrix proteins.^{14,19} Ligation of SIRP α by CD47 also promotes tyrosine phosphorylation of its cytoplasmic region.^{20,21} SIRP α thus functions as a docking protein for the recruitment and activation of Shp1 and Shp2 at the cell membrane in response to extracellular stimuli,¹⁶ with these phosphatases then being thought to play an important role in signaling downstream of SIRP α . SIRP α is especially abundant in neurons and in hematopoietic cells of the myeloid lineage such as macrophages, neutrophils, and DCs,^{22–26} whereas CD47 is expressed in most cell types.¹⁸

Signal regulatory protein α mutant mice, which express a mutant form of SIRP α that lacks most of the cytoplasmic region and thus fail to bind Shp1 and Shp2,²⁴ manifested mild anemia associated with a short lifetime of RBCs as a result of increased phagocytotic activity of splenic macrophages against RBCs,^{21,24} suggesting the importance of SIRP α for both the lifespan of RBCs and their number in the circulation. In addition, DC-specific SIRP α knockout mice showed a reduced number of DCs, as well as of fibroblastic reticular cells, a subset of stromal cells, in the spleen.²⁷ Moreover, SIRP α mutant mice, as well as CD47-deficient mice, were resistant to the development of autoimmune animal models, such

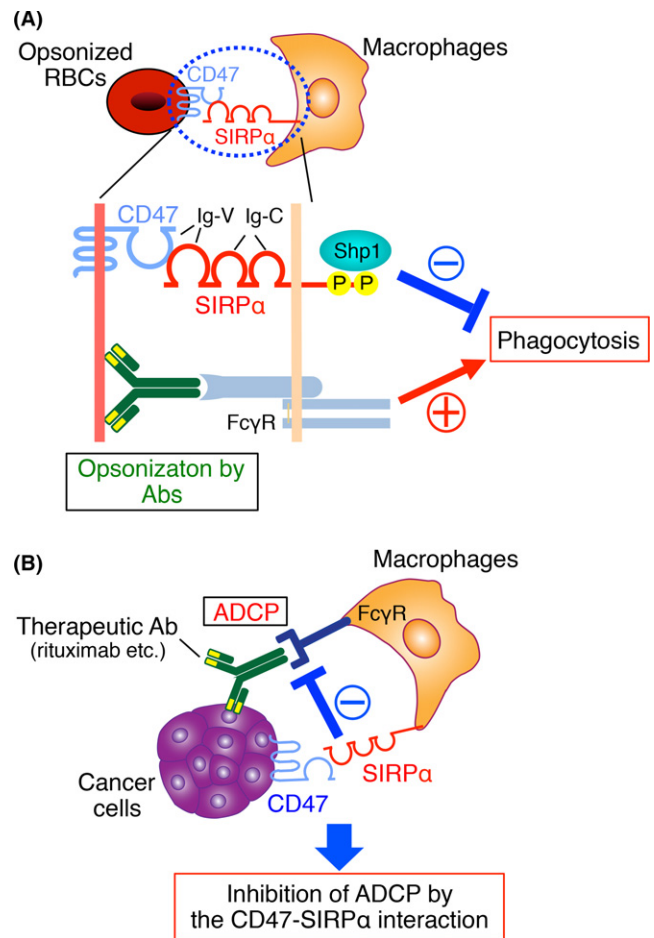


FIGURE 1 The CD47-signal regulatory protein α (SIRP α) signaling system and its role in the regulation of phagocytosis by macrophages. A, SIRP α is a transmembrane protein that contains 3 Ig-like domains (1 V-like and 2 C1-like Ig domains) in its NH₂-terminal extracellular region and 2 key tyrosine phosphorylation sites in its COOH-terminal cytoplasmic region. The tyrosine-phosphorylated sites of SIRP α bind and thereby activate the protein tyrosine phosphatases Shp1 and Shp2. The SIRP α ligand CD47 is also a member of the Ig superfamily, with an Ig-V-like extracellular domain, 5 membrane-spanning segments, and a short cytoplasmic tail. The Ig-V-like domain of CD47 interacts in trans with the NH₂-terminal Ig-V-like domain of SIRP α and thereby triggers the tyrosine phosphorylation of the latter protein. Ligation of SIRP α on macrophages by CD47 on opsonized red blood cells (RBCs) thus promotes tyrosine phosphorylation of SIRP α and its subsequent association with Shp1, resulting in inhibition of RBCs phagocytosis by the macrophages elicited by the interaction of the Fc region of RBC-bound Abs with the macrophage Fc γ receptor (Fc γ R). B, Interaction of CD47 on tumor cells with SIRP α on macrophages attenuates phagocytosis by the macrophages of the tumor cells triggered by opsonization with tumor antigen-specific therapeutic Abs such as rituximab. ADCP, antibody-dependent cellular phagocytosis

as experimental autoimmune encephalomyelitis,^{28,29} suggesting that the interaction of SIRP α with CD47 is involved in the development of autoimmune diseases. SIRP α and CD47 are also thought to play a role in the regulation of central nervous system functions. Both SIRP α mutant mice and CD47-deficient mice, indeed, showed prolonged immobility

(depression-like behavior) in the forced swim test.³⁰ The CD47-SIRP α signaling system is thus likely to act as a signaling platform for the brain response to stress and the regulation of depression-like behavior in the forced swim test.

By contrast, mutations associated with human diseases, including hematological disorders, autoimmune diseases and neurological disorders, have not been identified within *CD47* and *SIRPA* genes. Although the N-terminal IgV-like domain of human SIRP α , which is responsible for the interaction with CD47, is known to be highly polymorphic,³¹ any relationship between such polymorphisms of SIRP α and the incidence of diseases has not been reported so far.

3 | BINDING OF CD47 TO SIRP α PREVENTS PHAGOCYTOSIS IN MACROPHAGES

Oldenborg and colleagues^{20,32} first showed that the rate of clearance of transfused CD47-deficient RBCs from the bloodstream of WT mice was markedly increased compared with that of transfused WT cells.^{20,32} In addition, the phagocytosis of CD47-deficient RBCs by isolated splenic macrophages from WT mice *in vitro* was greatly enhanced compared with that of WT RBCs.²⁰ We also subsequently showed that the rate of clearance of transfused WT RBCs from the bloodstream was markedly increased in SIRP α mutant mice. Phagocytosis of antibody-opsonized WT RBCs by isolated macrophages from the SIRP α mutant mice was also enhanced compared with that seen with macrophages from WT mice.²¹ Collectively, these observations indicated that the binding of CD47 on RBCs to SIRP α on macrophages prevents the Fc γ receptor (Fc γ R)-dependent phagocytosis of the former cells by the latter (Figure 1A). The activity of Shp1, which binds to the tyrosine-phosphorylated cytoplasmic region of SIRP α , is thought to be important for this prevention of phagocytosis (Figure 1A).³²

4 | BLOCKADE OF THE CD47-SIRP α SIGNALING SYSTEM HAS ANTITUMOR EFFECTS

Two major groups of molecularly targeted drugs are currently in clinical use for cancer therapy. One group includes potent inhibitors of a variety of signaling molecules that are essential for the proliferation of tumor cells, such as inhibitors of various tyrosine kinases as well as of the Raf-MEK and PI3K-mTOR (mammalian target of rapamycin) signaling pathways.³³⁻³⁷ The other group consists of mAbs to surface molecules that are highly expressed in particular cancers, such as rituximab (to CD20 in lymphoma), trastuzumab (to HER2 in breast cancer), and cetuximab (to epidermal growth factor receptor [EGFR] in colon cancer). These mAbs are thought to bind to their targets on tumor cells and thereby induce the killing of these cells by complement-dependent cytotoxicity as well as via ADCP mediated by macrophages and ADCC mediated by NK cells, both of which require the interaction of Fc receptors on the effector cells with the Fc domain of the bound

mAbs.³⁸ Many studies have shown that the interaction of CD47 on cancer cells with SIRP α on macrophages serves to inhibit phagocytosis of the former cells by the latter cells (Figure 1B).^{9,39-41} Blockade of the CD47-SIRP α signaling system was thus shown to enhance the ADCP-mediated killing of Ab-opsonized tumor cells by macrophages (Figure 2A) as well as to suppress tumor growth and metastasis in preclinical mouse models of cancer.

4.1 | Targeting of CD47

CD47 was shown to be identical to the tumor antigen OV-3, which interacts with integrins and is markedly upregulated in ovarian carcinoma cells.⁴² Expression of CD47 was also found to be increased in AML stem cells, non-Hodgkin lymphoma, and various solid tumors compared with their normal counterparts,^{40,43,44} and such increased expression was associated with poor prognosis in patients with these malignancies.^{40,43-46} Incubation with a blocking Ab to CD47 either alone or in combination with mAbs to tumor antigens *in vitro* promoted the phagocytosis by macrophages of cancer cells such as AML stem cells, non-Hodgkin lymphoma cells, colorectal cancer stem cells, and breast cancer cells (Figure 2A).^{40,43,44} Such Ab treatment also attenuated tumor growth and metastasis in a variety of xenograft or syngeneic mouse models of AML, non-Hodgkin lymphoma, pediatric brain tumors, as well as ovarian, colon, breast, bladder, and small-cell lung cancers.^{40,43,44,47-49} Moreover, an engineered recombinant SIRP α protein, which has a higher affinity for CD47 than does the WT protein and prevents endogenous CD47-SIRP α interaction, was found to significantly enhance the efficacy of therapeutic Abs such as rituximab, trastuzumab, and alemtuzumab (an Ab to CD52) in mouse xenograft models of human cancer.⁵⁰

In addition to its effect on ADCP of tumor cells by macrophages, CD47 blockade promotes the activation of tumor-specific cytotoxic T cells by DCs or macrophages.^{40,44,51-53} Treatment of tumor-bearing mice with blocking Abs to CD47 thus promoted the recognition of tumor-derived DNA through the stimulator of interferon genes (STING) pathway.⁵² Such recognition increased type I interferon production by DCs and enhanced the cross-priming of tumor-specific cytotoxic T cells.⁵³ Tumor-infiltrating macrophages were also found to participate in the cross-priming of tumor-specific cytotoxic T cells.⁵¹ Blockade of CD47-SIRP α interaction is thus thought to promote the phagocytosis of tumor cells by macrophages and DCs, which, in turn, activate tumor-specific cytotoxic T cells at the site of tumor rejection. In contrast, given that CD47 is expressed on most cell types, off-target effects of CD47 blockade on normal cells—in particular, erythrocytes—is a potential concern with regard to treatment with drugs that target CD47. Indeed, treatment with Abs to CD47 induced in a dose-dependent way the development of transient anemia associated with reticulocytosis in cynomolgus monkeys.⁴⁷

4.2 | Targeting of SIRP α

Blockade of SIRP α in combination with Abs to tumor antigens is also a promising strategy for cancer therapy. Indeed, Abs to mouse SIRP α enhanced the rituximab-induced elimination of human Burkitt's

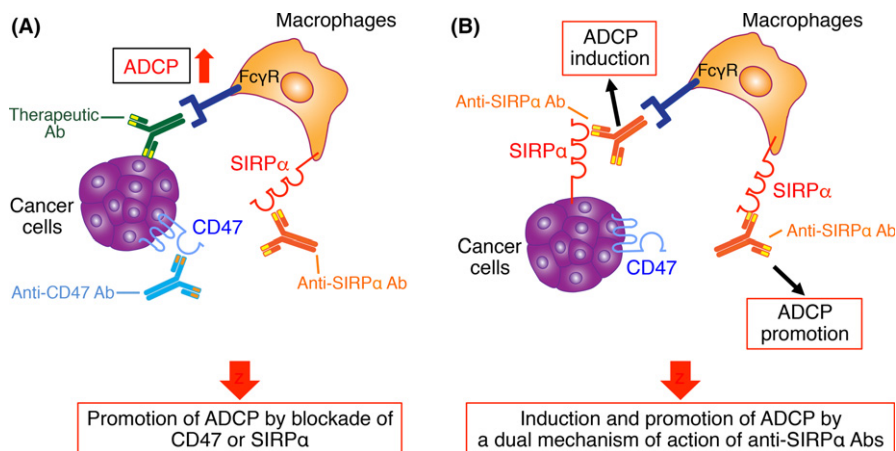


FIGURE 2 Suppression of tumor growth and metastasis by blockade of CD47-signal regulatory protein α (SIRP α) interaction and consequent promotion of macrophage-mediated antibody-dependent cellular phagocytosis (ADCP). A, Inhibition of the interaction of CD47 on tumor cells with SIRP α on macrophages by biological agents such as Abs to these proteins promotes macrophage-mediated ADCP of the tumor cells triggered by opsonization with therapeutic Abs to tumor antigens and thereby leads to suppression of tumor growth and metastasis. B, Blocking Abs to SIRP α bind to this protein on both macrophages and certain tumor cells such as melanoma and renal cell carcinoma cells. Such binding results in both direct induction of macrophage-mediated ADCP of the tumor cells as well as blockade of CD47-SIRP α signaling that negatively regulates such phagocytosis

lymphoma Raji cells transplanted into immunodeficient non-obese diabetic (NOD)/SCID mice,¹¹ whose endogenous SIRP α has a high affinity for human CD47.³¹ In addition, whereas tumor growth or metastasis did not differ between SIRP α mutant and WT mice injected with syngeneic melanoma cells, treatment with therapeutic Abs specific for a melanoma antigen eliminated tumor cells to a markedly greater extent in the mutant mice than in the WT animals.⁴⁵ Moreover, an Ab to human SIRP α that inhibits CD47-SIRP α interaction enhanced killing by human phagocytes of HER2-positive breast cancer cells opsonized with the HER2-specific mAb trastuzumab *in vitro*.⁴⁵ Abs to mouse SIRP α also enhanced the phagocytic activity of bone marrow-derived macrophages from NOD mice toward Raji cells opsonized with rituximab.¹¹ Collectively, these findings suggest that blocking Abs to SIRP α has the potential to promote tumor elimination *in vivo* by enhancement of macrophage-mediated ADCP of cancer cells opsonized by Abs to tumor antigens (Figure 2A). In addition to Abs to SIRP α , recombinant CD47 proteins that contain the NH₂-terminal Ig-V-like domain and block CD47-SIRP α interaction may also contribute to the killing of tumor cells. Indeed, an NH₂-terminal variant of CD47 with a higher affinity for SIRP α than WT CD47 was found to act synergistically with tumor-specific mAbs to promote macrophage-mediated phagocytosis of tumor cells *in vitro*,⁵⁴ although its potential antitumor effects *in vivo* have yet to be evaluated.

Blocking Abs to SIRP α might also have therapeutic effects as single agents in the case of SIRP α -expressing tumors, with the antitumor effects of such Abs being mediated by a dual mechanism of action: direct induction of ADCP of tumor cells by macrophages and blockade of CD47-SIRP α signaling that negatively regulates such phagocytosis (Figure 2B). Similar to the increased expression of CD47 apparent in many types of cancer, SIRP α is also more prominently expressed in tumor tissue from patients with renal cell carcinoma or melanoma compared with the surrounding normal tissue.¹¹ Of note, treatment with a blocking Ab to mouse SIRP α resulted in a marked reduction in

the tumor burden of immunocompetent mice injected with syngeneic renal cell carcinoma or melanoma cells, both of which highly expressed endogenous SIRP α .¹¹ This antitumor action of the Ab was significantly attenuated by selective depletion of macrophages. Moreover, the expression of SIRP α on tumor cells and the Fc region of the Ab to SIRP α were required for promotion of the phagocytosis of mouse renal cell carcinoma cells by macrophages *in vitro*, suggesting that opsonization of the tumor cells by the Ab induces ADCP through activation of the Fc receptor on macrophages.

In addition to macrophages, both cytotoxic T cells and NK cells may contribute to the antitumor effects of blocking Abs to SIRP α .¹¹ Such Abs might thus initiate cross-priming of tumor-specific cytotoxic T cells by macrophages and DCs. Moreover, the Abs might induce NK cell-mediated ADCC toward tumor cells directly and contribute to the activation of NK cells by tumor-infiltrating macrophages or DCs.⁵⁵⁻⁵⁷ Of interest, treatment with blocking Abs to SIRP α did not induce obvious adverse effects including anemia in mice.¹¹ Treatment of mice with Abs to SIRP α also did not cause any obvious neurotoxicity regardless of the high expression of SIRP α in the neuron.¹¹ Such Abs might thus be worth pursuing for cancer therapy, especially for the treatment of melanoma and renal cell carcinoma.

4.3 | Therapeutic drugs targeting CD47 or SIRP α

Several biological agents, including Abs and recombinant proteins, that are able to bind to human CD47 specifically and block the human CD47-SIRP α interaction have been developed for cancer treatment (Table 1). Hu5F9-G4 was the first drug to be developed as a humanized mAb to human CD47.⁴⁷ This agent enhances the phagocytosis of tumor cells by macrophages *in vitro* and eradicates human hematological malignancies and solid tumors in xenograft mouse models.⁴⁷⁻⁴⁹ Phase I or I/II clinical trials of Hu5F9-G4 and

TABLE 1 Therapeutic agents in preclinical or clinical development that target CD47 or SIRP α

Company	Country	Drug	Description	Phase	Disease	Strategy	Combination agent	ID
Forty Seven Inc.	USA	Hu5F9-G4	Anti-CD47 Ab (IgG4)	I	Solid tumors, NHL	Mono		NCT02216409
				I	AML, MDS	Mono		NCT02678338
				I/II	CRC, solid tumors	Combi	Cetuximab	NCT02953782
				I/II	NHL	Combi	Rituximab	NCT02953509
				I	AML, MDS	Mono/Combi	Azacitidine	NCT03248479
Celgene	USA	CC-90002	Anti-CD47 Ab (IgG4)	I	Solid tumors, MM, NHL	Mono/Combi	Rituximab	NCT02367196
				I	AML, MDS	Mono		NCT02641002
Trillium Therapeutics Inc.	Canada	TTI-621	SIRP α -Fc fusion protein (IgG1)	I	Hematological malignancies, solid tumors	Mono/Combi	Rituximab, nivolumab	NCT02663518
				I	Solid tumors	Mono/Combi	PD-1/PD-L1 inhibitor, PEG-IFN- α 2a, T-Vec, radiation	NCT02890368
Alexo Therapeutics	USA	ALX148	SIRP α V1-Fc fusion protein	I	Solid tumors, NHL	Mono/Combi	Pembrolizumab, trastuzumab, rituximab	NCT03013218
Novimmune SA	Switzerland	NI-1701	Anti-CD47/CD19 bispecific Ab	Preclinical				
		NI-1801	Anti-CD47/mesothelin bispecific Ab	Preclinical				
Arch Oncology	USA	AO-104, -108, -176	Anti-CD47 Ab	Preclinical				
Surface Oncology Inc.	USA	SRF231	Anti-CD47 Ab	Preclinical				
Hummingbird Bioscience	Singapore	HMBD004	Anti-CD47/CD33 bispecific Ab	Preclinical				
OSE Immunotherapeutics	France	OSE-172	Anti-SIRP α Ab	Preclinical				

Combi, combination therapy; CRC, colorectal carcinoma; ID, clinicaltrials.gov identifier; MDS, myelodysplastic syndrome; MM, multiple myeloma; Mono, monotherapy; NHL, non-Hodgkin lymphoma; PD-1, programmed cell death-1; PD-L1, programmed cell death-ligand 1; PEG-IFN- α 2a, pegylated interferon- α 2a; SIRP α , signal regulatory protein α ; T-Vec, talimogene laherparepvec.

another humanized mAb to human CD47, CC-90002, are now being conducted for both solid tumors and hematological malignancies. Recombinant SIRP α proteins, such as TTI-621 and ALX148, are also in phase I clinical trials for patients with hematological malignancies or solid tumors (Table 1). TTI-621 consists of the Ig-V-like domain of human SIRP α linked to the Fc region of human IgG1, and it was shown to enhance the phagocytosis of tumor cells by macrophages in vitro and to effectively control tumor growth in xenograft models of aggressive AML or B lymphoma as well as in a syngeneic mouse model of B lymphoma.^{58,59} Like TTI-621, ALX148 has a higher affinity for human CD47 than does WT SIRP α , but it comprises a variant of the Ig-V-like domain of human SIRP α fused to an inactive Fc domain. Both of these drugs are currently being tested in clinical trials as monotherapy or in combination either with molecularly

targeted agents including Abs to tumor antigens and immune checkpoint inhibitors or with radiotherapy. Various other CD47-targeting drugs including bispecific Abs that bind to both tumor-specific antigens and CD47 are also in preclinical development (Table 1).

5 | COMBINATION OF BLOCKING Abs TO CD47 OR TO SIRP α WITH IMMUNE CHECKPOINT INHIBITORS

Treatment with immune checkpoint inhibitors that target PD-1, PD-L1, or CTLA-4 provides substantial clinical benefit in patients with a wide range of malignancies including metastatic melanoma, renal cell carcinoma, and non-small-cell lung cancer.⁵⁻⁷ However, many

patients remain unresponsive to these therapies. Combinations of immune checkpoint inhibitors with standard chemotherapeutic drugs, small-molecule compounds, cancer vaccines, and immune-stimulatory agents are currently under evaluation in preclinical models and in clinical trials in attempts to increase the efficacy of immune checkpoint inhibition in such unresponsive patients.

Immune checkpoint inhibitors that target PD-1 or PD-L1 prevent the interaction of PD-1 on cytotoxic T cells with PD-L1 on cancer cells, which generates an inhibitory signal within the T cells. Blocking of this interaction is therefore thought to enhance the killing of tumor cells by cytotoxic T cells.^{4,6} Inhibition of both the CD47-SIRP α and PD-1-PD-L1 axes might therefore be expected to have a synergistic antitumor action, given that blockade of CD47 is thought to exert antitumor effects by promoting the phagocytosis of tumor cells and tumor cell-releasing substances by macrophages or DCs as well as by enhancing the cross-priming of tumor-specific cytotoxic T cells by these antigen-presenting cells (Figure 3).^{51,52} Indeed, a nanobody (an antigen-binding fragment of an Ab heavy chain) that reacts with CD47 and thereby inhibits the CD47-SIRP α interaction was found to synergize with PD-L1 antagonism to attenuate the growth of tumors formed in immunocompetent mice by s.c. injected syngeneic melanoma cells.^{60,61} The efficacy of combined therapy with Abs to PD-1 and to CTLA-4 in a mouse model of esophageal squamous cell cancer was also enhanced by blocking Abs to CD47.⁶² Moreover, combined blockade of SIRP α and PD-1 had a synergistic antitumor effect in a syngeneic mouse model of colon cancer.¹¹ The combination of

inhibitors of the CD47-SIRP α interaction with immune checkpoint inhibitors that target the PD-1-PD-L1 axis is thus a potential new approach to immunotherapy for a broad range of cancers.

6 | XENOGRAFT TUMOR MODELS FOR PRECLINICAL VALIDATION OF THE ANTITUMOR EFFECTS OF Abs TO HUMAN SIRP α

Animal models that mimic human diseases are important tools with which to investigate the potential therapeutic efficacy and adverse effects of biological agents in patients. However, the antitumor effects of Abs to human SIRP α in preclinical cancer models have remained unclear because such Abs have failed to bind to endogenous SIRP α expressed on macrophages of immunodeficient mice, likely because of differences in the amino acid sequence of the NH₂-terminal Ig-V-like domain between human and mouse SIRP α . In this regard, a blocking Ab to human SIRP α was recently shown to promote the antitumor effects of rituximab and of vorsetuzumab (an Ab to CD70) in human tumor-bearing *Rag2*^{-/-}/*Il2rg*^{-/-} immunodeficient mice in which the DNA sequence encoding the extracellular domain of mouse SIRP α was replaced with the corresponding human sequence (hSIRP α ^{K1} mice).^{63,64} With the use of *Rag2*^{-/-}/*Il2rg*^{-/-} mice expressing human SIRP α under the control of human regulatory elements (hSIRP α ^{Tg} mice),⁶⁵ we also showed that a blocking Ab to human SIRP α enhanced the inhibitory effect of rituximab on the growth of tumors formed by s.c. injected Raji cells.⁶⁶ This Ab to human SIRP α also increased the phagocytic activity of macrophages from hSIRP α ^{Tg} mice, but not that of those from *Rag2*^{-/-}/*Il2rg*^{-/-} mice, as measured in vitro with human cancer cells opsonized with Abs to tumor antigens, suggesting that the interaction of human CD47 on human cancer cells with human SIRP α on mouse macrophages generates an inhibitory signal for macrophage ADCP. Moreover, in both of these genetically modified mouse models transplanted with human tumor cells, the antitumor effects of the Abs to human SIRP α were found to be dependent, at least in part, on macrophages.^{64,66} These mice may thus serve as models for preclinical validation of Abs to human SIRP α in cancer immunotherapy (Figure 4).

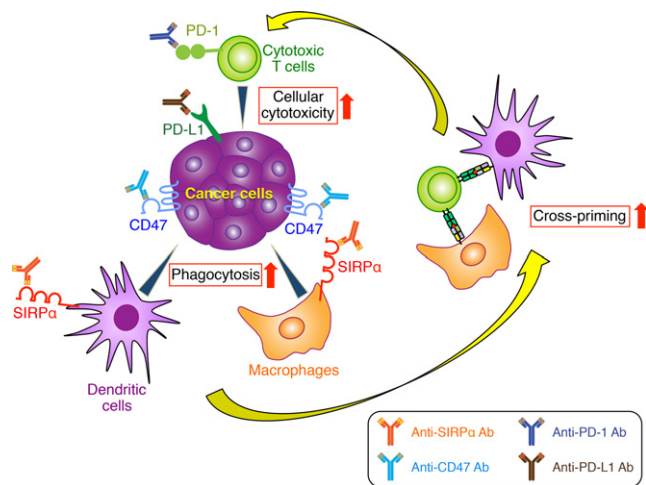


FIGURE 3 Synergistic antitumor effects of blockade of CD47-signal regulatory protein α (SIRP α) interaction combined with immune checkpoint inhibitors. Blockade of CD47 or SIRP α with corresponding specific Abs promotes the phagocytosis of tumor cells by macrophages or dendritic cells as well as the consequent cross-priming of tumor-specific cytotoxic T cells. Activity of cytotoxic T cells is also enhanced by immune checkpoint inhibitors, such as Abs to programmed cell death-1 (PD-1) or programmed cell death-ligand 1 (PD-L1) that prevent the interaction of these proteins. Blockade of CD47 or SIRP α together with giving immune checkpoint inhibitors may therefore have synergistic antitumor effects

7 | CONCLUSION

Attention has recently focused on modifying immune responses as a basis for new cancer treatments. Immunotherapy with immune checkpoint inhibitors that target PD-1, PD-L1, or CTLA-4, which enhance the antitumor activity of cytotoxic T cells, has shown clinical activity in a variety of cancer types. The CD47-SIRP α signaling system serves as an innate immune checkpoint that is thought to help tumor cells evade immune surveillance by preventing their phagocytosis by macrophages and other phagocytes. Numerous studies with preclinical mouse models of cancer have suggested

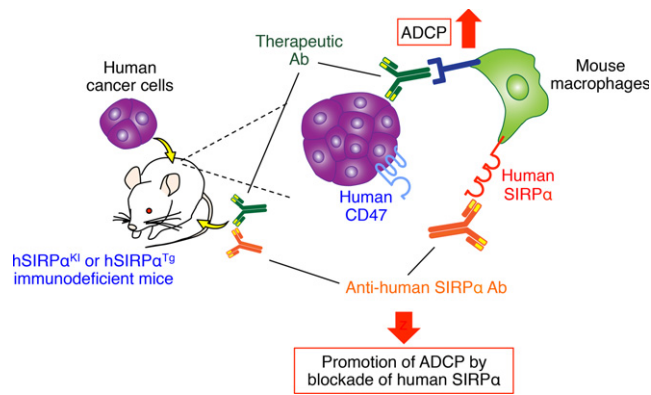


FIGURE 4 Xenograft tumor models for preclinical validation of the antitumor effects of Abs to human signal regulatory protein α (SIRP α). In xenograft tumor models in which human cancer cells are transplanted into human SIRP α knockin (hSIRP α ^{Kl}) or human SIRP α transgenic (hSIRP α ^{Tg}) immunodeficient mice, interaction of human CD47 on human cancer cells with human SIRP α on mouse macrophages inhibits phagocytosis of the former cells by the latter. Prevention of this interaction with Abs to human SIRP α thus enhances antibody-dependent cellular phagocytosis (ADCP) by macrophages of the cancer cells opsonized with therapeutic Abs to tumor-specific antigens

that blockade of CD47 or SIRP α with Abs or recombinant proteins, either alone or in combination with other agents such as Abs to tumor-specific antigens or immune checkpoint inhibitors, holds promise for the treatment of various types of cancer. However, the mechanisms underlying the initiation and consolidation of immune responses to tumor cells by CD47 or SIRP α blockade, as well as the possible adverse effects of such blockade *in vivo*, remain to be fully understood. In addition, xenograft models based on immunodeficient mice that lack NK, B, and T cells may not be sufficient for validation of the efficacy of agents that target human CD47 or SIRP α , given that the effects of such agents on immune cells in these mice are limited to myeloid cells such as macrophages and DCs. Immunodeficient mice engrafted with a human immune system by transplantation of human hematopoietic stem cells have been developed as an important new tool for cancer research.⁶⁷⁻⁶⁹ Future preclinical studies with such humanized and immunodeficient mice, as well as clinical trials, should show the potential of targeting the CD47-SIRP α axis as a new strategy for immunotherapy of human cancer.

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CONFLICTS OF INTEREST

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