



REVIEW ARTICLE

Targeting CD47-SIRP α axis for Hodgkin and non-Hodgkin lymphoma immunotherapy

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Abstract The interaction between cluster of differentiation 47 (CD47) and signal regulatory protein α (SIRP α) protects healthy cells from macrophage attack, which is crucial for maintaining immune homeostasis. Overexpression of CD47 occurs widely across various tumor cell types and transmits the “don’t eat me” signal to macrophages to avoid phagocytosis through binding to SIRP α . Blockade of the CD47-SIRP α axis is therefore a promising approach for cancer treatment. Lymphoma is the most common hematological malignancy and is an area of unmet clinical need. This review mainly described the current strategies targeting the CD47-SIRP α axis, including antibodies, SIRP α Fc fusion proteins, small molecule inhibitors, and peptides both in preclinical studies and clinical trials with Hodgkin lymphoma and non-Hodgkin lymphoma.

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Introduction

Lymphoma is a type of cancer that originates in lymphocytes. Lymphoma is the most common hematological malignancy, with an estimated 89,010 new cases in the United

States in 2022.¹ Lymphoma has several subtypes and complex terminology. Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) are the two main classifications.^{2,3} NHL is the fifth most common cancer in the United States and consists of indolent and aggressive subtypes with a 5-year overall survival ranging from 25% to 75%.¹ Depending on the type and stage of lymphoma, treatments for patients with lymphoma include surgery, chemotherapy, radiation therapy, and targeted drugs.⁴ Although these treatments can improve the survival rate, many patients relapse, and some experience drug resistance.

The malignant lymphoid cells impede the anti-tumor immune response due to the interactions between lymphoid

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cells with complexing tumor microenvironment⁵ (Fig. 1). Tumor microenvironment is essential for tumor origin, progression, and resistance to chemotherapy in lymphoma.⁶ Tumor microenvironment comprises tumor cells, immune cells, stromal cells, blood vessels, associated tissue cells, and a wide variety of cytokines and chemokines.⁷ The immune microenvironment consists of macrophages, lymphocytes, neutrophils, myeloid-derived suppressor cells, natural killer cells, and dendritic cells.⁸ Anti-tumor immune response can be formed when antigen-presenting cells (APCs), such as macrophages, dendritic cells, and B-cells, present antigens to effector cells.⁹ The major histocompatibility complex (MHC) class I and II, expressed on macrophages and other APCs, are required for the presentation of tumor antigens to immune cells. Lymphoma cells themselves act as APCs, and the MHC is induced on the surface of lymphoma cells, leading to a weakly immunogenic response.¹⁰ Lymphocyte-activation gene 3 (LAG-3), an inhibitory co-receptor, selectively interacts with MHC II and then suppresses the immune presentation process.¹¹ Signal regulatory protein alpha (SIRP α), expressed on the membrane of myeloid immune cells such as macrophages, and other APCs triggers a "don't eat me" signal by interacting with the cluster of differentiation 47 (CD47), and further enables immune evasion for lymphoma cells.¹² Moreover, overexpression of inhibitory ligands suppresses immune activation in lymphoma. High expression of programmed death-ligand 1 and 2 (PD-L1/2) in malignant lymphoma causes T-cell dysfunction, exhaustion, and subsequent apoptosis through the PD-1/PD-L1 signaling pathway.¹³ Other inhibitory ligands, such as B- and T-lymphocyte-

associated protein (BTLA),^{14,15} cytotoxic T-lymphocyte-associated protein 4 (CTLA-4),¹⁶ and CD160¹⁷ are also active in the tumor microenvironment of lymphoma and suppress immune cell function. In addition, myeloid-derived suppressor cells and immature monocytic myeloid cells promote regulatory T (Treg) cell differentiation and suppress effector T-cells in B-cells lymphoma¹⁸ (Fig. 1).

CD47-SIRP α signal pathway

The biological background of CD47

CD47, also named integrin-associated protein (IAP), is a transmembrane protein ubiquitously expressed on all normal cells and various types of cancer cells, such as leukemia, lymphoma, gastrointestinal cancer, prostate cancer, lung cancer, and hepatocellular carcinoma.^{19–21} CD47 was discovered in 1990 when researchers purified the integrin-associated protein.²² As an immunoglobulin superfamily member, it is composed of an N-terminus Ig-V domain attached to a five membrane-spanning domain and a short cytoplasmic C-terminus.^{23,24} CD47 regulates cell angiogenesis,²⁵ proliferation,²⁶ and apoptosis²⁷ through interacting with its ligands, including thrombospondin-1 (TSP-1), SIRP α , and integrin $\alpha v \beta 3$ or $\alpha 2 \beta 1$.²⁴ The expression of CD47 in cancer cells can be regulated at the transcriptional and post-translational modification (PTM) levels (Fig. 2).²⁸

Mechanism of CD47-SIRP α signal pathway

SIRP α (also named SHPS-1) is a receptor-like glycoprotein mainly expressed and functions in myeloid cells and is composed of an extracellular immunoglobulin domain and a transmembrane domain connected to an intracellular tail.²⁹ CD47-SIRP α interaction plays a crucial role in immune recognition and prevention of attacks on self-tissues. When the extracellular domain of CD47 in cancer cells binds to the NH2 terminal domain of SIRP α in macrophages, the Src family kinases phosphorylate the intracellular ITIMs domain of SIRP α ,³⁰ then recruited the cytosolic tyrosine phosphatase SHP-1 and SHP-2.³¹ SHP-1 and SHP-2 are typically inactive for its autoinhibitory mechanism involving the SH2 domain,³² and the recruitment by phosphorylated ITIMs causes a conformational change to relieve the suppression of tyrosine phosphatase activity.³⁰ Consequently, downstream molecules are dephosphorylated by activated SHP-1 and SHP-2,³³ resulting in the suppression of myosin-IIA accumulation at the phagocytic synapse and inhibition of phagocytosis of macrophages (Fig. 2).^{34,35} Binding of CD47 to SIRP α also inhibit the activation and maturation of dendritic cells (DCs).³⁶ Blocking of CD47 activates an innate immune response through phagocytosis mediated by macrophages. Moreover, inhibiting CD47 promotes NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) in lymphoma.¹² Except for affecting the innate immune response, CD47 suppression can enhance the antigen-specific CD8⁺ T cell response.³⁷ In addition, blockade of the CD47 suppresses cancer stem cells and induces cell apoptosis (Fig. 3).^{38,39}

Blockade of the CD47-SIRP α axis has exhibited anti-tumor activity in various cancers.^{40,41} A growing number of

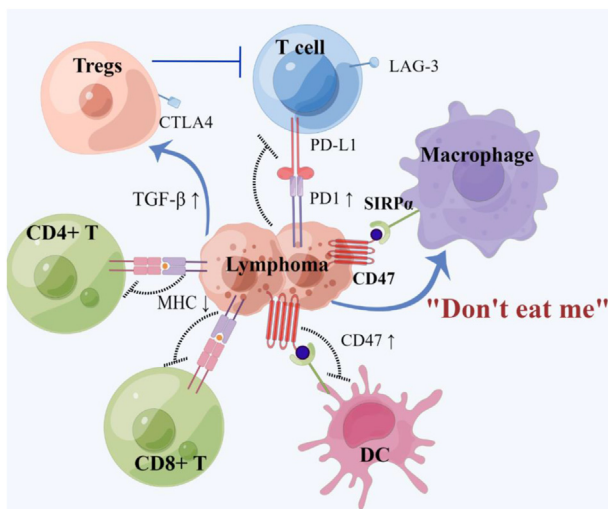


Figure 1 Tumor microenvironment and mechanisms of immune escape of lymphoma cells. Low expression of MHC on the surface of lymphoma cells leads to a weak immunogenic response of T cells; recruitment of Tregs by TGF- β from lymphoma cells suppresses T cell activity. The interaction of PD-1 and PD-L1 suppresses the activity of T cells. Up-regulated CD47 suppresses macrophage phagocytosis and antigen presentation of DC cells through interaction with SIRP α . The image was created using Figdraw.

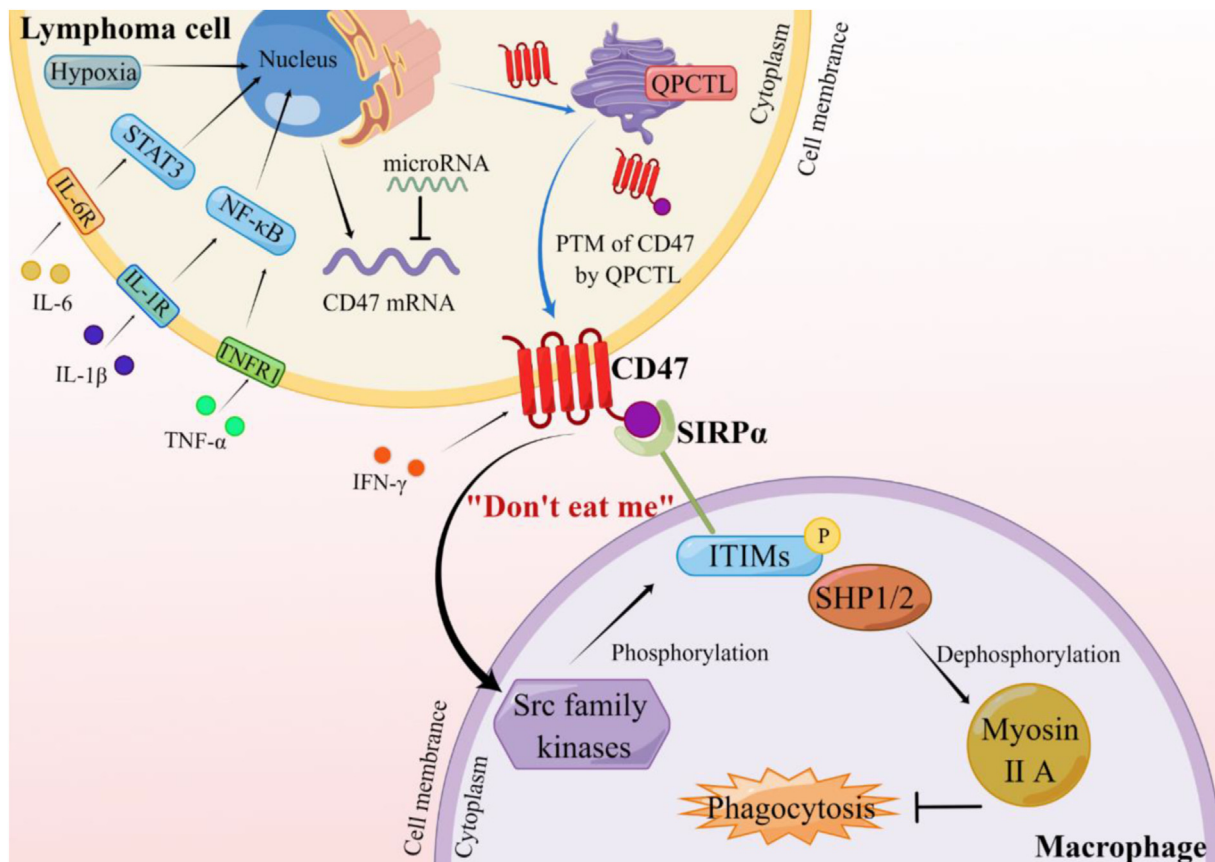


Figure 2 The regulation of CD47 expression and the "Don't eat me" signal. CD47 expression in lymphoma cells is regulated by cytokines, oncogenes, microRNAs, and enzymes both in transcriptional and PTM levels. TNF- α and IL-1 β stimulate CD47 through activating NF- κ B; CD47 expression is regulated by the activation of STAT3 signal induced by IL-6. MicroRNAs suppress CD47 expression by directly binding to CD47 mRNA. Hypoxia conditions increase the transcriptional expression of CD47. The Golgi-resident enzyme QPCTL catalyzes the cyclization of N-terminal glutamic acid and glutamine residues into a pyroglutamate residue (pGlu) on CD47, which is essential for CD47-SIRP α interaction and can be inhibited by QPCTL inhibitors. Interaction of lymphoma cell CD47 and macrophage SIRP α recruited protein tyrosine phosphatases SHP-1/2 to inhibit downstream phosphorylation signaling, then inhibiting the remodeling of the macrophage cytoskeleton and transmitting the "Don't eat me" signal. The image was created using Figdraw.

studies suggest that targeting CD47 is a promising immunotherapeutic strategy. In this review, we systematically discuss immunological approaches targeting CD47 to treat lymphoma.

Treatments for HL targeting CD47-SIRP α axis

HL, including classical Hodgkin lymphoma (cHL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), is a rare type accounting for approximately 15% of all lymphomas.⁴ Treatments targeting immune checkpoints, such as PD-1 and LAG-3, have achieved clinical efficacy in patients with relapsed/refractory HL. CD47 is overexpressed in Hodgkin Reed Sternberg cells (HRS),⁴² suggesting that CD47 is a potential therapeutic target for cHL. At present, a clinical trial (NCT04788043) studying the combination of Hu5F9-G4 with Pembrolizumab in relapsed/refractory classical HL is ongoing to recruit participants. TTI-621, a soluble fusion protein combined with the CD47-binding domain of human SIRP α with the Fc region of human

IgG1, is involved in a clinical trial (NCT02663518) as monotherapy for HLs (Table 2).

Treatments for NHL targeting CD47-SIRP α axis

NHL, a most common lymphoid malignancy, comprises mature B-cell, T-cell, and NK/T-cell lymphomas.⁴³ Mature B-cell neoplasms, including diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL), accounts for $\geq 80\%$ of NHLs.² T-cell lymphomas (TCLs) are uncommon types and account for approximately 12% of all lymphomas.⁴⁴ TCLs are a broad group of heterogeneous diseases, further categorized into peripheral T-cell lymphomas (PTCL) and cutaneous T-cell lymphomas (CTCL).⁴⁵ NK/T-cell lymphomas are aggressive EBV-related malignancies, mostly deriving from NK-cell.⁴⁶ CD47 is highly expressed in multiple NHL subtypes.^{12,47–49} Overexpression of CD47 is associated with tumor progression and dissemination^{50,51} and poor survival of NHL patients.⁵² Strategies blocking the CD47-SIRP α axis were demonstrated to treat NHLs in several studies (Fig. 3),

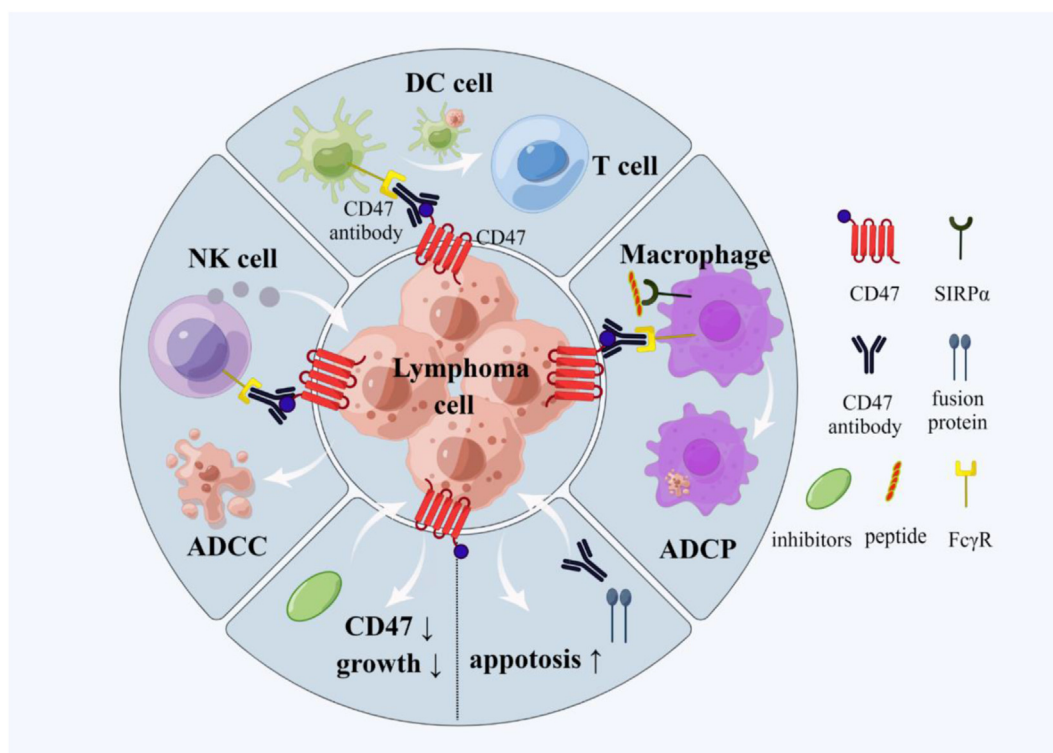


Figure 3 Strategies and mechanisms of blocking the CD47-SIRP α pathway in lymphoma. CD47 antibodies directly bind to CD47 in lymphoma cells and exert anti-tumor effects through ADCP of macrophages and ADCC of NK cells, and antigen presentation by DC cells. CD47 antibodies and Fc fusion proteins also directly promote apoptosis of lymphoma cells. Peptides targeting macrophage SIRP α promote phagocytosis. Small molecule inhibitors down-regulate CD47 expression and suppress PTM of CD47 and suppress lymphoma cell growth. The image was created using Figdraw.

highlighting the promising foreground of CD47-targeted therapies in NHLs. We will focus on the latest studies on NHL treatments, such as antibodies, fusion proteins, and small-molecule inhibitors targeting the CD47-SIRP α pathway.

Antibodies

Several anti-CD47 antibodies blocking the CD47-SIRP α axis were demonstrated to have significant therapeutic efficacy in both preclinical research (Table 1) and clinical trials (Table 2) for NHLs. These antibodies exert efficacy through several mechanisms, including (i) inducing apoptosis or suppressing proliferation of lymphoma cells; (ii) suppressing NHL hematogenous dissemination; (iii) Fc-mediated functions such as antibody-dependent cellular phagocytosis (ADCP), which is one of the most vital mechanisms of these antibodies, and ADCC in lymphomas; (iv) antigen presentation by DC and activation of T cells (Fig. 3).

CC-90002

CC-90002 is a humanized anti-CD47 IgG4 antibody that is the first-generation anti-CD47 antibody used in clinical research. The Fc function of CC-90002, which promoted hemagglutination, was detuned. CC-90002 exhibits high-affinity binding to CD47 in various cancer cells, including Raji lymphoma cells, and inhibits CD47-SIRP α interaction. Treatment with CC-90002 recruits F4/80-positive

macrophages into tumors and induces the expression of selected chemokines and cytokines of murine origin. A preclinical study showed that CC-90002 has acceptable pharmacokinetic properties and low toxicity in non-human primates.⁵³ In the first clinical study of CC-90002, the preliminary monotherapy data failed to provide sufficient evidence for a further dose increase, for which Celgene terminated the clinical trial in 2018 (NCT02641002).⁵⁴ Later, a phase I study (NCT02367196) in subjects with advanced solid and hematologic cancers was completed after modification of the dosing strategy and other procedures.

Hu5F9-G4 (Magrolimab)

Hu5F9-G4 (*abbr.* 5F9), a humanized IgG₄ antibody from a mouse monoclonal anti-human CD47 antibody, binds to monomeric human CD47 with an 8 nM affinity.⁵⁵ In a first-in-human, first-in-class phase I trial of 5F9 in patients with advanced cancers (NCT02216409), 5F9 was well tolerated at a priming dose of 1 mg/kg on the first treatment and maintenance dose of up to 45 mg/kg weekly. 5F9 can induce potent macrophage-mediated phagocytosis of cancer cells *in vitro* and synergize with rituximab to eliminate NHL engraftment in a Raji Luc-EGFP engrafted NSG mice model.⁵⁵ Currently, 5F9 is undergoing several clinical trials, including in patients with lymphoma. A phase I b study (NCT02953509) evaluated the efficacy of 5F9 combined with rituximab in patients with DLBCL and FL. Treatment

Table 1 Anti-CD47 antibodies and SIRP α fusion proteins in preclinical studies for lymphomas.

No.	Name	Type	Target	Tumor model	Administration route	Result	Reference
1	B6H12	Humanized IgG ₁	hCD47	Human NHL tumor-bearing NSG mice	i.p	Disease-free survival was extended	¹²
	B6H12.2			Human DLBCL tumor-bearing NSG mice	i.p	Hematogenous dissemination of NHL was inhibited	⁵¹
	B6H12			Human B-CLL cell line	<i>in vitro</i>	B-CLL cell apoptosis was induced	⁵⁸
	B6H12			Hut78 cells; tumor-bearing NSG mice	i.p	TCL burden was reduced and the survival rate was extended	⁴⁹
2	IBI188	Humanized IgG ₄	hCD47	BCL tumor-bearing NOD/SCID mice	i.p	BCL was eradicated	⁶⁰
3	CC90002	Humanized IgG ₄	hCD47	Raji lymphoma	<i>in vitro</i>	CD47-SIRP α interaction was blocked	⁵³
4	BRIC126	Mouse IgG _{2b}	hCD47	DLBCL B-CLL	<i>in vitro</i>	Phagocytosis of DLBCL was induced	¹² ⁵⁸
5	IMM0306	DVD-Ig	hCD20 and hCD47	B-CLL Raji NHL tumor-bearing NSG mice	<i>in vitro</i> i.p	Apoptosis of B-CLL cells was induced Phagocytosis was induced The lymphoma burden was reduced and survival was extended	⁶⁷
6	MABL scFv-5	mAb	hCD47	L1210-hCD47 cells	<i>in vitro</i>	Apoptosis was induced	³⁸
7	MABL sc(Fv)2	Mouse IgG ₁	hCD47	JOK-1 B-CLL tumor-bearing SCID mice	i.v	Medium survival time was improved	³⁸
8	TJC4	Fully human IgG ₄	hCD47	Raji cells; tumor-bearing NOD-SCID mice	i.p	Tumor growth suppression	⁶⁵
9	CV1	SIRP α monomer	hCD47	Canine lymphoma CLBL-1 cells; tumor-bearing NSG mice	i.h	Anti-tumor response was increased	⁷⁶
				Raji cell lymphoma; tumor-bearing NSG mouse	i.h	Phagocytosis was induced combined with rituximab or alemtuzumab	
10	CV1-hIgG4	SIRP α fusion protein	hCD47 mCD47	Canine lymphoma CLBL-1 cells; tumor-bearing NSG mouse	i.h	Phagocytosis was increased Tumor growth suppression	⁷⁶

Notes: i.p, intraperitoneal injection; i.h, hypodermic injection; i.v, intravenous injection.

with 30 mg/kg of 5F9 led to an approximately 100% CD47-receptor occupancy on circulating white and red blood cells; however, dose-related thrombocytopenia and neutropenia were not observed. The objective response rate was 50% of the patients in total, and the complete response rate was 36%. The rates of objective response and complete response were 40% and 33% in patients with DLBCL, and 71% and 43% in those with FL, respectively.⁵⁶ A platform study (NCT03527147) evaluating 5F9 in combination with acalabrutinib for the treatment of relapsed/refractory aggressive NHL was completed, but no treatment results have been reported so far. The potential adverse events of 5F9 treatment were transient and predictable anemia. In terms of safety, anemia could be mitigated by administering a priming dose of 1 mg/kg and a maintenance dose of 30 mg/

kg in a cycle.^{55,57} Used as a single drug or combined with chemotherapy and other anti-tumor antibodies, 5F9 can be an effective treatment for patients with aggressive and relapsed lymphomas.

B6H12

B6H12 is another humanized IgG1 anti-CD47 antibody. It can induce phagocytosis of macrophages and show synergy in combination with rituximab in multiple NHL cell types.¹² In a xenotransplant mouse model of human primary DLBCL, 8 out of 9 mice treated with B6H12.2 (intact CD47 purified from placenta was used as the immunogen for this antibody) combined with rituximab were cured, and disease-free survival was extended for 4 months after the treatment.¹²

Table 2 Anti-CD47 antibodies and SIRP α fusion proteins in clinical trials for lymphomas.

No.	Name	Type	Target	Indication	Clinical trial	Year	Status
1	Hu5F9-G4	Humanized IgG ₄	hCD47	CHL DLBCL FL DLBCL	Phase II (NCT04788043) Phase I (NCT02953509) Phase I (NCT03527147)	2021 Nov. 2016 Nov. 2018 Jun.	Recruiting Active, not recruiting Completed
2	IBI188	Fully human IgG ₄	hCD47	lymphomas	Phase I (NCT03763149)	2019 Feb.	Completed
3	SRF231	Humanized IgG ₄	hCD47	lymphomas	Phase I (NCT03512340)	2018 Mar.	Completed
4	NI1701 (TG-1801)	Human IgG1	hCD47 hCD19	BCL	Phase I (NCT04806035)	2021 Apr.	Recruiting
5	IMM0306	Bispecific ab	hCD20 hCD47	B-cell NHL	Phase I (NCT04746131)	2021 Feb.	Suspended
6	IBI322	Bispecific ab	hCD47 hPD-L1	Lymphomas	Phase Ia (NCT04338659)	2020 Apr.	Recruiting
7	HX009	Bispecific ab	hPD-1 hCD47	Relapsed/refractory Lymphoma	Phase I (NCT05189093)	2022 Jan.	Recruiting
8	TTI-621	SIRP α -IgG ₁ fusion protein	hCD47	CTCL, DLBCL, FL CTCL	Phase I (NCT02663518) Phase I (NCT02890368)	2016 Jan. 2016 Sep.	Active, not recruiting Terminated
9	TTI-622	SIRP α -IgG ₄ fusion protein	hCD47	DLBCL	Phase I (NCT03530683)	2018 May.	Recruiting
10	ALX-148	SIRP α -IgG ₁ fusion protein	hCD47	DLBCL, FL, and refractory MCL lymphomas	Phase I/II (NCT05025800) Phase I (NCT03013218)	2021 Oct. 2017 Feb.	Recruiting Active, not recruiting
11	Gentulizumab	mAb	hCD47	NHL	Phase I (NCT05221385)	2021 Apr.	Recruiting
12	SG2501	Bispecific ab	hCD47 hCD38	HL/NHL	Phase I (NCT05293912)	2022 Apr.	Not yet recruiting
13	CPO107	SIRP α fusion protein	hCD47 hCD20	CD20 positive NHL	Phase I/II (NCT04853329)	2021 Dec.	Recruiting
14	IMC-002	Humanized IgG ₄	hCD47	R/R lymphomas	Phase I (NCT04306224)	2020 Jun.	Recruiting
15	TJC4	Fully human IgG ₁	hCD47	CD20 positive NHL	Phase I (NCT03934814)	2019 May	Recruiting
16	GS-0189	Humanized IgG ₁	SIRP α	NHL	Phase I (NCT04502706)	2020 Nov.	Active, not recruiting

Furthermore, B16H12.2 can efficiently inhibit hematogenous dissemination of primary human NHL in a DLBCL xenotransplant NSG mice model.⁵¹ Interestingly, blocking CD47 with B6H12 can induce apoptosis of primary human B-CLL cells in a caspase-independent manner.^{58,59} CD47 inhibition in T-cell lymphoma can suppress tumor formation of Hut78 cells *in vivo* and markedly reduce lymphoma burden.⁴⁷ The survival rate was prolonged when mice were treated with B6H12 in human TCL patient-derived xenografts or immunocompetent murine TCL model.⁴⁹

IBI188 (Letaplimab)

IBI188 is a human IgG₄ CD47 antibody that specifically blocks the CD47-SIRP α interaction in different cell lines such as Raji and MDA-MB-231.⁶⁰ Preclinical study indicated that IBI188 can promote phagocytosis of the human tumor cell line CCRF-CEM through CD47-SIRP α blockade *in vitro*. B-cell lymphoma was eradicated in a NOD/SCID mouse model after IBI188 was intraperitoneally administered every other day for two weeks.⁶⁰ A clinical study (NCT03763149) was performed to evaluate the safety, tolerability, and initial efficacy of IBI188 in lymphomas. However, no treatment results have been reported to date.

SRF231

SRF231 is a high-affinity human IgG₄ anti-CD47 monoclonal antibody. It elicits anti-tumor activity by inducing phagocytosis in a CD32a-dependent manner.⁶¹ When cooperated with the rituximab, SRF231 enhances ADCP efficiency in malignant B-cell lymphoma Raji and SU-DHL-4 cell lines without causing hemagglutination or RBC phagocytosis, and induces the macrophage cytokines and MIP-1 α (macrophage inflammatory protein 1 alpha) in tumor-bearing mice.⁶¹ A clinical study (NCT03512340) to evaluate the safety and tolerability of SRF231 was completed, but it has been deprioritized owing to significant hematologic toxicity.⁶²

BRIC126

BRIC126 is a mouse IgG2b CD47 antibody. Blockade of CD47 with BRIC126 induced more efficient phagocytosis of NHL cells than IgG1 isotype control *in vitro*. The synergistic phagocytosis by the combination of BRIC126 with rituximab was proved in the NHL-17-DLBCL cell line.¹² In addition to inducing phagocytosis, BRIC126 can induce apoptosis of primary human B-CLL cells in a caspase-independent manner.^{58,59}

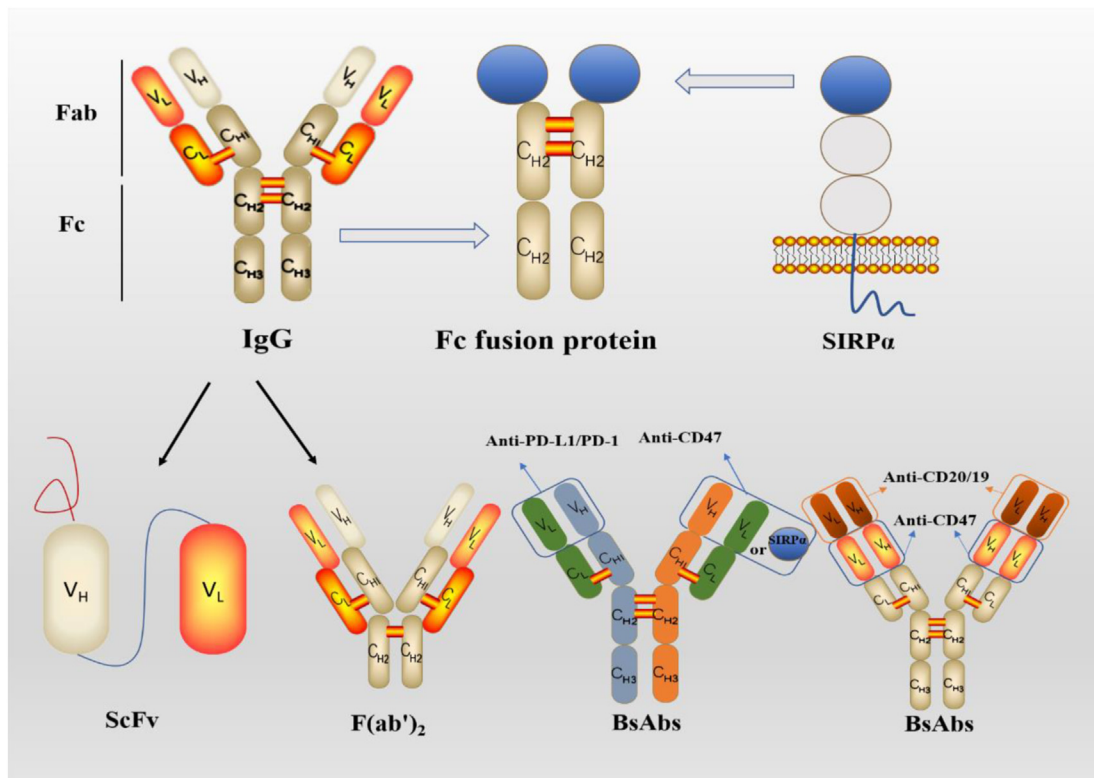


Figure 4 Schematic diagram of antibodies and fusion protein. The structure of IgG represents antibodies such as Hu5F9-G4, IBI188, CC90002, SRF231, and MABL; the model of the fusion protein is similar to CV1, TTI-621, and ALX-148; scFv and F(ab')₂ is designed from MABL. BsAbs specifically bind to two different antigens, such as CD47 and CD20.

MABL

MABL is a murine monoclonal antibody against an extracellular domain of human CD47 that can induce cellular apoptosis against CD47-positive leukemic cells.³⁸ Based on the MABL antibody, MABL scFv-5 diabody and MABL sc(Fv)₂ single-chain antibody fragments were designed (Fig. 4), and were more stable and sustained the ability to induce apoptosis in L1210-hCD47 cells.³⁸ The median survival time for mice model intravenously inoculated with B-CLL JOK-1 cells was significantly prolonged by the treatment of MABL scFv-5 diabody and MABL sc(Fv)₂.^{38,63} Additionally, MABL sc(Fv)₂ was confirmed to strongly induce apoptosis of B-CLL cells.

TJC4

TJC4 (Lemzoparlimab or TJ011133) is a CD47 IgG4 antibody targeting a distinct CD47 epitope that enables unique red blood cell sparing. It possesses novel anti-tumor activity and weakly binds to human erythrocytes minimizing hematological toxicity.⁶⁴ Macrophage phagocytosis of different human B-cell lymphoma cell lines such as SU-DHL-16, Raji, OCI-LY-8, and SU-DHL-6 was induced by TJC4 *in vitro*.⁶⁵ Monotherapy of TJC4 exhibited a significant dose-dependent response in NOD/SCID mice engrafted with Raji cells, a remarkable reduction in tumor volume was observed in OCI-LY-8 cells, DLBCL PDX, and Raji cell CDX tumor-bearing mouse models when mice were treated with

TJC4 combined with Venetoclax (a BCL-2 inhibitor).⁶⁵ A phase I study of TJC4 (NCT03934814) indicated acceptable safety, tolerability, pharmacokinetics, pharmacodynamics, and anti-tumor activity in patients with relapsed and refractory (R/R) lymphoma. A clinical study of TJC4 in patients with CD20-positive NHL is undergoing in the phase I study (NCT03934814). Among the seven efficacy-evaluable patients dosed with 20 or 30 mg/kg TJC4 in combination with rituximab, one DLBCL and three FL complete responses, and one partial response were observed.⁶⁶

Bispecific antibodies

Bispecific antibodies (BsAbs) are novel antibody therapeutics that specifically bind to two different antigens (Fig. 4). BsAbs may provide a lower-cost solution for patients than two monoclonal antibody combination therapies. Therefore, the development of BsAbs will provide a novel, comprehensive, effective, and cost-saving option for patients.

IMM0306 (CD20-CD47 SL)

IMM0306 is a bispecific recombinant antibody receptor fusion protein that targets both CD47 and CD20 in B cells. IMM0306 can activate the phagocytic capacity of macrophages and trigger antigen-specific T cells through tumor antigen presentation.⁶⁷ *In vitro* studies have shown that IMM0306 binds CD47 and CD20 with a 3–8 fold lower affinity

than individual target molecules. However, it has stronger phagocytic activity against CD47-positive target cells and even stronger ADCC activity than rituximab. Interestingly, IMM0306 had no binding activity against human erythrocytes.⁶⁸ Treatment of mice implanted with lymphoma tumors with IMM0306 significantly inhibited tumor growth and cleared lymphomas at a low dose (1.5 mg/kg).⁶⁹ IMM0306 is currently in phase I clinical trial (NCT04746131) to assess its safety and efficacy in patients with B-cell NHL. A clinical trial (IND no. CTR20192612) of IMM0306 in China is being evaluated in patients with refractory or relapsed CD20-positive B-cell NHL. To date, no clinical data have been reported for IMM0306.

IBI322

IBI322 is a novel BsAb that inhibits both the PD-1/PD-L1 axis and the CD47/SIRP- α axis for the treatment of patients with advanced malignancies.⁷⁰ Preclinical studies have shown that IBI322 effectively inhibits CD47/SIRP- α interaction and induces macrophage phagocytosis of CD47-expressing tumor cells. IBI322 can more selectively bind to tumor cells than anti-CD47 monoclonal antibodies due to PD-L1 expression in tumor cells, which consequently reduces the erythrocyte toxicity associated with anti-CD47 monoclonal antibodies.⁷¹ Phase I clinical trial (CIBI322A101) of IBI322 in China assessed the pharmacokinetics, safety, and target engagement of IBI322 in humanized transgenic animal models bearing MC38 tumors, suggesting that preliminary pharmacodynamics study with 0.34 mg/kg was rational.⁷² IBI322 is currently in phase I a study evaluating the safety, tolerability, and preliminary efficacy of IBI322 in patients with advanced malignant tumors lymphomas (NCT04338659) and phase I dose-escalation trials in China (NCT04328831). To date, no clinical data have been reported.

HX009

HX009 is an anti-PD-1/CD47 BsAb used for the treatment of patients with advanced solid tumors, including gastric, colorectal, and hepatocellular carcinomas, and lymphoma.⁷³ It consists of a monoclonal antibody against the IgG4 subtype of PD-1 and the extracellular structural domain of SIRP- α , consequently achieving a synergistic anti-tumor effect by simultaneously activating innate and acquired immune responses to inhibit tumor immune escape. HX009 is currently in phase I clinical trial in patients with relapsed/refractory lymphoma (NCT05189093) to evaluate its dose escalation and efficacy. To date, no clinical data have been reported on the safety and efficacy of HX009.

NI1701 (TG1080)

NI1701 is another first-in-class, fully human IgG1 BsAb that co-targets CD47 and CD19. Treatment with NI-1701 had no deleterious effects on hematological parameters following single or multiple administrations in non-human primates. Furthermore, phagocytosis was significantly induced following treatment with NI-1701 in different subtypes of NHL cells derived from patients including marginal zone lymphomas (MZL), Waldenstrom macroglobulinemia (WM),

mantle cell lymphomas (MCL), FL, and DLBCL. When NOD/SCID mice transplanted with Raji cells were intravenously administrated NI-1701 (10 mg/kg) once a week for 4 or 5 weeks, tumor growth was remarkably inhibited and the median survival time was prolonged.^{74,75} NI1701 is currently in a clinical trial (NCT04806035), recruiting participants to evaluate the efficacy used alone or in combination with ublituximab in subjects with B-CLL.

In summary, both humanized antibodies and fully human antibodies targeting CD47 are used in clinical experiments for a variety of lymphomas. Fully human antibodies have an edge over humanized antibodies in terms of lower immunogenicity and higher safety resulting from the absence of murine sequences, while humanized antibodies contain the mouse complementarity-determining region (CDR) sequences.⁷⁷ CD47-blocking antibodies directly binding to SIRP- α may have some advantages in avoiding anemia, resulting in limited tissue expression of SIRP- α . The anti-tumor activity of antibodies is almost dependent on the interaction of Fc fragment and Fc γ R on immune cells,⁷⁸ the affinities of different IgG subtypes for Fc γ R are different even though the identity in amino acid level is more than 90% among them.⁷⁹ IgGs including IgG₁, IgG₂, and IgG₄ have been used for developing CD47 antibodies (Table 1, 2), among the three subtypes, IgG1 demonstrates the highest affinity for all Fc γ Rs and mediated potent ADCC and ADCP. IgG4 and IgG2 are weaker in inducing Fc-mediated functions, but IgG4 is the most common type of CD47 antibody that erodes the side effects, such as anemia. In this regard, bispecific antibodies seem to have some advantages in avoiding anemia due to the co-targeting ability of CD47, and another tumor-specific antigen limits their binding to tumor antigen-positive cells to some extent. Bispecific antibodies may have reduced drug efficacy because the affinity of targeting CD47 is usually weaker than that of binding to tumor-specific antigens. In general, it is important to balance the relationship among IgG subtypes, structure, and efficacy when developing antibodies.

SIRP- α fusion protein

In addition to monoclonal antibodies, high-affinity SIRP- α monomers (Fig. 4) and fusion proteins, such as CV1, TTI-621, and ALX-148, were reported to block the CD47-SIRP- α axis in patients with NHLs (Table 1, 2).

CV1

CV1, a high-affinity SIRP- α protein, antagonizes CD47 on cancer cells with up to a 50,000-fold increase in affinity for human CD47 relative to wild-type SIRP- α .⁷⁶ CV1 has no obvious inhibition in tumor growth and does not stimulate phagocytosis when it is used alone, but increased phagocytosis *in vivo* was found when it was combined with tumor-specific monoclonal antibodies.⁷⁶ CV1 was further optimized to CV1-hlgG4 which blocks both human and mouse CD47. CV1-hlgG4 demonstrated potent binding affinity to canine CD47 with an EC₅₀ of 741 pM and exerts significantly increased phagocytosis of CLBL-1 cells by canine macrophages.⁸⁰ In a mouse xenograft model of canine DLBCL CLBL-1 cells, tumor growth was suppressed when mice were

treated with 250 μ g/daily CV1-hIgG4 for 1 week. The combination of the CV1 monomer and 1E4-clgGB (anti-canine CD20) elicited cures in 100% of mice bearing canine lymphoma.⁸⁰

TTI-621

TTI-621 is a soluble fusion protein combining the CD47-binding domain of human SIRP α with the Fc region of human IgG1 Fc, which plays a crucial role in potent macrophage activation and ADCC but avoids side effects.⁸¹ In a phase I study (NCT02663518), TTI-621 was well-tolerated when it was combined with rituximab in patients with B-NHL or with nivolumab in patients with HL and demonstrated promising therapeutic activity as monotherapy in patients with R/R B-NHL and T-cell NHL. The overall response rate of 0.2 mg/kg TTI-621 ranged from 17% to 25% in cutaneous T-cell lymphoma, peripheral T-cell lymphoma, and DLBCL.⁸² It is underway to evaluate the safety and activity of TTI-621 for patients with DLBCL and FL.⁸³ In another phase I study (NCT02890368), patients with CTCL were intravenously administered TTI621 as a single intra-tumoral injection demonstrated rapid reductions in both circulating lymphoma burden and severity of skin lesions, and no treatment-related adverse events over grade 3 and no treatment-related deaths were found, and no dose-limiting toxicities occurred.⁸⁴ Another CD47-blocking fusion protein TTI-622 (SIRP α -IgG4 Fc) achieved PR by week 8 and CR by week 36 in patients with advanced R/R lymphoma, and a phase I clinical study (NCT03530683) for the dose-escalation and expansion trial of TTI-622 in patients with DLBCL is underway.

ALX148

ALX148, a fusion protein consisting of a CD47 blocker linked to an inactive human immunoglobulin Fc region, efficiently reduces ADCC and exerts anti-tumor activity combined with PD-L1 or CD20 antibodies.⁸⁵ A phase I/II trial (NCT05025800) is underway to find out the optimal dose, possible benefits, and side effects of ALX148 in combination with rituximab and lenalidomide for treating patients with DLBCL, FL, and refractory MCL. Another clinical phase I trial (NCT03013218) is ongoing for patients with lymphoma.

JMT601 (CPO107)

JMT601 is the first bi-specific SIRP α fusion protein in clinical development, with a synergistic targeted binding effect. It synergistically binds to CD20 and CD47 on lymphoma cells and induces strong ADCC and complement-dependent cytotoxicity (CDC).⁸⁶ Especially, no obvious binding of JMT601 to CD20-negative lymphoma cells and RBCs was observed, which would be an advantage in avoiding anemia. JMT601 was more effective in multiple human B-cell lymphoma models than conventional CD20-targeted antibodies. The first-in-human, phase I study (NCT04853329) recruited patients with advanced CD20-positive NHL to evaluate the safety, pharmacokinetics, and preliminary efficacy of JMT601.

The SIRP α fusion protein was developed as an alternative to CD47 antibody. It enhances phagocytosis of lymphoma cells and suppresses tumor growth by binding to CD47, and could induce the ADCC effect by activating NK cell Fc γ R.⁸⁷ In comparison to CD47 antibodies, it can activate more potent immune activation such as activation of macrophages and ADCC via the IgG1 Fc region, although the fusion protein has a lower affinity for blocking CD47. Meanwhile, SIRP α Fc fusion proteins have demonstrated considerable effects in clinical trials, but they still need to be combined with other therapeutic antibodies or chemotherapeutic drugs for lymphomas.

Small molecule inhibitors and peptides

Due to the widespread expression of CD47 in various types of cells, including red blood cells, the anti-CD47 antibodies may cause adverse reactions, such as anemia. In contrast, small-molecule compounds or peptides have certain advantages in some respects, such as shorter metabolic half-life and small molecular weight, making it easier for them to enter tumor cells and avoid erythrocyte toxicity. They block the CD47 axis through several mechanisms, including the disruption of the interaction between CD47-SIRP α and modulators of CD47 at the transcriptional, translational, and PTM levels (Fig. 3). Herein, we review small-molecule inhibitors and peptides studied in lymphoma (Table 3).

Small molecule inhibitors

JQ1, 4-methylumbelliferone, is a BET bromodomain inhibitor that can reduce the expression of CD47 in DHL/THL cells.⁸⁸ JQ1 can inhibit the proliferation of CTCL by inducing G1 phase stagnation, and down-regulation of c-myc expression. Additionally, intraperitoneal injection of JQ1 inhibits tumor growth in HH cell tumor-bearing SCID mice.⁸⁹

9-cis-retinoic acid (RA)

Dolcetti et al developed a dendritic cell (DC)-based vaccination protocol for aggressive and/or refractory lymphomas, which combines the interferon-conditioned DC (IFN-DC) with highly immunogenic tumor cell lysates (TCL) obtained from lymphoma cells undergoing immunogenic cell death.⁹⁰ RA combined with IFN α induced early membrane exposure of calreticulin, HSP70, and HSP90, down-regulation of CD47, and enhanced HMGB1 secretion in MCL and DLBCL cell lines *in vitro*. Furthermore, the RA/IFN α -TCL pulsed-DC vaccine inhibited lymphoma growth in immunodeficient NOD/SCID mice transplanted with human lymphoma Mino cells. This study proved that vaccine may be a new immunotherapeutic strategy for lymphoma.

QPCTL inhibitors

Glutamyl-peptide cyclotransferase-like protein (known as isoQC or QPCTL) was identified to regulate the PTM of CD47, and disturb CD47-SIRP α interaction.⁹¹ It catalyzes the cyclization of N-terminal glutamic acid and glutamine residues into a pyroglutamate residue (pGlu) on CD47

Table 3 Small molecule inhibitors and peptides used in lymphoma.

No.	Name	Type	Target	Tumor model	Administration route	Result	Reference
1	JQ1	Small molecule inhibitor	BET bromo-domain	CTCL cells; tumor-bearing SCID mice	i.p	Proliferation was inhibited	89
2	9-cis-retinoic acid (RA)	Small molecule inhibitor	Not known	MCL and DLBCL cell lines	<i>in vitro</i>	Down-regulation of CD47 was induced	90
				Human lymphoma tumor-bearing NOD/SCID mice	Vaccinated	Lymphoma growth was inhibited	
3	SEN177	Small molecule inhibitor	QPCTL	Raji lymphoma cells	<i>in vitro</i>	ADCP was enhanced	94
4	D4-2	Macrocyclic peptide	mouse SIRP α	Raji lymphoma tumor-bearing NOD/SCID mice	i.v	Anti-tumor response of rituximab was increased	98

Notes: i.p, intraperitoneal injection; i.v, intravenous injection.

(Fig. 2).⁹² Both genetic depletion and inhibitors such as SEN177 and PQ912 interference with QPCTL can block the binding of tumor CD47 to human SIRP α .^{93,94} The ADCP activity of human macrophages was enhanced after treatment with SEN177 in Raji lymphoma cells combined with anti-CD20 antibody *in vitro*.⁹⁴ Recently, it was reported that QPCTL genetic depletion can reshape myeloid infiltration to augment tumor immunity by limiting chemokine pGlu formation⁹⁵ and alter the tumor-infiltrating monocyte-to-macrophage ratio to promote tumor-specific immunity.⁹⁶ In particular, QPCTL is absent from mature RBCs, which may avoid side effects triggered by CD47-blocking.⁹⁷ Those studies indicate that treatment with QPCTL inhibitors is a promising strategy with strong clinical benefits for blocking the CD47-SIRP α axis in lymphoma therapy.

Peptides

D4-2, consisting of 15 amino acids, binds to the mouse SIRP α ectodomain and blocks the CD47-SIRP α interaction in an allosteric manner (Fig. 3). The IC₅₀ of D4-2 was 180 nM in disturbing the binding of mouse CD47-Fc to NOD SIRP α and the K_d value is 8.22 nM.⁹⁸ It had no obvious effect using D4-2 alone in inhibiting the growth of Raji cells, nevertheless, the ADCP effect on Raji lymphoma cells was significantly enhanced by combining D4-2 with rituximab *in vitro* and *in vivo*. Additionally, no adverse effects on hematologic and blood biochemical parameters in immunocompetent mice were observed after treatment with D4-2.⁹⁸

Conclusions and perspectives

Lymphoma is a hematological malignancy with complex terminology and variable clinical outcomes and continues to be an area of unmet need. Over the last two decades, our understanding of the landscape of HL and NHL has been significantly improved. Molecular and genetic therapeutics have been used in patients, including those with relapsed/refractory diseases. Treatments with agents that block the CD47-SIRP α axis have shown promising results in some lymphomas. This review systematically summarizes

preclinical and clinical therapeutic strategies using antibodies, fusion proteins, small molecule inhibitors, and peptides targeting the CD47-SIRP α axis in lymphoma patients. The different characteristics, advantages, and mechanisms of these strategies are also discussed. The CD47 antibody is commonly used in clinical trials for lymphomas, and its anti-tumor activity depends on the IgG subtype, which binds Fc γ R to active Fc-mediated functions. The fully human antibody has lower immunogenicity and higher safety than humanized antibody, but side effects such as anemia often occur. The SIRP α antibody is still in the early stages of lymphoma treatment, but SIRP α Fc fusion proteins have demonstrated considerable effects in clinical trials combined with tumor-specific antibodies. Small-molecule inhibitors and peptides have advantages such as easier entry into deep tissue and lower immunogenicity. In conclusion, as a promising immunotherapeutic strategy, the blockade of the CD47-SIRP α axis produces a significant anti-tumor effect and benefits lymphoma patients.

Author contributions

P. Zhao and L. Xie drafted and reviewed the manuscript. P. Wang and L. Yu conceived of the study, and reviewed and edited the manuscript. All authors read and agreed to the publication of the final manuscript.

Conflict of interests

The authors declare no conflict of interests.

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