# Progress in cancer research on the regulator of phagocytosis CD47, which determines the fate of tumor cells (Review)

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Received December 14, 2023; Accepted March 21, 2024

DOI: 10.3892/ol.2024.14389

**Abstract.** Cluster of differentiation 47 (CD47) is a transmembrane protein that is widely and moderately expressed on the surface of various cells and can have an essential role in mediating cell proliferation, migration, phagocytosis, apoptosis, immune homeostasis and other related responses by binding to its ligands, integrins, thrombospondin-1 and signal regulatory protein α. The poor prognosis of cancer patients is closely associated with high expression of CD47 in glioblastoma, ovarian cancer, breast cancer, bladder cancer, colon cancer and hepatocellular carcinoma. Upregulation of CD47 expression facilitates the growth of numerous types of tumor cells, while downregulation of its expression promotes phagocytosis of tumor cells by macrophages, thereby limiting tumor growth. In addition, blocking CD47 activates the cyclic GMP-AMP (cGAMP) synthase/cGAMP/interferon gene stimulating factor signaling pathway and initiates an adaptive immune response that kills tumor cells. The present review describes the structure, function and interactions of CD47 with its ligands, as well as its regulation of phagocytosis and tumor cell fate. It summarizes the therapeutics, mechanisms of action, research advances and challenges of targeting CD47. In addition, this paper provides an overview of the latest therapeutic options for targeting CD47, such as chimeric antigen receptor (CAR) T-cells, CAR macrophages and nanotechnology-based delivery systems, which are essential for future clinical research on targeting CD47.

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*Key words:* CD47, SIRPα, phagocytosis, macrophages, tumor immunotherapy

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## 1. Introduction

Cluster of differentiation 47 (CD47) expression levels are influenced by an organism's physiological state and cell type (1). Under normal physiological conditions, the expression level of CD47 has a vital role in maintaining homeostasis. For instance, young erythrocytes have high CD47 expression on their surface. By contrast, senescent erythrocytes have low CD47 expression on their surface, which allows macrophages to eliminate CD47 for erythrocyte renewal (2). As previously reported, binding different ligands to CD47 also results in different biological effects. For example, CD47 binds to signal regulatory protein α (SIRPα) to activate a signaling pathway that inhibits phagocytosis and the killing of tumor cells by macrophages in the tumor microenvironment (TME) by modulating the immune response (3). Under pathological conditions, CD47 is highly expressed in hematological tumors, and by binding to its ligand SIRPα, CD47 transmits a series of inhibitory signals to macrophages; consequently, the phagocytosis of tumor cells by macrophages is prevented (4). The expression level of CD47 and blockade of the signaling pathway activated by CD47 also significantly impact the fate of tumor cells, and the upregulation or downregulation of CD47 expression and blockade of CD47 signaling can determine the fate of tumor cells. Blocking CD47 signaling can also determine the survival of tumor cells. In recent years, blocking CD47 has emerged as a potential therapeutic strategy for tumor immunotherapy (5), and immunotherapies targeting CD47 have achieved significant success in certain cancer patients. However, remission rates vary; not all individuals benefit

from current treatments (6). Various drugs targeting CD47, such as monoclonal antibodies (mAbs), SIRPα fusion proteins (SIRPα-Fc), bispecific antibodies (BsAbs), small-molecule inhibitors and nanotechnology-based delivery systems, are being developed (7,8). Preclinical studies and early clinical trials have demonstrated that CD47-targeted therapies have a promising future for application. In addition, CD47-targeted therapies have potential limitations and challenges, including adverse reactions such as anemia and thrombocytopenia, as well as resistance to drugs (9).

## 2. CD47

In 1990, Brown *et al* (10) first identified CD47 as a cell surface protein associated with αvβ3 integrins in placenta and platelets. This protein was subsequently shown to regulate integrins and leukocyte responsiveness to extracellular matrix proteins; hence, it was named integrin-associated protein (IAP) (11,12).

CD47 has a molecular weight of 45-55 kDa and is a member of the immunoglobulin (Ig) superfamily (IgSF) (4,13,14). Its molecular structure includes one N-terminal extracellular IgG-like domain, five highly hydrophobic transmembrane segments and one hydrophilic cytoplasmic tail at the C-terminus. Hatherley *et al* (15,16) investigated the crystal structure of the IgG structural domain. They showed that the structure of CD47 has a typical IgV-like fold and is similar to that of myelin oligodendrocyte glycoprotein. CD47 mediates vascular smooth muscle cell (VSMC) proliferation and migration (17), as well as platelet activation and spreading (18), and recruits granulocytes and T cells to the site of infection (11).

Although an existing review (19) has summarised the structure and function of CD47, a description of the structure and function of CD47 isoforms is lacking. This review refers to previous studies reporting that CD47 has four traditional isoforms in human cell lines and tissues, all of which have different amino acid lengths in the cytoplasmic tail (19). In a recent study, sequence analysis of cDNA cloned from human skeletal muscle revealed that, in addition to the four traditional isoforms, human CD47 has a new isoform 5, which features an entirely different cytoplasmic tail amino acid length and amino acid sequence compared with the four traditional isoforms (20,21).

The distribution and functions of different isoforms within tissues vary. bladder, ovarian and breast cancer cells are examples of keratinocytes and tumor cells expressing type 1 CD47 (22,23). The most extensively expressed form, type 2 CD47, is mainly involved in signaling between astrocytes and is primarily expressed in hematopoietic cells, epithelial cells and vascular endothelial cells. Neuronal, testicular and intestinal mucosal cells explicitly express type 3 and 4 CD47 (24). Types 3 and 4 are thought to be closely related to memory mechanisms because their expression is markedly elevated in the brains of rats with good memory (25,26). Protein linking IAP with cytoskeleton 1 (PLIC-1) cytoplasmic protein regulates cyclic adenosine monophosphate (cAMP) signaling by CD47 by binding to the cytoplasmic tails of types 2 and 4, recruiting heterotrimeric G proteins to CD47 (27), inhibiting chemotactic signaling induced by the Gi-coupled receptor C-X-C motif chemokine receptor 4 (28) and activating the PI3K/Akt pathway in astrocytomas (29). More research is required to determine the functional distinctions between the cytoplasmic tails of the various CD47 isoforms, as the studies on this aspect of CD47 isoforms have been minimal in recent years, resulting in a limited understanding of the regulatory mechanisms and roles of the cytoplasmic tails of different isoforms of CD47.

In addition, certain cells can adapt to various physiological and pathological changes by switching their subtype, e.g., Reinhold *et al* (23) used PCR to detect mRNA expression and found that primary mouse endothelial cells cultured *in vitro* predominantly expressed CD47 type 2 mRNA, and endothelial cells transformed with intermediate T antigen expressed all four types of mRNA. However, certain researchers dispute this; for instance, Mateo *et al* (30) observed no change in the expression of CD47 isoforms. These studies indicate that the role of the CD47 types in tumorigenesis and development and the mechanism of interconversion require further investigation.

## 3. CD47 receptors

CD47 receptors include integrin, thrombospondin-1 (TSP-1) and SIRPα. Based on published reviews, it may be summarized that CD47 affects multiple biological functions of target cells by binding to these ligands (31,32). In addition, the gene expression of the three ligands of CD47 under physiological conditions and in tumors may be summarized through the GEPIA database (http://gepia.cancer-pku.cn/index.html) and the Human Protein Atlas (https://www.proteinatlas.org/), as elaborated below.

*Interaction with integrins*. Integrins are transmembrane ligands that bridge the gap between cells and the extracellular matrix and regulate signaling processes such as the cell cycle, morphology and motility (26,32,33). CD47 was initially found to interact with αvβ3 intergrin, hence the designation IAP. Under normal physiological conditions, αvβ3 integrins are mainly expressed in cardiomyocytes, oligodendrocytes and astrocytes, while under pathological conditions, they may be widely expressed mainly in cancers, such as glioblastoma, esophageal, thyroid and pancreatic cancers. The CD47-integrin complex may activate multiple heterotrimeric G proteins by linking IAP to PLIC-1, thereby inducing CD47 to activate cAMP signaling (34). Lindberg *et al* (35), through a study using a CD47-deficient human cell line, showed that CD47 is required for αvβ3 integrin-mediated binding of hyaluronan to encapsulated microbeads. In addition to αvβ3 integrin, CD47 binds to αIIbβ3 integrin and induces platelet aggregation and increased adhesion spot kinase tyrosine phosphorylation (18). In addition, CD47 binds to α4β1 integrin and mediates reticulocyte adhesion (36); CD47 binds to α5β1 integrin and is involved in chondrocyte mechanotransduction (37); and CD47 binds to α6β1 integrin and has a role in fibrillar β-amyloid-mediated activation and phagocytosis of microglia (38).

*Interaction with TSP*. TSP is an extracellular matrix calcium-binding glycoprotein that is highly expressed on monocytes, mucus cells and macrophages under normal physiological conditions and is widely expressed in cancers, such

as breast adenocarcinoma carcinoma, lung adenocarcinoma, pancreatic adenocarcinoma and gastric adenocarcinoma, mainly under pathological conditions. There are currently five known isoforms of TSP, i.e., TSP-1-5 (39). TSP-1 is the first identified endogenous ligand of CD47 and it has a variety of biological functions, including the inhibition of angiogenesis, activation of transforming growth factor-β and participation in tissue repair (40). Protein-related studies have shown that TSP-1 binds to the CD47 extracellular IgV structural domain through its C-terminal structural domain peptide 4N1K and has a role in several biological processes, including inflammation, immune response, cell proliferation, apoptosis, adhesion and migration (41). The mechanism of CD47-TSP-1 interaction has not been studied in detail because the crystal structure of the CD47-TSP-1 complex still needs to be clarified. Early experiments have shown that CD47 affects signaling through heterotrimeric Gi proteins in a pertussis toxin-sensitive manner (28), thereby modulating TSP-1-induced cell spreading and platelet activation. Isenberg *et al* (42) measured cGMP levels by immunoassay, indicating that binding of CD47 to TSP-1 inhibits nitric oxide signaling in endothelial and VSMCs, thereby promoting platelet aggregation. To date, we have found that CD47-TSP-1 expression serves as a marker for predicting patient response to immune checkpoint blockade therapy, but there is no targeted therapy for the CD47-TSP-1 axis. It is hypothesized that this may be because CD47 has little effect on the adaptive immune response through its interaction with TSP-1, and therefore, blocking the CD47-TSP-1 axis has little clinical therapeutic significance. However, a novel immunotherapeutic drug, TAX2 peptide, which acts as an orthosteric antagonist of the interaction between TSP-1 and CD47, has shown a good safety profile in mouse models of ovarian cancer and is effective in killing tumor cells (43).

*Interaction with SIRPα*. SIRPα, the ligand with the highest affinity for CD47, is a member of the SIRP family and was first identified by Kharitonenkov *et al* (44) in the 1990s. Belonging to the IgSF, under normal physiological conditions, SIRPα is extensively expressed on the surface of cells such as monocytes, macrophages, neutrophils, dendritic cells (DCs) and microglia. Under pathological conditions, it is widely expressed in cancers such as glioblastoma, melanoma, renal cancer and head and neck cancer (45,46).

The intracellular region of SIRPα contains four tyrosine phosphorylation sites and two immunoreceptor tyrosine inhibition motifs (ITIMs), and the extracellular region has three IgSF structural domains, namely, one N-terminal IgV-like domain and two C-like domains (47,48). The crystal structure of the N-terminal IgV-like domain of SIRPα suggested an IgV-like fold and four-loop structure (BC, CD, DE and FG loops) with an overall structure similar to that of the T-cell receptor (16,49).

SIRPα binds to CD47 through its N-terminal FG and BC loop, thus forming a highly entangled, well-fitted complex structure (15). The long disulfide bond between Cys33 of the IgV structural domain and Cys263 of the transmembrane structural domain in CD47 is essential for enhancing binding to SIRPα (50-52). According to X-ray computational crystallography calculations and analysis, when CD47 interacts with SIRPα, the total distance between the two cell types approximates the entire distance of the immune synapse (~14 nm) (53). Therefore, the binding of SIRPα to CD47 may occur via an antigen receptor rather than through the usual cell-cell structural domain binding interaction (16).

The binding of SIRPα to CD47 promotes the phosphorylation of the intracellular region of the ITIM (15,47). Phosphorylated ITIM recruits and activates Src homology region 2 (SH2)-containing tyrosine phosphatase-1 (SHP-1) and SHP-2 (54), which affects cytoskeletal function by inactivating motor myosin IIA (55), thereby blocking tyrosine phosphorylation-dependent signaling pathways and limiting phagocytosis by macrophages and others (47). Although SHP-1 and SHP-2 are typically inactive, phosphorylated ITIM recruits the SH2 structural domain to the cell membrane, and a change in its conformation activates SHP-1 and SHP-2. SHP-1 is present mainly in hematopoietic and epithelial cells and is selectively expressed in myeloid cells, which function as a negative regulator of phagocytosis. By contrast, SHP-2 is widely expressed and promotes cell proliferation, growth and migration mainly by regulating the GTP-binding proteins RAS and Rho (26).

CD47-SIRPα interactions not only regulate the maintenance of lymphocyte homeostasis (56), DC maturation and activation (57), the correct localization of DC subpopulations in sub-lymphoid organs and cell migration (58) but also have an essential role during remodeling of the nervous system and bone tissues (59). The cellular responses regulated by CD47-SIRPα interactions depend upon bidirectional signaling between CD47 and SIRPα: CD47 on host cells acts as a 'self-tag' (60) and regulates phagocytosis by binding to SIRPα. How this regulates phagocytosis will be further discussed in a later section.

## 4. CD47 and SIRPα: Bidirectional regulation of the immune system

Complex cellular communication systems in multicellular organisms have evolved to ensure adequate intercellular communication, which is crucial for cell differentiation, tissue and organ formation, individual development in multicellular organisms and immune function regulation (61).

The interaction between CD47 and SIRPα constitutes an intercellular communication system whose role in regulating immune system function is bidirectional (62). The CD47-SIRPα signaling pathway negatively regulates DC activation. The fusion protein of CD47, when bound to SIRPα, inhibits the phenotype and function of immature DCs and the production of cytokines by mature DCs (63). However, considering its role in antigen presentation, SIRPα has a positive regulatory effect. SIRPα is abundantly expressed on the surface of mature DCs. When the immune system responds to pathogens, SIRPα helps DCs present relevant antigens to T cells and costimulatory molecules associated with initiating T cells, thus promoting T-cell activation and proliferation (1,19).

## 5. CD47-SIRPα regulates phagocytosis

Phagocytosis is the process by which tissue cell debris and apoptotic cells are engulfed and digested, and this process helps maintain a stable balance in the body's internal environment. CD47 has a vital role in regulating phagocytosis. This

regulatory function is mediated by binding to the inhibitory receptor SIRPα on phagocytes to activate the CD47-SIRPα signaling pathway. CD47 binds to SIRPα and sends an inhibitory 'do not eat me' signal to phagocytes, thus limiting phagocytosis (3,64) (Fig. 1).

The most characterized function of the CD47-SIRPα signaling pathway *in vivo* is the clearance of senescent and apoptotic erythrocytes. Okazawa *et al* (65) reported that the primary site of erythrocyte macrophage clearance is the red pulp of the spleen, suggesting that erythrocyte clearance is mediated by splenic red pulp macrophages. SIRPα is abundant in these macrophages and Ishikawa-Sekigami *et al* (66) demonstrated that erythrocyte clearance was significantly increased in SIRPα mutant mice injected with normal erythrocytes. This increase is because the mutated form of SIRPα expressed by SIRPα mutant mice cannot bind to SHP-1 or SHP-2 due to the lack of cytoplasmic domains, and SIRPα fails to exert an inhibitory effect on the CD47-SIRPα signaling pathway. As a result, the phagocytosis of red blood cells by splenic red pulp macrophages is enhanced (65). This phenomenon is observed not only in erythrocytes but also in platelets. Previous studies have demonstrated that SIRPα mutant mice lacking the cytoplasmic structural domains exhibit thrombocytopenia in the SIRPα mutant mouse model and clear platelets from the blood of the mutant mice at a more rapid rate when compared to wild-type mice (13,67).

CD47-SIRPα signaling also has an essential regulatory role in hematopoietic stem cell (HSC) transplantation. HSCs upregulate CD47 expression to protect themselves from phagocytosis by macrophages, thus achieving successful implantation (68,69). In general, the CD47 of one species has little interaction with the SIRPα of another species. However, higher-polymorphism SIRPα on macrophages was observed in a nonobese diabetic (NOD)-severe combined immunodeficiency xenograft mouse model when compared with other mouse lines. These cells have an exceptionally high affinity for human CD47, even higher than the mouse-mouse or human-human affinity of CD47 and SIRPα (70,71). Theocharides *et al* (72) demonstrated that implantation of normal human HSCs in NOD mice was also dependent on the interaction of human CD47 with SIRPa in NOD mice by implanting HSCs into a NOD mouse model. These studies demonstrated that the interaction between CD47 on human HSCs and SIRPα on macrophages is critical for the successful implantation of HSCs. In addition, human SIRPα is polymorphic and each polymorphic variant has a different affinity for human CD47 *in vitro*. This finding suggested that the human SIRPα polymorphism is critical for successfully implanting HSCs (73,74).

The 'do not eat me' signal from the CD47-SIRPα signaling pathway is also used to maintain homeostasis in body. The body must remove various cells, including those that are overproduced, damaged or aged. One removal mechanism is apoptosis, through which macrophages clear apoptotic cells precisely and efficiently. This mechanism is a key 'do not eat me' signal from CD47 that occurs on the surface of healthy cells, and binds to SIRPα inhibitory receptors on macrophages to prevent them from being eaten by macrophages. CD47 expression on the surface of apoptotic cells is downregulated, thereby attenuating the inhibitory signal generated by CD47 binding to SIRPα. By contrast, low IgG or C3b opsonization levels can cause the phagocytosis of apoptotic cells by macrophages (75).

## 6. CD47 signaling regulates tumor cell fate

Malignant tumors like glioblastoma, acute lymphoblastic leukemia, as well as ovarian, breast, gastric and lung cancers express high levels of CD47 (76-78). Liu *et al* (79) used flow cytometry to detect the expression of CD47 in isolated primary lung cancer cells and adjacent normal cells and the results showed that the expression level of CD47 in tumor cells was higher than that in normal cells. There were apparent differences between subtypes of lung cancer, with the highest expression of CD47 in small-cell lung cancer, followed by lung adenocarcinoma, and the lowest in lung squamous carcinoma (79). Furthermore, compared to normal myeloid cells from healthy individuals, acute myeloid leukemia (AML) and chronic myeloid leukemia cells expressed higher levels of CD47. Furthermore, a positive association was found between high levels of CD47 expression and poor treatment response and patient prognosis (80). A study confirmed that CD47 mRNA and protein levels were higher in leukemic stem cells of patients with AML than in normal healthy stem cells (81). In a study on Epstein-Barr virus (EBV)-associated gastric cancer (EBVaGC), the expression of CD47 in EBVaGC was higher than that in EBV-negative gastric cancer tissue samples, which also indicated that high expression of CD47 was associated with poor prognosis in EBVaGC (82). Yu *et al* (83) detected the expression of CD47 in ovarian cancer tissues by immunohistochemistry, which showed that the prognosis of patients with low expression of CD47 was better than that of patients with high CD47 expression. The above studies indicate that CD47 expression levels are closely related to the prognosis of patients with cancer.

Recent research has demonstrated that controlling the expression of CD47 in tumor cells and inhibiting the signaling pathway that CD47 activates have crucial regulatory roles in determining the fate of tumor cells. The mechanisms of action include the following: i) Upregulation of CD47 expression, which binds to the macrophage surface receptor SIRPα and transmits the 'do not eat me' signal to promote phagocytosis of tumors by macrophages; ii) blockade of CD47 enhances the phagocytosis of tumor cells by DCs and promotes antigen delivery from DCs to T lymphocytes, initiating an antitumor adaptive immune response; iii) blockade of CD47 is capable of clearing tumor cells through natural killer cell-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) to clear tumor cells; iv) blockade of CD47 also activates the apoptotic pathway and directly induces apoptosis in tumor cells.

The expression level of CD47 is regulated by transcription factors such as nuclear factor κB (NF-κB), the MYC oncogene and hypoxia-inducible factor-1 (HIF-1), which regulate the phagocytosis of tumor cells by upregulating or downregulating the expression of CD47 (4). In a T-cell acute lymphoblastic leukemia xenograft model, MYC directly binds to the CD47 promoter and upregulates its expression, thus promoting the growth of tumor cells. By contrast, inactivation of MYC downregulates the expression of CD47 and

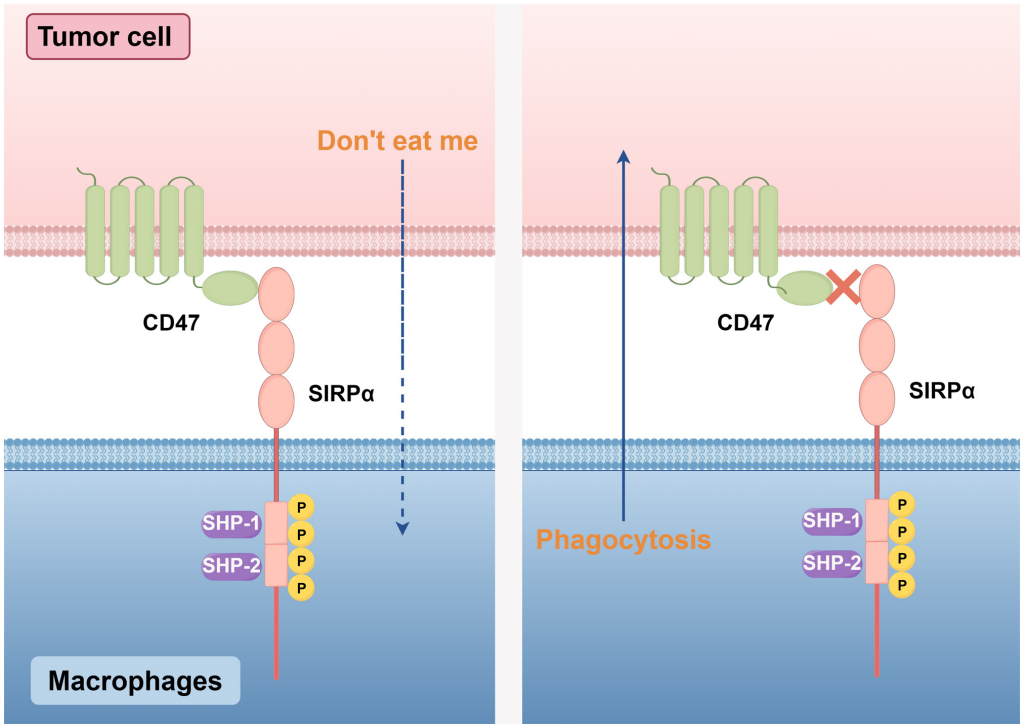


Figure 1. Regulation of phagocytosis by the CD47-SIRPα signaling pathway. SIRPα is the receptor with the highest affinity for CD47. When CD47 on the surface of tumor cells binds to SIRPα, an inhibitory receptor on macrophages, it sends a 'do not eat me' signal to macrophages, preventing immune surveillance. When the CD47-SIRPα signaling pathway is blocked, phagocytosis of tumor cells by macrophages is promoted (schematic generated with figdraw). CD47, cluster of differentiation 47; SIRPα, signal regulatory protein α; SHP-1, Src homology region 2-containing tyrosine phosphatase-1; P, phosphate.

enhances macrophage infiltration and phagocytosis, thereby inhibiting tumor cell growth (84). In addition, activated NF-κB directly binds to specific enhancer components of CD47 and upregulates CD47 expression in breast cancer cells, thereby promoting tumor growth (85). In a clinical analysis of thousands of patients with breast cancer, Zhang *et al* (78) reported a strong correlation between CD47 and HIF-1. Under hypoxic conditions, HIF-1 binds to the CD47 promoter and upregulates its expression, thereby inhibiting the phagocytosis of breast cancer cells (84). In addition, ERK signaling inhibits tumor-cell phagocytosis by activating nuclear respiratory factor-1 and upregulating CD47 expression in melanoma cells. Conversely, microRNA (miRNA)-mediated downregulation of CD47 expression promotes tumor-cell phagocytosis. MiR-708 is inversely associated with CD47 expression and its binding to the 3'-untranslated region of CD47 induces tumor-cell phagocytosis by suppressing CD47 expression (86). In multiple myeloma, CD47 expression on the surface of myeloma cells can be inhibited by upregulating the expression of the tumor suppressor gene miRNA-155, thereby inducing phagocytosis of tumor cells by macrophages (87) (Fig. 2).

Reputable reviews (64,88) have shown that transcription factors, oncogenes and miRNAs may control CD47 expression in tumor cells; however, regulation of CD47 expression occurs in the tumor immune microenvironment and the immune response of other cell types. A recent study found that blocking CD47-SIRPα signaling enhances antitumor immune responses (89). Tumor DNA in DCs activates the cell membrane DNA sensor cGMP-AMP (cGAMP) synthase (cGAS), which subsequently exerts potent antitumor effects by binding to the second messenger cGAMP and activating interferon gene stimulating factor (STING) (89). By contrast, CD47 inhibits this signaling pathway and aids in the immune escape of tumor cells. For instance, in treating glioblastoma, blocking CD47 not only enhances DC phagocytosis but also promotes the initiation of the adaptive immune response by T cells by activating the cGAS-cGAMP-STING signaling pathway (90). In addition, a study by Xu *et al* (91) found that in mouse models of colon cancer, lymphoma and melanoma, blocking CD47-SIRPα signaling activates NADPH oxidase in DCs to inhibit the degradation of tumor-derived mitochondrial DNA (mtDNA), which leads to an increase in the level of mtDNA and its recognition by cGAS in the cytoplasm of DCs. As a result, the cGAS-cGAMP-STING signaling pathway is activated, which releases interferon-γ to initiate the CD8+ T-cell mediated adaptive immune response, thereby killing tumor cells (47); i.e., the tumor-killing effect of T cells is dependent on the blockade of the CD47-activated cGAS-cGAMP-STING signaling pathway (4) (Fig. 3).

In addition, blocking CD47 induces tumor cell death only when endogenous activation signals are present (64). A study by Chen *et al* (92) revealed the presence of an endogenous activation signal on the surface of tumor cells called SLAM family member 7 (SLAMF7), a prophagocytic signal of SLAMF7, which is a prophagocytic 'eat-me' signal and usually interacts with the macrophage-1 antigen, promoting the phagocytosis of tumor cells by macrophages. Furthermore, they contended that CD47-mediated phagocytosis requires SLAMF7. However, He *et al* (93) refuted this view by finding that phagocytosis was also effectively induced in SLAMF7-negative diffuse large B-cell lymphomas cells after they blocked CD47 by the CD47 antibody Inhibrix. Further studies are needed to determine whether SLAMF7 is required to mediate CD47.

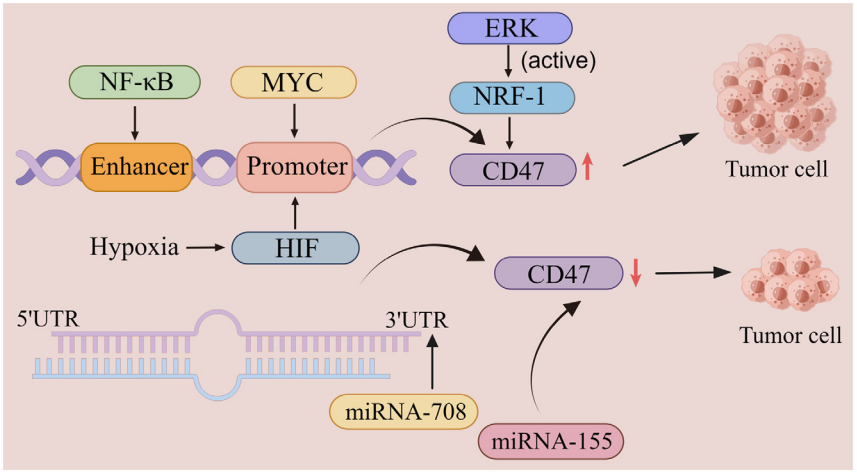


Figure 2. CD47 expression levels regulate tumor cell fate. Multiple transcription factors regulate CD47 expression levels. Nuclear factors bind to specific enhancers to promote CD47 expression. MYC and HIF enhance CD47 expression by directly binding to promoters, ERK signaling activates NRF-1 and upregulates CD47 expression, and upregulation of CD47 expression promotes tumor cell growth. By contrast, miRNA-708 and miRNA-155 promote phagocytosis of tumor cells by downregulating CD47 expression (schematic generated with figdraw). NF-κB, nuclear factor κB; HIF, hypoxia-inducible factor; NRF-1, nuclear respiratory factor-1; miRNA, microRNA.

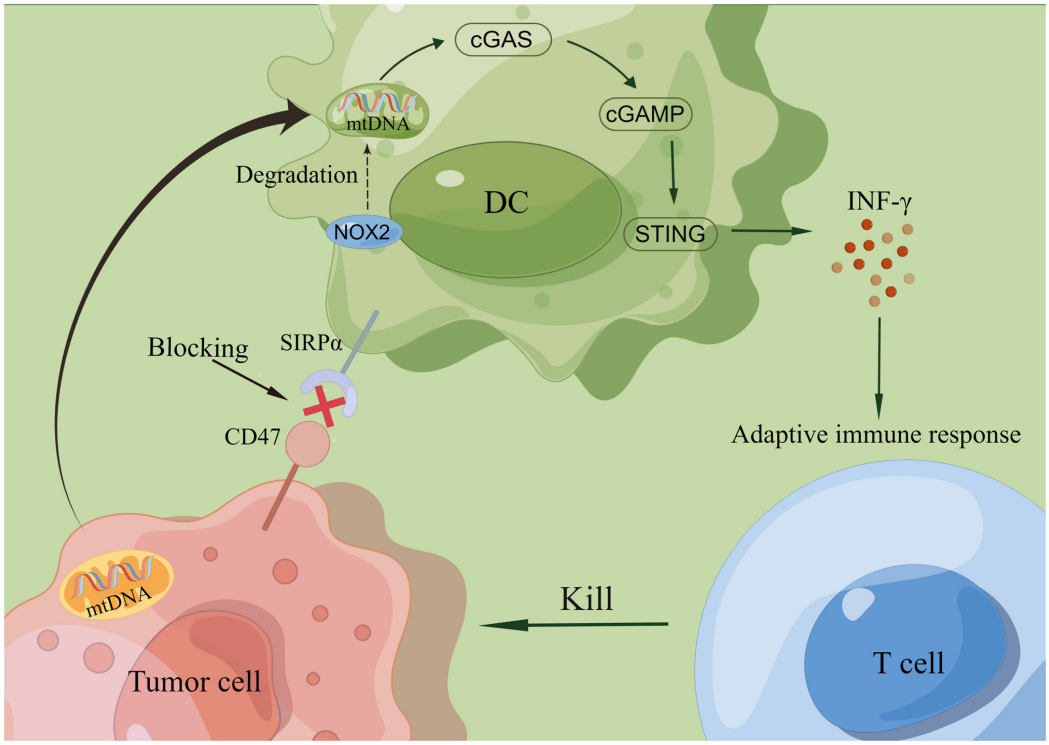


Figure 3. Blockade of the CD47-SIRPα signaling pathway initiates an adaptive immune response to kill tumor cells. Blockade of the CD47-SIRPα signaling axis inhibits the degradation of tumor-derived mtDNA through the activation of NOX2, which, once in the cytoplasm of the DC, is recognized by cGAS, further activating the cGAS-cGAMP-STING signaling pathway to release INF-γ, thus promoting T cells to initiate adaptive immune response and kill tumor cells (schematic generated with figdraw). mtDNA, mitochondrial DNA; DC, dendritic cell; NOX, NADPH oxidase; cGAMP, cyclic GMP-AMP; cGAS, cGAMP synthase; INF, interferon; STING, INF gene stimulating factor.

## 7. Therapeutic strategies targeting CD47

Clinical research has shown that CD47 is an intrinsic immune checkpoint with high clinical development value and promising application prospects. Numerous domestic and foreign companies are actively developing drugs targeting CD47, particularly mAbs, BsAbs, fusion proteins and small-molecule antibodies, and many of them have already entered the clinical research stage. A search of the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) and online open resources from the US National Clinical Trials Registry system (www.clinicaltrials.gov) was performed as part of the present review. Compared with previously published reviews (48,94,95), not only the names, structures and clinical trials of various CD47-targeted representative drugs were summarized, but the current state of clinical research and the results of clinical trials were also outlined (Table I).

Table I. Summary of representative CD47-targeted drugs in clinical trials.

| Drug name | Format | Target | Forms of treatment | Indications | Phase | Start date | Status | NCT no. |
|---|---|---|---|---|---|---|---|---|
| Magrolimab (Hu5F9-G4) | CD47 mAb | CD47 | Monotherapy | Solid tumor | I | November 2, 2016 | Completed | NCT02953782 |
| | | | Monotherapy | AML, MDS | I | November 2015 | Completed | NCT02678338 |
| | | | Obinutuzumab | Follicular lymphoma | I | December 16, 2021 | Active, not recruiting | NCT04599634 |
| | | | Avelumab | Ovarian cancer | I | May 23, 2018 | Completed | NCT03558139 |
| | | | Rituximab | R/R B-NHL | I/II | November 21, 2016 | Active, not recruiting | NCT02953509 |
| Lemzoparlimab (TIJC4) | CD47 mAb | CD47 | Pembrolizumab, Rituximab | Solid tumor/ lymphoma | I | April 16, 2019 | Completed | NCT03934814 |
| | | | Monotherapy | AML, MDS | I/II | March 25, 2020 | Completed | NCT04202003 |
| | | | Toripalimab | R/R advanced solid tumor | I/II | December 30, 2021 | Terminated | NCT05148533 |
| | | | Dexamethasone, Pomalidomide, Daratumumab | Multiple myeloma | I | January 17, 2022 | Terminated | NCT04895410 |
| Ligufalimab (AK117) | CD47 mAb | CD47 | Monotherapy | Malignant neoplasms | I | April 23, 2020 | Completed | NCT04349969 |
| | | | Azacitidine | AML | I/II | August 13, 2021 | Recruiting | NCT04980885 |
| | | | Azacitidine | MDS | I/II | June 18, 2021 | Recruiting | NCT04900350 |
| AO-176 | CD47 mAb | CD47 | Monotherapy, Paclitaxel, Pembrolizumab | Solid tumor | I/II | February 4, 2019 | Completed | NCT03834948 |
| | | | Dexamethasone, Bortezomib | R/R multiple myeloma | I/II | November 30, 2020 | Completed | NCT04445701 |
| CC-90002 | CD47 mAb | CD47 | Monotherapy | AML, MDS | I | March 1, 2016 | Terminated | NCT02641002 |
| | | | Rituximab | Hematological cancer | I | March 12, 2015 | Completed | NCT02367196 |
| TII-621 | SIRPa-Fc mAb | CD47 | Monotherapy, Rituximab and Nivolumab | Hematologic malignancies | I | January 28, 2016 | Terminated | NCT02663518 |
| | | | Monotherapy, PD-1/PD-L1 inhibitor | R/R solid tumors | I | September 2016 | Terminated | NCT02890368 |
| | | | Daratumumab Hyaluronidase-fihj | Multiple myeloma | I | October 28, 2021 | Active, not recruiting | NCT05139225 |
| TII-622 | SIRPa-Fc mAb | CD47 | Monotherapy, Rituximab, PD-1 inhibitor, proteasome-inhibitor regimen | Hematological malignancies | I | June 7, 2018 | Active, not recruiting | NCT03530683 |

Table I. Continued.

| Drug name | Format | Target | Forms of treatment | Indications | Phase | Start date | Status | NCT no. |
|---|---|---|---|---|---|---|---|---|
| Evorpacept (ALX-148) | SIRPa-D1 mAb | SIRPa | Monotherapy, Pembrolizumab, Trastuzumab, Rituximab | Solid tumor | I | February 3, 2017 | Active, not recruiting | NCT03013218 |
| | | | Lenalidomide, Rituximab | Indolent and aggressive B-NHL | I/II | October 13, 2021 | Recruiting | NCT05025800 |
| | | | Azacitidine | Higher-risk MDS | I/II | February 2020 | Active, not recruiting | NCT04417517 |
| | | | Venetoclax, Azacitidine | AML | I/II | May 5, 2021 | Active, not recruiting | NCT04755244 |
| IMM-306 | BsAb | CD47, CD20 | Rituximab | B-cell NHL | I | January 15, 2021 | Suspended | NCT04746131 |
| IBI-322 | BsAb | CD47, PD-L1 | Azacitidine | Advanced solid tumor | I | July 21, 2021 | Completed | NCT04912466 |
| | | | | Hematologic malignancy | I | May 7, 2021 | Recruiting | NCT04795128 |
| | | | | Myeloid tumor | I | December 28, 2021 | Terminated | NCT05148442 |
| HX009 | BsAb | CD47, PD-L1 | | Advanced solid tumor | I | June 12, 2019 | Completed | NCT04097769 |
| | | | | R/R lymphoma | I/II | December 31, 2021 | Unknown | NCT05189093 |
| NI1701 | BsAb | CD47, CD19 | Rituximab | B-cell lymphoma | I | March 5, 2019 | Active, not recruiting | NCT03804996 |
| SL-172154 | BsAb | SIRPa-Fc, CD40L | | Ovarian cancer | I | June 29, 2020 | Completed | NCT04406623 |

NCT, US National Clinical Trials Registry (www.clinical trials.gov); mAb, monoclonal antibody; CD47, cluster of differentiation 47; SIRPa-Fc, signal regulatory protein α fusion proteins; BsAb, bispecific antibodies; AML, acute myeloid leukemia; MDS, myelodysplastic syndromes; PD-1, programmed cell death 1; PD-L1, PD-1 ligand 1; R/R, relapsed/refractory; B-NHL, B-cell non-Hodgkin's lymphoma.

*mAbs targeting CD47 or SIRPα*. Immunotherapies targeting CD47 can be divided into two categories: First, blocking or inhibiting the 'do not eat me' signal with SIRPα via antibodies to promote the phagocytosis of tumor cells by macrophages (96); second, the activation of innate and adaptive immune responses. Tumor cells are recognized, taken up by antigen-presenting cells (APCs) and delivered to the initial T cells, activating T cells. T cells activate when APCs identify, pick up and transfer tumor cells to initial T cells. Antibodies targeting CD47 can kill tumor cells by inhibiting protein kinase A (97,98).

Closure of CD47 on tumor cells using mAbs targeting CD47 or soluble SIRPα-Fc structures triggers macrophage antibody-dependent cellular phagocytosis *in vitro*. It significantly promotes the killing of tumor cells (99). In addition, the CD47-targeted fusion protein SIRPαD1-Fc was found to inhibit the Akt/mTOR signaling pathway, upregulate reactive oxygen species production and promote autophagy in non-small cell lung cancer cells, thereby enhancing the antitumor effect (100).

More than 10 antibodies targeting CD47 have entered clinical trials (Table I), among which Magrolimab (Hu5F9-G4) was the first CD47 antibody to enter clinical trials and is already in clinical trials for various types of cancers, including AML, myelodysplastic syndromes (MDS) and solid tumors (45,101,102).

Furthermore, CD47-targeting antibodies can synergize with various mAbs, and combining the two can provide a better antitumor effect. Commonly used combinations include combination therapy with other therapeutic antibodies, chemotherapy or radiation therapy. A phase I clinical study revealed that the combination of a CD47 mAb and Rituximab resulted in an objective response rate (ORR) of 40% and a complete response rate (CRR) of 33% in patients with diffuse large B-cell lymphoma, with an ORR of 71% and a CRR of 43% in patients with follicular lymphoma (45).

In addition, several clinical trials have evaluated the safety and efficacy of CD47-targeted drugs in different stages and types of tumors. For instance, Lemzoparlimab (TJC4), which targets CD47, was screened using a phage display system. A phase I clinical trial is evaluating the efficacy effects of TJC4 alone or in combination with Pembrolizumab or Rituximab in the treatment of relapsed or refractory (R/R) advanced solid tumors and lymphomas (103). The results of the preclinical study demonstrated a favorable safety profile and clinical efficacy in five patients with AML and high-risk MDS who had received at least two treatments. Of particular note, one patient with R/R AML achieved a morphologic leukemia-free status after treatment with TJC4 (104). Humanized CD47 antibody Ligufalimab (AK117) is an anti-CD47 mAb with a unique structure, which not only has anti-tumor effects but also eliminates erythrocyte agglutination and significantly reduces phagocytosis of erythrocytes by macrophages. Phase I trials have been completed in Australia and phase II trials are underway in China and Australia. Results from a clinical trial enrolling 15 patients with advanced solid tumors showed that AK117 was safe and well tolerated, with no infusion- or treatment-related adverse effects observed (105). AO-176, a mAb targeting CD47, is being evaluated in a phase I clinical trial for treating R/R multiple myeloma (106,107). AO-176 binds preferentially to tumor cells (rather than normal cells), can bind tumor cells more efficiently in an acidic microenvironment and can kill tumor cells directly in a cell-autonomous manner (108). Current clinical data show that of the 27 patients treated with AO-176, one patient with endometrial cancer did not respond to its treatment regimen and seven patients had the best response of stable disease (SD) (109). CC-90002 is the first generation of humanized CD47 antibody to enter clinical studies that block CD47-SIRPα binding to achieve the killing of hematological tumor cells (96). Clinical trials for AML and MDS revealed that CC-90002 had poor efficacy and safety, which led to its forced discontinuation. Researchers restarted clinical trials after improving the CC-90002 treatment regimen and safety (110,111). In a mouse transplantation tumor model of multiple myeloma, CC-90002 showed significant dose-dependent antitumor activity. In addition, in non-primate animals, CC-90002 exhibited favorable pharmacokinetic properties and toxicity (96). TTI-621 and TTI-622 are SIRPs-Fc fusion proteins that have been used in the treatment of hematologic malignancies, solid tumors and mycosis fungoides (112), and such agents are currently being evaluated in a phase I clinical trial for R/R B-cell lymphomas (113). In 164 patients with B-cell non-Hodgkin's lymphoma (B-NHL), TT-621 plus rituximab was used to treat the disease in a phase I trial. The study showed that TT1-621 was well tolerated and that monotherapy is a promising therapeutic option. The ORR for all patients treated with TTI-621 monotherapy was 13%, while it was 29% for diffuse large B-cell lymphoma and 25% for T-cell NHL (113). Clinical studies of TTI-622 in patients with advanced R/R lymphomas showed that one patient with non-growth center B cells who had received five prior therapies achieved partial remission (PR) at week 8 and overall response at week 36 (114). The SIRPs-Fc fusion protein Evorpacept (ALX148) is presently undergoing evaluation in several programs (95), such as a phase I/II trial for patients with advanced solid tumors and a phase I trial for patients with aggressive and indolent NHL (115). PR rates were 22% with trastuzumab combination therapy in patients with Her2-positive gastric cancer and 16% with pembrolizumab combination therapy in patients with head and neck squamous cell carcinoma (116).

*BsAbs*. BsAbs are genetically engineered artificial antibodies that contain two specific antigen-binding sites. The BsAb backbone has two binding arms, one blocking the CD47-SIRPα pathway and the other binding tumor-specific antigens, thus ensuring the killing of tumor cells by BsAbs (99). Compared with combination therapy, using BsAbs also reduces the cost of drug development and clinical trials.

Several CD47-related BsAbs are in early clinical trials. For instance, IMM0306, a BsAbs targeting CD20 and CD47, avoids binding to CD47 in normal cells due to its high affinity for CD20, thus reducing the toxicity associated with the CD47 target. IMM0306 has demonstrated vigorous antitumor activity in a mouse model of human NHL transplantation tumor (117). It is currently being evaluated in a phase I clinical trial in B-NHL (118). IBI322 is a drug that inhibits both the programmed cell death 1 (PD-1)/PD-1 ligand 1 and CD47-SIRPα signaling pathways for treating intermediate to advanced malignancies. Repeated weekly

injections of IBI322 showed good tolerability in non-human primates (119). IBI322 is currently being evaluated in a phase I clinical trial for advanced malignancies. HX009 is a BsAb targeting PD-1 and CD47 for treating advanced tumors such as gastric, colorectal and hepatocellular carcinomas and is currently being evaluated in a phase I trial for advanced solid tumors (120). Clinical studies demonstrated that of the 18 patients with at least one post-baseline tumor assessment, three patients achieved a PR and six achieved SD (121). CC-96673, a humanized BsAbs co-targeting CD47 and CD20, was able to efficiently promote phagocytosis by macrophages by blocking CD47-SIRPα interactions and mediated the selective removal of CD20-expressing tumor cells by ADCC and CDC to selectively clear CD20-expressing tumor cells. A phase I clinical trial is presently assessing it for R/R NHL. NI-1701 is a novel BsAb constructed using spinopore technology to target CD47 and CD19 (122). Previous studies have found that NI-1701 selectively binds to CD47 and CD19 co-expressing cells and has poor binding ability with normal cells by interacting poorly with normal cells, avoiding binding to normal cells and thus improving biosafety (121,123). SL-172154, a fusion protein targeting SIRPs-Fc and CD40L, is being evaluated in a phase I clinical trial for solid tumors (124).

*Other treatment strategies*. Chimeric antigen receptor T cell (CAR T cell) immunotherapy has made significant progress in oncology, and combining CD47 blockade therapy with CAR T-cell therapy has become a hot research topic. A previous review (125) described CAR T cells and their future prospects and directions in detail; however, there is a lack of description of the role of CD47-CAR T cells in various types of tumor. CAR T-cell therapy is a cell-over-cell immunotherapy that does not depend on major histocompatibility complex (126). Beckett *et al* (127) examined the role of CD47 in CAR T-cell function by knocking down CD47 in T cells for downstream functional analysis. They showed that CD47 expression is critical for CAR T-cell survival *in vivo* and is required for successful overt T-cell therapy. Golubovskaya *et al* (128) reported that CD47 CAR T cells had antitumor activity and significantly inhibited the growth of transplanted pancreatic cancer tumors. Shu *et al* (129) constructed a CAR T cell targeting both CD47 and tumor-associated glycoprotein 72 (TAG-72), which showed vigorous antitumor activity in both *in vitro* and *in vivo* models of ovarian cancer. The specific targeting of TAG-72 could reduce its killing of normal cells. Chen *et al* (130) developed a SIRPα-Fc fusion protein CAR T cell, which promoted the phagocytosis of macrophages, recruited more DCs into tumor tissues, inhibited the apoptosis of CAR T cells themselves and reduced the expression of PD-1 on the surface of CAR T cells, thus enhancing the antitumor effect.

The understanding of chimeric antigen receptor macrophages (CAR-Ms) is minimal. CAR-Ms is the engineering of macrophages to modify CARs in order to enhance macrophage antigen-specific phagocytosis and tumor clearance (131,132). Klichinsky *et al* (133) first proposed the CAR-M concept, constructed CAR-Ms and reported that CAR-Ms have strong antitumor effects and can promote the secretion of proinflammatory factors, promote M2-type to M1-type polarization and increase T-lymphocyte antigen presentation (134).

In addition, a new therapeutic strategy for targeting CD47 has emerged in recent years, namely reprogramming the immunogenicity of cancer cells, whereby specific chemotherapeutic agents or radiation therapy stimulate tumor cells to undergo tumor immunogenic cell death (ICD), which is a form of apoptosis that activates the immune system (9). Abdel-Bar *et al* (135) developed nucleic acid lipid particles for the delivery of ICD-inducing Adriamycin and CD47 proteins, which could enhance phagocytosis by macrophages by increasing the amount of cell surface calreticulin.

## 8. Challenges of antitumor therapy targeting CD47

Due to its high expression on the surface of tumor cells, CD47 has become an ideal target for tumor immunotherapy, and antitumor drugs targeting CD47 were shown to have promising applications. However, chemotherapeutic drugs targeting CD47 have numerous adverse effects, a limitation that makes targeted CD47 therapy a significant challenge. First, CD47 is widely expressed on the surface of tumor cells and normal cells, leading to inevitable injury to normal red blood cells in the process of killing tumor cells. Many red blood cells will become the best 'cover' for tumor cells, and red blood cells will be exhausted by targeted drugs before tumor cells, resulting in adverse effects such as red blood-cell aggregation, anemia and thrombocytopenia (100,136). The degree of toxicity is dose-, time- and patient-specific and can be reduced by optimizing the dosage and combining drugs with erythropoietin. Second, there may be differences in the level of CD47 expression on the surface of different tumor cells, resulting in different sensitivities to targeted CD47 therapy (137,138). Finally, due to the presence of multiple immunosuppressive cells in the human body, such as myeloid-derived suppressor cells, tumor-associated macrophages and tumor-associated DCs, tumor cells may evade the surveillance of immune cells by upregulating the expression other immune checkpoint molecules (139-141), thus altering the therapeutic efficacy of targeting CD47 (6).

Challenges in immunotherapy targeting CD47 have led to the proposal of new therapeutic regimens to improve the effectiveness of treatment. One such approach is to combine CD47-targeting drugs with other immune checkpoint inhibitors to reduce immune escape by tumor cells (142,143). Furthermore, the development of BsAbs has provided new ideas for achieving improved specificity of targeted therapy (144,145). In addition, solutions to modulate the TME to enhance the efficacy of CD47-targeted therapies are also being explored (141,146,147). These solutions are expected to improve the efficacy of CD47-targeted therapies and reduce resistance.

## 9. Conclusion and prospects

In recent years, an increasing number of studies on CD47 have been conducted, and this topic has become a significant hotspot in various research fields. CD47 binds to SIRPα to activate a signaling pathway that regulates DC activation and antigen presentation in both directions and regulates macrophage phagocytosis during erythrocyte

and HSC transplantation. Upregulating or downregulating the expression of CD47 has an essential regulatory role in tumor-cell growth or death, and blocking CD47 expression also initiates an adaptive immune response that kills tumor cells (148). Although the combination of targeted CD47-SIRPα axis blockade therapy with other antibody drugs or therapies has shown good antitumor efficacy, CD47 is widely expressed in erythrocytes, myeloid cells and other hematopoietic cells, and anemia remains the most significant challenge associated with CD47-targeted drug therapy (149); furthermore, relevant antibody drugs have shown good efficacy. These drugs effectively attenuate the adverse effects of CD47-SIRPα blockade and significantly improve safety (117,143). However, much progress is needed before immunotherapy targeting the CD47-SIRPα axis can be applied in the clinic. To date, numerous clinical studies have shown that metabolic reprogramming has an essential role in the regulation of macrophage activation and study of the regulation of phagocytosis by the CD47-SIRPα axis from the point of view of metabolic reprogramming will be a promising direction; however, the underlying mechanisms of metabolism during phagocytosis, which are associated with the CD47-SIRPα axis, remain elusive (9). Further scientific research will clarify the mechanisms of action.

**Acknowledgements**

Not applicable.

**Funding**

This work was supported by the Natural Science Research Project of the Anhui Educational Committee (grant no. KJ2020ZD49) and the 512 Talent Cultivation Program of Bengbu Medical College (grant no. by51201103).

**Availability of data and materials**

Not applicable.

**Authors' contributions**

FW was involved in writing of the original draft and searching the literature. HP, FL and MH performed the literature search and reviewed the draft. JT and CS were involved in supervision, writing and editing. All of the authors discussed the article, and have read and approved the final version of the manuscript. Data authentication is not applicable.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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