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REVIEW

Building on the backbone of CD47-based therapy in cancer: Combination strategies, mechanisms, and future perspectives

Zi-Han Ye[a], Wei-Bang Yu[a], Mu-Yang Huang[a], Jun Chen[b], Jin-Jian Lu[a,c,d,e,*]

[a]*State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao 999078, China*
[b]*Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China*
[c]*Department of Pharmaceutical Sciences, Faculty of Health Sciences, University of Macau, Macao 999078, China*
[d]*MoE Frontiers Science Center for Precision Oncology, University of Macau, Macao 999078, China*
[e]*Guangdong-Hong Kong-Macau Joint Lab on Chinese Medicine and Immune Disease Research, University of Macau, Macao 999078, China*

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Abstract Described as a "don't eat me" signal, CD47 becomes a vital immune checkpoint in cancer. Its interaction with signal regulatory protein alpha (SIRPα) prevents macrophage phagocytosis. In recent years, a growing body of evidences have unveiled that CD47-based combination therapy exhibits a superior anti-cancer effect. Latest clinical trials about CD47 have adopted the regimen of collaborating with other therapies or developing CD47-directed bispecific antibodies, indicating the combination strategy as a general trend of the future. In this review, clinical and preclinical cases about the current combination strategies targeting CD47 are collected, their underlying mechanisms of action are discussed, and ideas from future perspectives are shared.

*Corresponding author. Tel.: +853 88224674; fax: +853 28841358.
E-mail address: jinjianlu@um.edu.mo (Jin-Jian Lu).

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1. Introduction

CD47 is one of the most important anti-phagocytic signals in the immune system. It is a ligand for signal regulatory protein alpha (SIRPα) expressed on phagocytes such as macrophages, neutrophils, and dendritic cells (DCs)[1−3]. The interplay between CD47 and SIRPα leads to activation of immune receptor tyrosine-based inhibition motifs (ITIMs), subsequently causing recruitment of inhibitory molecules including Src homology 2 (SH2) domain-containing protein tyrosine phosphatase (SHP)-1 and SHP-2[4−6], and deactivation of proteins such as non-muscle myosin IIA[7], thus limiting phagocytosis by phagocytes[8]. The blockade of CD47−SIRPα axis by targeting CD47 or SIRPα could be the first and foremost step in re-activating phagocytosis, and the focus of this review is targeting CD47 (CD47-based). The ubiquitous expression of CD47 in normal cells protects from elimination by phagocytes, while cancer cells also hijack this function to evade immunosurveillance[9]. Elevated expression of CD47 is found in solid tumor and hematological malignancies[10,11], resulting in poor prognosis of patients with cancer but equally a potential therapeutic target for cancer treatment[12].

Various types of CD47-based agents including antisense morpholino, anti-CD47 antibody, and SIRPα fusion protein have been developed in the past few years. Before antibodies become widely available, *CD47* gene suppression with an antisense morpholino (CD47 morpholino) was a relatively common agent used in some preclinical studies[13]. Meanwhile, anti-CD47 antibodies and SIRPα fusion proteins are the mainstays of CD47-based agents with the fastest development and relatively abundant clinical evidence. However, encouraging clinical outcomes are rarely to find after an overview of the efficacy of these agents as monotherapy in cancer treatment. Monotherapy of magrolimab (NCT02216409), the first-in-class anti-CD47 antibody with IgG4 portion, presented that only 2 of 62 patients with advanced solid cancer had reduced tumor lesions after the treatment[14]. A clinical project (NCT02641002) of another anti-CD47 antibody named CC-90002 was terminated for its poor efficacy in patients with hematological malignancies[15]. Moreover, ALX148 (NCT03013218), a SIRPα−Fc fusion protein, also showed limited anti-cancer efficacy as monotherapy[16]. By contrast, no matter from clinical or preclinical evidence, CD47-based combination strategies obtained more intriguing therapeutic effects than monotherapy. Considering that many excellent prior reviews summarized CD47-based therapies mainly according to different types of single agents[12,17], a systematic review collecting and analyzing these strategies purely from a combinatorial perspective is needed.

The combination regimens are divided into four sections, CD47-targeted therapy combined with chemotherapy, radiotherapy, targeted therapy, and immunotherapy. Since no drug targeting CD47 has been approved for clinical use, preclinical data about CD47-based combination strategies are of equal value as clinical data during the process of drug discovery. The preclinical data was first illustrated in accordance with different rationales, followed by the clinical information (if available) in each section. A notable detail is that bispecific antibodies or fusion proteins are also viewed as one type of combination regimen and included in this summary. After all data were collected, detailed working mechanisms underpinning the combinational effect were summarized in the discussion part. Lastly, future insights were provided from preclinical and clinical aspects.

2. Agents targeting CD47 with chemotherapy

Considering its remarkable anti-cancer effect, conventional chemotherapy maintains its irreplaceable role on the cancer treatment option list. With the development of immunotherapy in the past decade, the chemo-immunotherapy combination, especially the combination between chemotherapeutic agents and anti-PD-L1/PD-1 antibodies, has made great progress in certain types of cancer[18−20]. The distinct immune landscape built by chemotherapy is the key reason for its synergy with immunotherapy[18]. The same principle could be used in agents targeting CD47 with chemotherapy. Additionally, in a few cases, agents targeting CD47 are introduced to overcome the chemo-resistance.

Chemotherapeutic agents have been historically thought to kill cancer cells directly[21]. However, depth understanding of the immunological function of chemotherapy is more than simply killing cancer cells; it renders them to “antigenicity” (the property of being recognized by immune cells) and “adjuvanticity” (the property of delivering activating signals to immune cells) within the process of so-called immunogenic cell death (ICD)[22,23]. Calreticulin (CRT) is a marker of ICD and a pro-phagocytic signal in mediating macrophage phagocytosis[24,25]. Exposure of CRT on the surface of cancer cells caused by chemotherapeutic agents is viewed as one critical rationale for the combination with CD47-targeted therapy (Fig. 1A). Preclinical data observed that chemotherapeutic agents, including doxorubicin, temozolomide, and cisplatin, induced the CRT translocation to the cancer cell surface, all of which in combination with anti-CD47 antibody accounted for enhanced phagocytosis[26,27]. Notably, more detailed information about these data including experimental model, administration design, and key findings were mentioned in Table 1.

In the tumor microenvironment (TME), the direct immunostimulatory effect of chemotherapy on the immune cells in the combination is also crucial (Fig. 1). Tumor-associated macrophages (TAMs) is canonically divided into M1 and M2[28]. The M2 type of macrophage consists of a larger portion in TAMs and plays a tumor-promoting role, while the M1 type of macrophage plays a tumor-suppressive role[29]. By utilizing a screening system, Cao et al.[30] identified that cabazitaxel repolarized macrophage to M1-like phenotype and enhanced its phagocytotic ability in breast cancer, possibly *via* NF-κB signaling pathway. Combination between cabazitaxel with anti-CD47 antibody exerted a stronger anti-cancer activity. The researchers recently used the same screening model and found that paclitaxel increased a certain population of TAMs and enhanced phagocytosis through Src family tyrosine kinase signaling, which potentiated the effect of anti-CD47 antibody in non-Hodgkin’s lymphoma (NHL)[31]. Moreover, DNA damage caused by chemotherapeutic drugs potentially influences the innate sensing by propagating the stimulator of interferon genes (STING) pathway, which, in turn, enhances the type I IFN response, consequently arousing antigen presenting cell (APC)- and T cell-mediated immune response[25,32]. Based on this feature, treatment with cyclophosphamide, paclitaxel or temozolomide plus anti-CD47 antibody exerted potent tumor-fighting efficacy by augmenting APC infiltration and subsequently leading to more antigen presentation and T cell priming[27,33].

Some chemotherapeutic agents induced the expression of CD47 on cancer cells and consequently caused these cancer cells to evade attacking from immune cells[34]. The countertherapeutic induction of CD47 by these agents provides opportunity to

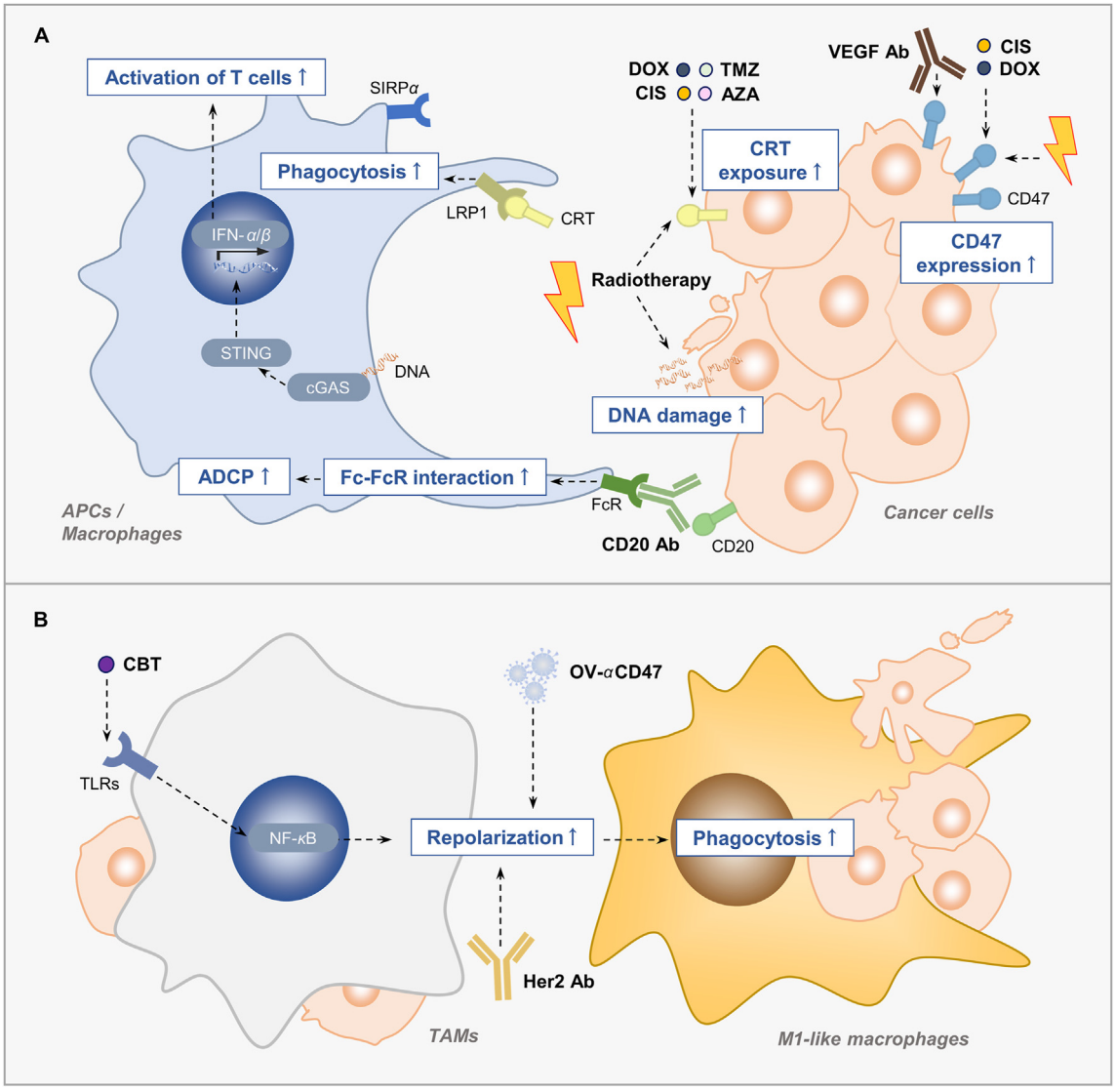

Figure 1 Combination strategies enhance macrophage phagocytosis. (A) The combination treatment enhances phagocytosis by increasing the CRT on the cell surface, or enhancing Fc−FcR interaction. Chemotherapeutic drugs or radiotherapy enhance phagocytosis by increasing the surface expression of CRT. Some of them induces the ICD of cancer cells, cause more DNA damage, activate the cGAS−STING pathway of APC and finally leading to T cell activation. Anti-CD20 antibody augments ADCP through Fc−FcR interaction after combining with anti-CD47 antibody. Moreover, the up-regulation of CD47 on cancer cells by chemotherapy, radiotherapy or anti-VEGF antibody provides the opportunities to combine the agents targeting CD47. (B) Some combination strategies enhance macrophage phagocytosis by repolarizing the macrophage phenotype from TAMs to M1-like macrophage. CIS, cisplatin; TMZ, temozolomide; AZA, azacytidine; DOX, doxorubicin; CD20 Ab, anti-CD20 antibody; HER2 Ab, anti-HER2 antibody, CBT, cabazitaxel; OV-αCD47, the oncolytic virus expressing an anti-CD47 antibody.

conduct the combination strategy (Fig. 1A). In both human cancer samples and murine model, cisplatin had been identified to up-regulate CD47 expression[35,36]. After cisplatin was combined with CD47 antibody, enhanced macrophage phagocytosis and tumor suppression were shown[35]. In addition, cancer stem cells (CSCs) in hepatocellular carcinoma (HCC) and pancreatic cancer are thought to be one of the main culprits of their chemo-resistance[37,38]. Elevated expression of CD47 was detected in these two types of CSCs after chemotherapy. Hence, targeting CSCs by CD47 morpholino, or anti-CD47 antibody exerted chemo-sensitizing effect in HCC and pancreatic cancer[39−41].

Chemotherapeutic agents, including azacitidine (AZA), paclitaxel, doxorubicin, and docetaxel combined with different CD47 inhibitor, are under clinical trials (Table 2). Among all chemotherapy-related combination strategies, magrolimab plus AZA obtained preliminary phase Ib clinical trial results (NCT03248479). AZA is used to treat patients with high-risk myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) patients. It has been identified to synergize with magrolimab by up-regulating the expression of CRT[42]. The phase Ib results demonstrated that the objective response rate (ORR) of patients with MDS was 91% (30/33), and its complete remission (CR) rate reached 56% (14/33) after six-month combination treatment. In all patients with AML, the ORR and CR/CRi (complete remission/complete remission with incomplete count recovery) of the combination group were 64% (16/25) and 56%, respectively. Especially in *TP53 mutant* AML, the CR/CRi reached

Table 1 Pre-clinical trials targeting CD47-based combination strategies in cancer.

Agents targeting CD47	Additional combinational regimen	Experiment model	Administration design	Key findings of combination regimen	Ref.
Chemotherapy					
MIAP301	Cyclophosphamide or Paclitaxel	A20 B cell malignancy C57BL/6 mice	i.p. Paclitaxel (40 mg/kg) or Cyclophosphamide (60 mg/kg) was administered 1 day before i.t. anti-CD47 antibody	Enhanced the anti-tumor effect; Preserved the memory immune response to tumor re-challenge	33
MIAP410	Cisplatin	LLC lung cancer C57BL/6 mice	Cisplatin (2.5 mg/kg) and anti-CD47 antibody (2.5 mg/kg) for 3 times	Enhanced anti-tumor effect; Prolonged survival time	35
B6H12	Doxorubicin	MNNG/HOS osteosarcoma NSG mice	i.v. Doxorubicin (1 mg/kg) on Days 0, 2, 4 i.p. anti-CD47 antibody (10 mg/kg) on Days 1, 3, 5	Reduced the tumor bioluminescence flux; Inhibited pulmonary metastases; Improved survival rate	26
B6H12	Doxorubicin	Patient-derived xenograft liver cancer NOD/SCID mice	i.p. anti-CD47 antibody (400 μg/mouse) i.v Doxorubicin (2 mg/kg) simultaneously	Enhanced anti-tumor effect	40
CD47 morpholino	Doxorubicin	PLC/PRF/5, or patient-derived xenograft hepatocellular carcinoma Nude mice	i.p. Doxorubicin (2 mg/kg) i.p. CD47 morpholino	Triggered complete eradication of tumors in PLC/PRF/5 tumor models; Enhanced anti-tumor effect in patient-derived tumor xenograft models	39
MIAP410	Temozolomide	GL261 glioblastoma or CT-2A astrocytoma C57BL/6 mice	i.p. Temozolomide (20 mg/kg) on Days 7, 8, 9 i.p. Temozolomide (80 mg/kg) and MIAP410 (100 μg) on Days 11, 13, 15	Inhibited tumor growth; Prolonged survival	27
B6H12	Gemcitabine	Patient-derived xenograft pancreatic cancer NU-*Foxn1*^{nu} nude mice	i.p. Gemcitabine (125 mg/kg) from Day 14 to 56, twice a week i.p. anti-CD47 (500 mg/mouse) from Day 14 to 35, every day	Resulted in sustained tumor regression; Prevented disease relapse	41
B6H12	Abraxane	Patient-derived xenograft pancreatic cancer NU-*Foxn1*^{nu} nude mice	i.v. Abraxane (50 mg/kg) from Day 14 to 28, every 4 days i.p. anti-CD47 (500 mg/mouse) from Day 14 to 35, every day	Resulted in sustained tumor regression; Prevented disease relapse	41
B6H12	Cabazitaxel	MDA-MB-231 breast cancer RAG2$^{-/-}$, γc$^{-/-}$ BALB/c mice	i.v. anti-CD47 antibody (4 mg/kg) once every week i.t. Cabazitaxel (40 μg/kg) once every week	Inhibited tumor development	30

B6H12	Paclitaxel	Daudi, or SUDHL-2 B cell malignancy RAG2$^{-/-}$, $\gamma c^{-/-}$ BALB/c mice; A20 B cell malignancy BALB/c mice	i.v. or i.t. Nab-paclitaxel in combination with anti-CD47 antibody	Triggered tumor burden reduction; Prolonged survival times	31
Radiotherapy					
Hu5F9-G4	Irradiation	Patient-derived orthotopic xenograft glioblastoma NSG mice	Anti-CD47 antibody followed irradiation (10 Gy)	Inhibited tumor growth and increased median survival times (combo 137 days *vs.* anti-CD47 antibody 93.5 days *vs.* irradiation 116 days)	53
Pep-20-D12	Irradiation	CT26 colon cancer C57BL/6 mice	Locally irradiation (20 Gy) i.p. Pep-20-D12 (2 mg/kg) every day for 2 weeks	Triggered tumor regression	47
CD47 morpholino	Irradiation	15-12RM fibrosarcoma BALB/c mice	i.p. CD47 morpholino (750 μL of 10 mmol/L), 48 h later, locally irradiation (10 Gy)	Reduced tumor growth (96% reduction in combo *vs.* saline alone)	51
Targeted therapy					
Anti-CD47 F (ab′) fragments	Trastuzumab + Irradiation	4T1 breast cancer BALB/c mice	i.t. anti-CD47 F (ab′) fragments (100 μg/mouse) i.t. Trastuzumab (5 mg/kg) Locally irradiation (5 Gy per day for 2 days)	Enhanced tumor inhibition; Increased necrotic area	52
MIAP410	Trastuzumab	MM3MG, breast cancer BALB/c mice; KPL-4 breast cancer SCID-beige BALB/c mice	i.p Trastuzumab (200 μg/mouse) i.p anti-CD47 antibody (300 μg/mouse)	Prolonged survival rate; Delayed tumor growth	67
Hu5F9-G4	Trastuzumab	BT474 breast cancer NSG mice	i.p. Trastuzumab (100 μg/week) i.p. Hu5F9-G4 (250 μg) every other day	Enhanced anti-tumor effect Prolonged survival rate in ADCC-tolerant cancer cells-xenograft model	70
B6H12	Blinatumomab	Raji B cell malignancy NOD/SCID mice	i.v. anti-CD47 antibody (10 mg/kg) every 3 days, 6 times in total i.v. Blinatumomab (3 mg/kg) daily, 16 times in total	Enhanced anti-tumor effect	96
Immunotherapy					
A4	Anti-PD-L1 antibody	B16F10 melanoma C57BL/6J mice	i.p. A4 (200 μg) for 14 days i.p. anti-PD-L1 (250 μg/dose) every other day for 14 days	Delayed tumor growth; Prolonged the survival	112
A4	Anti-PD-L1 antibody + anti-TRP-1 antibody	B16F10 melanoma C57BL/6J mice	i.p. A4 (200 μg/dose) for 14 days i.p. anti-PD-L1 antibody (250 μg/dose) every other day for 14 days i.p. anti-TRP-1 antibody every other day for 14 days	Delayed tumor growth; Improved the disease-free survival; Presented a durable immune memory response	112

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Table 1 (*continued*)

Agents targeting CD47	Additional combinational regimen	Experiment model	Administration design	Key findings of combination regimen	Ref.
CD47 morpholino	Ipilimumab + Irradiation	B16F10 melanoma C57BL/6J mice	i.p. CD47 morpholino (10 μmol/L) 10 Gy local irradiation i.p. anti-CTLA4 antibody (100 μg)	Increased tumor necrosis; Increased survival	48
A4	GVAX + anti-CTLA-4 antibody	B16F10 melanoma C57BL/6J mice	s.c. irradiated GVAX cells (5×10^5) in 250 μL of HBSS i.p. A4 (200 μg/dose) for 14 days i.p. anti-CTLA-4 (200 μg/dose) every other day for 14 days	Prolonged the survival; Enhanced the immune response after local delivery of A4; Caused considerable toxicity	120
MIAP301	Poly(I:C)	MC38 colon cancer C57BL/6J mice	i.p. anti-CD47 antibody (200 mg/mouse) every 3 days, began on Day 8 and stopped on Day 23 i.p. Poly(I:C) (50 μg) on Days 8, 11, 14, 17, 20, 23	Suppressed tumor growth; Prolonged survival	127
MIAP301	cGAMP	E0771 breast cancer C57BL/6J mice	i.t. anti-CD47 antibody (5 μg/mouse) i.t. cGAMP (0.5 μg/mouse)	Suppressed tumor growth (5/9 mice were completely cured); Prolonged survival time; Induced immune memory response	140
αCD47-IgG1	Oncolytic virus (OV-αCD47-G1)	A2780 ovarian cancer Athymic nude mice	i.v. 2×10^5 PFU OV-αCD47-G1 in 10 μL of saline	Slowed tumor progression	142
αmCD47-IgG2b	Oncolytic virus (OV-αmCD47-G2b)	ID8 ovarian cancer C57BL/6 mice	i.p 1×10^6 PFU OV-αmCD47-G2b in 100 μL of saline	Showed the strongest anti-tumor effect	142
αCD47-IgG1	Oncolytic virus (OV-αCD47-G1)	GBM43 glioblastoma Athymic nude mice; CT2A-hCD47 glioblastoma C57BL/6 mice	i.c. 2×10^5 PFU OV-αCD47-G1 in 3 μL of saline	Prolonged survival time in immunodeficient mice; Improved anti-tumor outcome in immunocompetent mice	143
A4-IgG2b	Oncolytic virus (OV-A4-IgG2b)	CT2A glioblastoma C57BL/6 mice	i.c. 2×10^5 PFU OV-A4-IgG2b in 3 μL of saline	Improved anti-tumor outcome	143
Bispecific antibody or fusion protein					
CD47/CD20		Raji B cell malignancy NSG mice	i.p. (300, 500, or 1000 μg)	Prolonged survival significantly	87
CD47/CD19		Raji Non-Hodgkin lymphoma NOD/SCID mice	i.p. (400 μg) 3 times per week for 3 weeks	Induced tumor regression; Reduced "off-target" toxicity	92
NI-1701		Raji B cell malignancy NOD/SCID mice	i.v. (4 or 5 doses, 10 or 20 mg/kg) once a week for 4 or 5 weeks.	Controlled tumor growth	91

NI-1701	Patient-derived xenograft B cell malignancies Humanized NSG mice	i.v. (20 mg/kg) on Days 7, 14, 21, 28, and 35	Eradicated tumor burden	91
CD47/Glypican3	Hep3B hepatocellular carcinoma NOD/SCID mice	i.p. (10 mg/kg) twice a week for 3 weeks	Exerted superior antitumor effects by macrophages and neutrophils	101
Bi-SP	A431 squamous cell carcinoma NOD/SCID mice	i.p. (10 or 40 mg/kg) on Days 0, 5, 10, 15	Delayed tumor growth	63
VEGFR1D2−SIRPαD1 fusion protein	U87 glioblastoma nude mice	i.p. SIRPα−VEGFR1 (10 mg/kg) twice a week	Reduced tumor volume	77
PF-07257876	B16F10 melanoma C57BL/6J mice	i.p. (20 mg/kg) three times a week for a total for 9 doses	Inhibited tumor growth	111
PF-07257876	MC38 colon cancer C57BL/6 mice	i.p. (10, 20, and 40 mg/kg) for a total of 6 doses	Inhibited tumor growth	111
PF-07257876	CT26 colon cancer BALB/c mice	i.p. (5 mg/kg) every 3−4 days for a total for 3 doses	Inhibited tumor growth; Prolonged the survival times (75% combo *vs*. 12.5% monotherapy)	111
Bispecific anti-PD-L1−SIRPα	MC38 colon cancer C57BL/6 mice	i.t. (50 μg/mouse) on Days 11 and 14 after tumor inoculation	Inhibited tumor growth; Extended mouse survival	107
Bispecific anti-PD-L1−SIRPα	CT26 colon cancer BALB/c mice	i.t. (100 μg/mouse) on Days 10 and 14 after tumor inoculation	Extended mouse survival	107
IBI322	Raji B cell malignancy NOD-SCID mice	i.p. (0.17 mg/kg) dosage once every 2 days for 6 continuous doses	Presented rapid and nearly completed tumor regression	110
IBI322	A375 melanoma NOG mice	i.p. (1.1 mg/kg) dosage once every 2 days for 6 continuous doses	Inhibited tumor growth	110
Anti-CTLA4−SIRPα fusion protein	Patient-derived xenograft lung cancer Humanized NSG mice	i.p. (30 μg) Day 12 i.p. after (200 μg) twice a week for a total of four times	Inhibited tumor growth *via* tumor Treg depletion	122
Anti-CTLA4−SIRPα fusion protein	MC38 colon cancer C57BL/6 mice	i.p. (20 μg) once on Day 13	Inhibited tumor growth *via* tumor Treg depletion	122
Anti-CTLA4−SIRPα fusion protein	CT26 colon cancer BALB/c mice	i.p. (50 μg) once on Day 6	Inhibited tumor growth *via* tumor Treg depletion	122
DSP107	Diffuse large B cell lymphoma Humanized NSG mice	i.p. 250 μg or 150 μL DSP107 every other day for 6 consecutive treatments	Inhibited tumor growth (2 out of 6 mice being tumor-free at the end of the experiment)	139
SIRPα−Fc−CD40L fusion protein	CT26 colon cancer; A20 B cell malignancy; WEHI-3 acute myelomonocytic leukemia BALB/c mice	i.p. mSIRPα−Fc−CD40L fusion protein	Enhanced the anti-tumor effect; Enhanced a type I interferon response	141

i.c., intracranial injection; i.p., intraperitoneally injection; i.t., intratumoral injection; i.v., intravenously injection; s.c., subcutaneous injection.

Table 2 Clinical trials targeting CD47-based combination strategies in cancer.

Agent targeting CD47	Additional combinational regimen	Status[a]	Phase	Condition	Sponsor	CT number
Chemotherapy						
Magrolimab	Azacitidine	Recruiting	III	Myelodysplastic syndromes	Gilead Sciences	NCT04313881
Magrolimab	Azacitidine	Recruiting	III	Acute myeloid leukemia	Gilead Sciences	NCT04778397
Magrolimab	Docetaxel	Recruiting	II	Solid tumor	Gilead Sciences	NCT04827576
Magrolimab	Pembrolizumab + 5-Fluorouracil + Platinum/[b] Docetaxel	Recruiting	II	Head and neck squamous cell carcinoma	Gilead Sciences	NCT04854499
Magrolimab	Venetoclax + Azacitidine/ Mitoxantrone + Etoposide + Cytarabine/CC-486	Recruiting	II	Myeloid malignancies	Gilead Sciences	NCT04778410
AK117	Nab paclitaxel/Paclitaxel	Recruiting	II	Metastatic triple-negative breast cancer locally advanced triple-negative breast cancer	Akeso	NCT05227664
TTI-621	Doxorubicin	Recruiting	II	Leiomyosarcoma	Pfizer	NCT04996004
Magrolimab	Azacitidine + Venetoclax	Recruiting	I/II	Acute myeloid leukemia recurrent acute myeloid leukemia refractory acute myeloid leukemia	M.D. Anderson Cancer Center	NCT04435691
Evorpacept	Azacitidine	Recruiting	I/II	Higher risk myelodysplastic syndromes	ALX Oncology Inc.	NCT04417517
AK117	Azacitidine	Recruiting	I/II	Myelodysplastic syndrome	Akeso	NCT04900350
AK117	Azacitidine	Recruiting	I/II	Acute myeloid leukemia	Akeso	NCT04980885
Evorpacept	Venetoclax + Azacitidine	Active, not recruiting	I/II	Acute myeloid leukemia	ALX Oncology Inc.	NCT04755244
AO-176	Paclitaxel	Active, not recruiting	I/II	Solid tumor	Arch Oncology	NCT03834948
Magrolimab	Azacitidine	Active, not recruiting	I	Hematological malignancies	Gilead Sciences	NCT03248479
IBI188	Azacitidine	Recruiting	I	Myelodysplastic syndromes	Innovent Biologics (Suzhou) Co., Ltd.	NCT04485065
Lemzoparlimab	Azacitidine + Venetoclax/Azacitidine	Recruiting	I	Acute myeloid leukemia myelodysplastic syndrome	AbbVie	NCT04912063
TTI-622	Azacitidine/Azacitidine + Venetoclax	Recruiting	I	Lymphoma multiple myeloma acute myeloid leukemia	Pfizer	NCT03530683
DSP107	Venetoclax + Azacitidine	Recruiting	I	Acute myeloid leukemia myelodysplastic syndromes chronic myelomonocytic leukemia	Kahr Medical	NCT04937166
IBI188	Azacitidine	Suspended	I	Myelodysplastic syndromes	Innovent Biologics (Suzhou) Co., Ltd.	NCT04511975
Targeted therapy						
Evorpacept	Trastuzumab + Ramucirumab + Paclitaxel	Recruiting	II/III	Gastric cancer gastroesophageal junction adenocarcinoma gastric adenocarcinoma	ALX Oncology Inc.	NCT05002127
Magrolimab	Daratumumab	Recruiting	II	Multiple myeloma	Gilead Sciences	NCT04892446
Magrolimab	Cetuximab	Completed	I/II	Solid tumor colorectal cancer	Gilead Sciences	NCT02953782
Magrolimab	Rituximab/Rituximab + Gemcitabine + Oxaliplatin	Active, not recruiting	I/II	Non-Hodgkin lymphoma	Gilead Sciences	NCT02953509

CC-90002	Rituximab	Completed	I	Hematologic neoplasms	Celgene	NCT02367196
Hu5F9-G4	Acalabrutinib + Rituximab	Completed	I	Non-Hodgkin lymphoma diffuse large B cell lymphoma	Acerta Pharma BV	NCT03527147
Magrolimab	Obinutuzumab + Venetoclax	Recruiting	I	Follicular lymphoma marginal zone lymphoma mantle cell lymphoma etc.	National Cancer Institute	NCT04599634
TG-1801	Ublituximab	Recruiting	I	B-cell lymphoma	TG Therapeutics, Inc.	NCT03804996
TG-1801	Ublituximab	Recruiting	I	Chronic lymphocytic lymphoma small lymphocytic lymphoma follicular lymphoma etc.	TG Therapeutics, Inc.	NCT04806035
Magrolimab	Dinutuximab	Recruiting	I	High risk neuroblastoma recurrent neuroblastoma recurrent osteosarcoma etc.	National Cancer Institute	NCT04751383
TTI-621	Rituximab	Active, not recruiting	I	Hematologic malignancies solid tumor	Pfizer	NCT02663518
Lemzoparlimab	Pomalidomide + Dexamethasone/ Daratumumab + Dexamethasone	Terminated (Strategic considerations)	I	Multiple myeloma	AbbVie	NCT04895410
Immunotherapy						
Evorpacept	Pembrolizumab + Platinum + 5-Fluorouracil	Recruiting	II	Head and neck cancer head and neck squamous cell carcinoma	ALX Oncology Inc.	NCT04675333
Magrolimab	Pembrolizumab	Recruiting	II	Head and neck squamous cell carcinoma	Gilead Sciences	NCT04854499
Magrolimab	Pembrolizumab	Recruiting	II	Hodgkin lymphoma classic Hodgkin lymphoma relapsed classical hodgkin lymphoma, etc.	Stanford University	NCT04788043
Evorpacept	Pembrolizumab	Recruiting	II	Head and neck cancer head and neck squamous cell carcinoma	ALX Oncology Inc.	NCT04675294
Anti-CD47 antibody	SHR2150	Recruiting	I/II	Solid tumor	Chinese PLA General Hospital	NCT04588324
DSP107	Atezolizumab	Recruiting	I/II	Advanced solid tumor non-small cell lung cancer	Kahr Medical	NCT04440735
AO-176	Pembrolizumab	Active, not recruiting	I/II	Solid tumor	Arch Oncology	NCT03834948
Magrolimab	Avelumab	Completed	I	Ovarian cancer	Gilead Sciences	NCT03558139
IBI188	Sintilimab/GM-CSF	Recruiting	I	Solid tumors lung adenocarcinoma osteosarcoma	Innovent Biologics (Suzhou) Co., Ltd.	NCT04861948
Evorpacept	Pembrolizumab/ Pembrolizumab + 5-Fluorouracil + Cisplatin	Active, not recruiting	I	Metastatic cancer solid tumor advanced cancer etc.	ALX Oncology Inc.	NCT03013218
TTI-621	Nivolumab	Active, not recruiting	I	Hematologic malignancies solid tumor	Pfizer	NCT02663518
Magrolimab	Mogamulizumab	Suspended (Other - Amendment Request)	I/II	Recurrent mycosis fungoides recurrent mycosis fungoides and sezary syndrome recurrent sezary syndrome etc.	National Cancer Institute	NCT04541017

(*continued on next page*)

Table 2 (*continued*)

Agent targeting CD47	Additional combinational regimen	Status[a]	Phase	Condition	Sponsor	CT number
TTI-621	PD-1 or PD-L1 inhibitor/ pegylated interferon-α2a/ T-Vec/Radiation	Terminated	I	Solid tumors mycosis fungoides melanoma Merkel-cell carcinoma etc.	Trillium Therapeutics Inc.	NCT02890368
Bispecific antibody or fusion protein						
HX009		Active, not recruiting	II	Advanced solid tumor	Waterstone Hanxbio Pty Ltd.	NCT04886271
DSP107		Recruiting	I/II	Advanced solid tumor non-small cell lung cancer	Kahr Medical	NCT04440735
CPO107		Recruiting	I/II	Non-Hodgkin lymphoma	Conjupro Biotherapeutics, Inc.	NCT04853329
IBI322		Recruiting	I	Hematologic malignancy	Innovent Biologics (Suzhou) Co., Ltd.	NCT04795128
IBI322		Recruiting	I	Advanced malignancies	Innovent Biologics (Suzhou) Co., Ltd.	NCT04338659
IBI322		Recruiting	I	Advanced malignancies	Innovent Biologics (Suzhou) Co., Ltd.	NCT04328831
PF-07257876		Recruiting	I	Non-small cell lung cancer head and neck squamous cell carcinoma Ovarian cancer	Pfizer	NCT04881045
IMM0306		Recruiting	I	Non-Hodgkin lymphoma	ImmuneOnco Biopharmaceuticals (Shanghai) Co., Ltd.	NCT04746131
IMM2902		Recruiting	I	Advanced solid tumor; advanced breast cancer; advanced gastric cancer	ImmuneOnco Biopharmaceuticals (Shanghai) Co., Ltd.	NCT05076591
TG-1801		Recruiting	I	B-cell lymphoma	TG Therapeutics, Inc.	NCT03804996
IBI322		Not yet recruiting	I	Advanced solid tumor	Innovent Biologics (Suzhou) Co., Ltd.	NCT04912466
HX009		Active, not recruiting	I	Advanced solid tumor	Waterstone Hanxbio Pty Ltd.	NCT04097769
SG2501		Not yet recruiting	I	Hematological malignancy lymphoma	Hangzhou Sumgen Biotech Co., Ltd.	NCT05293912
SL-172154		Terminated (Sponsor decision)	I	Cutaneous squamous cell Carcinoma squamous cell Carcinoma of head and neck	Shattuck Labs, Inc.	NCT04502888

[a]Data from https://clinicaltrials.gov/ (collected on August 22, 2022).
[b]"/" is used to distinguish the different study arms of the clinical trial.

75%. The combination therapy exhibited a better therapeutic efficacy than treatment with AZA alone and a similar tolerability[43]. Thus, it was approved by FDA as a breakthrough therapy for the treatment of MDS and AML (NCT04313881 and NCT04778397). As a special note, FDA has recently placed partial clinical hold on the aforementioned clinical trials (NCT04313881, NCT04778397, and NCT03248479) and other four trials (NCT05079230, NCT04778410, NCT04892446, and NCT02953509) due to an apparent imbalance in suspected unexpected serious adverse reactions between study arms, but soon lifted the hold on most of them after reviewing the comprehensive safety data from each clinical trial. The clinical trial results of the combination between magrolimab plus AZA are still meaningful. However, its safety should not be neglected in the future investigation. In addition, several other clinical studies about agents targeting CD47 plus chemotherapy are attempting to prove the feasibility of this kind of strategy. For example, high CR/CRi (94%) and CR (81%) rates were noted in newly diagnosed patients who are older/unfit or with high-risk AML patients after they received triplet combination regimen that included AZA, BCL-2 inhibitor venetoclax and magrolimab (NCT04435691). Another fully human anti-CD47 IgG4 antibody with lower red blood-cell binding affinity than magrolimab, namely, lemzoparlimab (TJC4), is in phase I clinical trials with the same triplet combination regimen in treating patients with AML or MDS (NCT04912063). Previously the initial result of a phase I/IIa study of lemzoparlimab showed that it was well-tolerated and caused moderate hematological adverse effects[44].

3. Agents targeting CD47 with radiotherapy

In line with chemotherapy, radiotherapy also induces ICD and remodels the TME to an inflammatory state[45,46]. Its potential to combine with agents targeting CD47 had been identified by many preclinical cases[47−53]. Moreover, the re-sensitization of radio-resistant cancer cells by agents targeting CD47 is another important rationale for the combination.

In various cancer types, the enhanced anti-cancer effect of radiotherapy plus the CD47 blockade was possibly through increasing the “eat me” signals such as CRT on the irradiated cancer cells to mediate more phagocytosis[47,50,53,54]. Meanwhile, the modulation effect of the combination therapy on immune cells, particularly macrophages and T cells, was another main factor that contributed to the synergy (Fig. 1A). A peptide targeting CD47 to block the CD47−SIRPα interaction synergized with irradiation through the increased proportion of tumor-infiltrating macrophages and enhanced phagocytosis[47]. CD47 played a negative role in T cell response, possibly due to its interaction with thrombospondin-1[55,56]. Regardless of macrophage-mediated immune response, one study using CD47 morpholino to suppress CD47 expression in $CD8^+$ T cells in the context of radiotherapy was identified to activate T cells for combating fibrosarcoma and melanoma[51]. Enlightened by this finding and the T cell-targeting strategy, a triple combination consisting of anti-CTLA4 antibody, CD47 morpholino, and radiotherapy was conducted. The outcome suggested that the CD47 and CTLA4 blockade in the context of irradiation significantly shrank the tumor volume and prolonged the survival of tumor-bearing mice[48].

In addition, up-regulation of CD47 expression after radiation was often accompanied by elevation of other factors such as PD-L1, HER2, or fatty acid oxidation enzymes, which is thought to cause radio-resistance and limit the efficacy of radiotherapy[49,50,52]. Therefore, anti-CD47 antibodies, in combination with inhibitors targeting these factors, respectively, are capable to re-sensitize the cancer cell resistance to radiotherapy[49,50,52].

Clinical trial about radiotherapy-related combination strategies of CD47 is relatively insufficient. The efficacy and safety of TTI-621 (SIRPα-IgG1 Fc) plus radiation was accessed in a previous clinical trial (NCT02890368) but discontinued by the sponsor. The relevant clinical data were not disclosed.

4. Agents targeting CD47 with targeted therapy

The identification of specific mutation of oncogenic kinase in cancer has led to the advent of targeted therapy[57]. In recent years, its combination with immunotherapy has achieved encouraging clinical development[58]. The reshaping effect on the tumor immune microenvironment by targeted therapy make it an optimal assistant to couple with immunotherapy[58]. As for the combination with agents targeting CD47, reprogramming of macrophages or Fc receptor-dependent function of antibody-dependent cellular phagocytosis (ADCP) by some targeted therapy is one critical rationale for the enhanced efficacy (Fig. 1). Meanwhile, the generation of most bi-specific antibodies in the section is based on the co-expression of CD47 and other targets in certain types of cancer. Notably, CD47 blockade is used to overcome the drug resistance of anti-HER2 antibody in a few studies.

4.1. *EGFR-targeted therapy*

EGFR is usually mutated or overexpressed in various malignant tumors[59]. Meanwhile, higher expression of CD47 is ubiquitous in cancer[9]. Co-expression of CD47 and EGFR was found in some solid tumors, and it has been shown to be a poor prognostic factor in patients with cancer[60]. These features make it possible to design the two targets-based bi-specific antibody. Importantly, it is speculated that most CD47-based bispecific antibodies reduced the “off-target” rate, thereby eliciting a more potent ADCP effect than anti-CD47 antibody alone (Fig. 2). A CD47/EGFR (IgG1 with effector function) bispecific antibody was identified to selectively target different EGFR/CD47-double positive cancer cells. Compared with CD47/Mock-IgG1, the *in vitro* co-culture experiment identified that CD47/EGFR-IgG1 promoted macrophage-mediated ADCP and antigen cross-presentation, thus arousing adaptive immune response[61]. Another bispecific antibody fusion protein named Bi-SP (IgG1 with effector function) conjugated anti-EGFR IgG1 antibody and SIRPα variant-IgG1 (SIRPαV−Fc) together. An important detail is that the SIRPα variant without Fc portion previously showed enhanced phagocytosis to colon cancer cells when combined with anti-EGFR antibody cetuximab[62]. In the study of Bi-SP, potent macrophage phagocytosis and stronger anti-cancer effect were shown after the bispecific antibody treatment. Meanwhile, less hematotoxicity of Bi-SP than that of SIRPαV−Fc was found[63].

A complete phase Ib/II clinical trial (NCT02953782) among subject with colorectal cancer demonstrated at most 45% (18/40) with stable disease (SD) after the combination treatment of magrolimab and cetuximab[64]. Currently, no CD47/EGFR bispecific antibody entered the clinical research.

4.2. *HER2-targeted therapy*

HER2, a driven gene usually overexpressed in breast cancers ($\sim$20%), is one of the important indicators for metastasis and poor

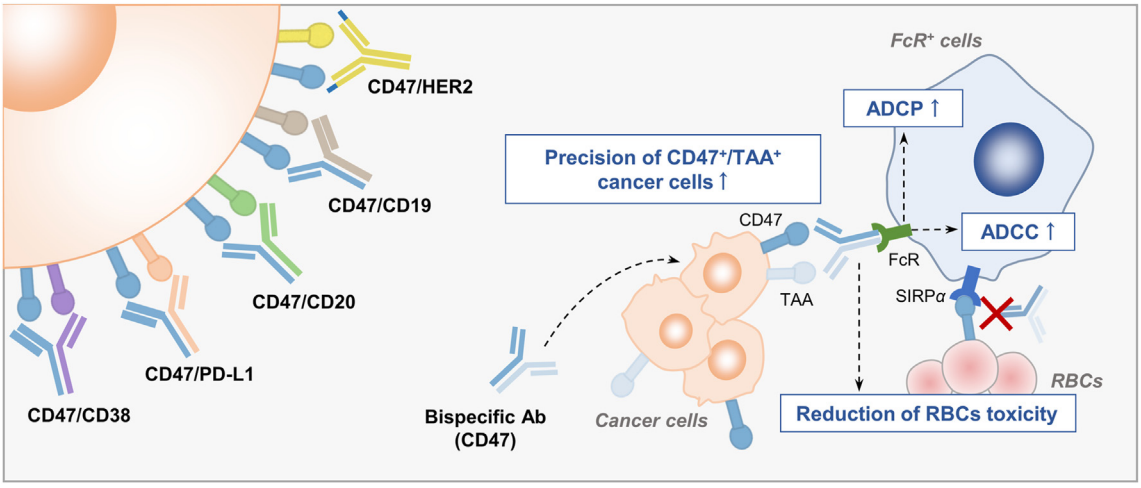

Figure 2 CD47-based bispecific antibodies or fusion proteins enhance cancer cell-specific targeting. Several CD47-based bispecific antibody or fusion protein had already entered clinical investigation, including bispecific antibodies or fusion proteins targeting CD47/PD-L1, CD47/CD20, CD47/CD19, CD47/HER2 and CD47/CD38. These agents show great targeting precision to the dual antigens-expressing cells ($CD47^+/TAA^+$). They bind less to other type of cells such as RBCs and have less "off-target" toxicity. Meanwhile, the bi-specific antibodies or fusion protein induces strong ADCP or ADCC through Fc−FcR interaction. The FcR^+ cells refer to macrophages (ADCP) or NK cells (ADCC).

prognosis[65,66]. The close correlation between macrophage and antibody targeting HER2 provides opportunities to combine with agents targeting CD47. Macrophage-mediated ADCP *via* Fc−Fcγ receptor (FcγR) has been identified to be another anti-cancer mechanism of anti-HER2 antibody in addition to the antibody-dependent cell-mediated cytotoxicity (ADCC) effect by natural killer (NK) cells or neutrophils[67,68]. Monotherapy with the anti-HER2 antibody trastuzumab was observed to affect the infiltration and the phenotype of TAMs. Combination with anti-CD47 antibody further expanded TAMs infiltration and enhanced ADCP. Gene expression analysis of these TAMs showed that combination treatment polarized TAMs to a pro-inflammatory and anti-cancer phenotype, which is close related to M1 macrophage[67].

Notably, the drug resistance of anti-HER2 therapy in some cases was caused by dysfunction of trastuzumab-mediated ADCC[69]. In a HER2 positive ADCC-tolerant breast cancer cells model, the addition of anti-CD47 antibody re-sensitized trastuzumab and prolonged the survival rate of mice *via* Fc−FcγR dependent ADCP[70]. Besides, as depicted in radiotherapy, correlation was established between CD47 and HER2 after irradiation, as both proteins were upregulated to abolish the efficacy of irradiation, and NF-κB was identified to be the common transcription factor of the two proteins. Inhibition of HER2 and CD47 simultaneously promoted the phagocytic effect of macrophage on radiation-resistant cells[52].

In clinic, different agents targeting HER2 have remarkably improved the patient outcomes, among which, trastuzumab is a universally recommended agent by the current guidelines for stages I−III HER2-positive breast cancer[66]. However, the response rate of trastuzumab is still heterogeneous and resistance appears[67]. ALX148 (inactive Fc domain with no binding to Fcγ receptor) combined with trastuzumab enhanced the anti-cancer effect significantly in HER2-positive gastric cancer in a NOD-SCID mouse model[71]. On the basis of the results, the combination therapy was evaluated in phase I clinical trial (NCT03013218), and 21.1% (4/19) overall response rate and 26.3% (5/19) disease control rate were observed in patients with HER2-positive gastric or gastroesophageal junction cancer[72]. The treatment-related adverse effects were mostly in low grade, suggesting excellent safety and tolerability of this combination treatment[72]. Moreover, a CD47/HER2 bispecific antibody named IMM2902 (unknown subset of IgG with functional Fc portion) is ongoing a phase Ib study to test its safety, tolerability, and preliminary efficacy in patients with HER2-positive solid tumor (NCT05076591), and recently, it completed the first patient dosing.

4.3. *VEGF-targeted therapy*

VEGF, a signal protein that binds to VEGF receptor (VEGFR), maintains homeostasis in angiogenesis in normal tissues[73]. However, high levels of VEGF inhibit the functions of macrophages, DCs and T cells, leading to an immunosuppressive environment and promoting the tumor growth[74]. Tumor vascular normalization by agents targeting VEGF pathway ameliorated the immunosuppressive TME, which has been developed against several malignancies[75]. During anti-angiogenic therapy, upregulation of CD47 was found in a non-small cell lung cancer mouse model *via* the TNFα−NF-κB pathway. Compared with other VEGFR family members, VEGFR1 has a higher binding affinity to VEGF[76]. Researchers designed a VEGFR1−SIRPα fusion protein and found that co-targeting VEGFR1 and CD47 by the fusion protein induced more macrophage infiltration and showed synergetic effect in lung cancer and glioblastoma[77,78].

4.4. *CD20-targeted therapy*

CD20 is a protein with a restricted expression starting from late pre-B cell but its expression could be lost in differentiated plasma cells, targeting CD20 by antibody provided potential therapeutic effect to B-cell malignancies[79]. In the combination context, FcγR activation by Fc portion of anti-CD20 antibody is thought to be the main rationale in exerting the additional anti-cancer effect in the presence of anti-CD47 antibody[80,81]. The anti-CD47 antibody could trigger phagocytosis without Fc portion, but once the rituximab had no Fc portion, the synergism was lost[80]. Chao et al.[80] and Advani et al.[81]

found that the macrophage killing effect and the superior ADCP induced by two agents but not by the NK cells-mediated ADCC, were the predominant factors in combination therapy. However, several recent studies observed that the ADCC effect by anti-CD20 antibody could not be excluded in the synergism[82–84].

In addition to integrating two separate agents together, several bispecific antibodies targeting CD47 and CD20 was also under preclinical investigation[85–87]. The CD20/CD47 bispecific antibodies had stronger binding affinity to the CD20/CD47 double-positive cancer cells and weaker binding to RBCs than rituximab or anti-CD47 antibody[86,87]. *In vivo* test also indicated that the bispecific antibody had a better tumor control and longer survival period than monotherapy[86,87].

As the first generation of monoclonal antibody targeting CD20, rituximab combined with chemotherapy significant prolongs the patient's overall survival and the PFS in "mature" B-cell leukemias and diffuse large B-cell lymphoma (DLBCL)[88]. However, neither rituximab alone nor the already existing combo treatment could not fully control the tumor growth of many patients with NHL. Progress was made by rituximab combined with magrolimab (Hu5F9) in the treatment with DLCBL patients who received more than four prior therapies and are resistant to rituximab. Twenty-two patients (15 with DLBCL and 7 with follicular lymphoma) were enrolled in the test of Hu5F9 in combination with rituximab. After the combination treatment, 50% of patients had objective response with 8 complete responses. Additionally, at a median follow-up of 6.2 months in the DLBCL group and 8.1 months in the follicular lymphoma group, 91% of patients continued to respond[81]. The clinical evidence suggested the impressive efficacy of the combination strategy. Besides, the CD47/CD20 bispecific antibody, IMM0306 (IgG1 with effector function) utilized the dual-variable-domain immunoglobulin format. At present, it is ongoing phase I trial to study the safety and efficacy in patient with NHL (NCT04746131).

4.5. CD19-targeted therapy

CD19 has a more stable expression from pre-B cells till the differentiated plasma cells than CD20. Targeting CD19 was identified to be one of the alternative strategies when drug resistance occurred after anti-CD20 treatment, because some CD20-negative B-cell malignance expressed CD19[89]. Anti-CD19 antibody was more efficient in $CD47^{-/-}$ and the existence of CD47 limited the ADCP in the treatment of B-cell malignance *in vitro*[90]. The main combination pattern between CD47 and CD19 was the bispecific antibody. NI-1701, a CD47/CD19 antibody with a fully human IgG1 in its fully functional Fc portion[91], was reported to target and enhance its ADCP to CD47/CD19 double-positive cells only, which accounted for weak antigen sink and RBCs clearances[92]. Compared with rituximab, NI-1701 had a better tumor control in Raji cells bearing NOD/SCID mouse. Different drug targets even made the combination between rituximab and NI-1701 have the best tumor regression. Safety evaluation in non-human primates indicated no drug related side effect such as hemagglutination and platelet aggregation occurred after treatment with NI-1701. Apart from enhanced the ADCP, NI-1701 demonstrated its potential to repolarize the M2 type of macrophages[91]. Another study also showed that co-engaging CD47 and CD19 by bi-specific antibody inhibited the B cell receptor (BCR)-mediated cell proliferation[93]. Moreover, MT103 (blinatumomab) is a clinical-used CD19/CD3-bispecific T cell engager antibody for treating patients with NHL and precursor B-ALL[94,95]. Co-treatment with MT103 and anti-CD47 caused persistent control of lymphoma[96], possibly providing distinct ideas about the combination strategy in targeting CD47 and CD19, which build a closer connection between the innate and adaptive immune response.

In clinical, the CD47/CD19 bispecific antibody TG-1801 (its previous name is NI-1701) aimed at patients with aggressive lymphoma, DLBCL, CLL, SLL, and other types of B-cell lymphoma. It is ongoing phase I clinical trials and has not yielded results currently (NCT03804996 and NCT04806035).

4.6. Others

Researchers have never stopped discovering new combination schemes between CD47 and other targets. Those schemes shared some common features, one of which is that the selected targets to cooperate with CD47 are already identified TAAs and have even entered the clinic. A recent study demonstrated that anti-GD2 synergized with anti-CD47 antibody in GD2 positive neuroblastoma[97]. GD2 is a TAA for neuroblastoma and osteosarcoma, and anti-GD2 antibody dinutuximab has been implicated in treatment for patients with neuroblastoma[98]. One rationale for the regimen is that the tumor cell death and CRT exposure caused by the binding of GD2 to its antibody enhances the phagocytosis and anti-cancer activity of anti-CD47 antibody[97]. Currently, the regimen (magrolimab plus dinutuximab) is taking a phase I clinical trial in children and young adults with relapsed or refractory neuroblastoma or relapsed osteosarcoma (NCT04751383).

CD38 is a transmembrane glycoprotein highly expressed in many hematologic malignancies including T-ALL[99]. A newly published study proved that CD47 also overexpressed in most T-ALL patient samples[100]. The blockade of CD47−SIRPα axis had been identified to work with anti-CD38 antibody daratumumab to eliminate T-ALL cells *in vitro* and in patient derived xenograft mouse model, respectively. Elevated ADCP was presented after the combination, probably due to more Fc−FcγR interaction induced by daratumumab[100]. It is worth mentioning that two clinical trials (NCT04892446 and NCT05293912) were carried out to evaluate the safety and efficacy of this combination regimen, including a CD47/CD38 bispecific antibody named SG2501. Furthermore, some dual-targeted agents such as CD47/Glypican-3, CD47/mesothelin, and SIRPα−αCD123 fusion antibodies, also had compelling preclinical data to show their combinational anti-cancer efficacy[92,101–104].

5. Agents targeting CD47 with immunotherapy

Although CD47 is a vital signal to directly control macrophage phagocytosis, or indirectly increases antigen presentation by APC, and T cells activation, accumulating evidence indicates that solely blocking CD47 signal transduction is unable to trigger an intense immune response[9]. Therefore, further activation of the immune system, no matter further enhancing the innate immune response or the adaptive immune response, is the main rationale for agents targeting CD47 to combine with immunotherapy. Likewise, most of the bispecific agents in this section are acted as a bridge to connect macrophage phagocytosis and the further immune response, the related mechanisms were depicted in Fig. 3.

5.1. Anti-PD-1 or anti-PD-L1 therapy

Antibodies targeting the PD-1−PD-L1 axis have been identified as the forefront immune checkpoint inhibitors in cancer treatment in

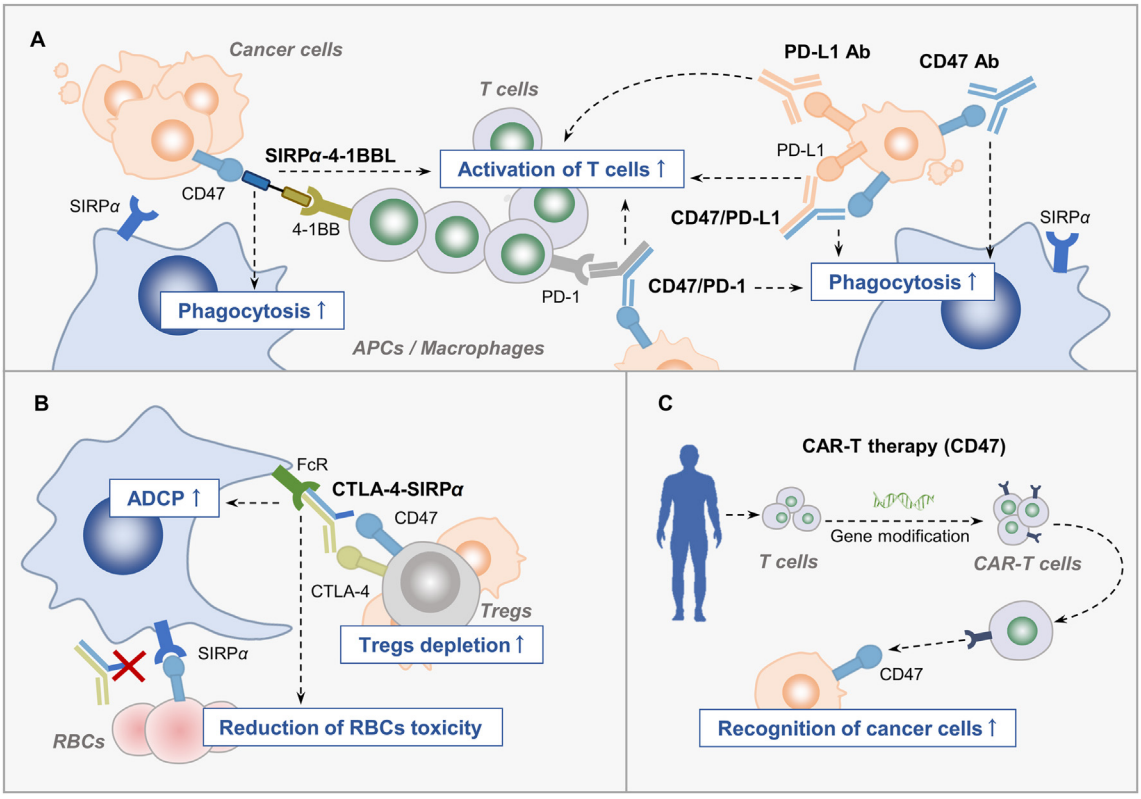

Figure 3 Combination strategies regulate T cell-mediated response. (A) The SIRPα−4-1BBL fusion protein or CD47/PD-1 bispecific antibody could not only block CD47 to enhance phagocytosis but also activate T cell-mediated immune response. Besides, the combination effect of co-targeting CD47 and PD-L1 relies on T cell activation. (B) Unselective depletion of Tregs in periphery by anti-CTLA-4 antibody contributes to immune-related adverse effects. The anti-CTLA-4−SIRPα heterodimer is developed to reduce the binding of agents targeting CD47 to RBCs and precisely deplete tumor Tregs. (C) Selection of CD47 as the TAA for CAR-T therapy increases T cell recognition to cancer cells. CD47 Ab, anti-CD47 antibody; PD-L1 Ab, PD-L1 antibody; CD47/PD-L1, bispecific antibody targeting CD47 and PD-L1; CD47/PD-1, bispecific antibody targeting CD47 and PD-1; CTLA-4-SIRPα: anti-CTLA-4−SIRPα heterodimer.

clinic[105]. PD-L1 interacts with PD-1 to cause the dysfunction of T cells. Therefore, the blockade of PD-1−PD-L1 interaction induces a strong T cell-mediated adaptive immune response[106]. A positive correlation was speculated to exist between CD47 and PD-L1 for two reasons, namely that CD47 and PD-L1 have been found to be co-expressed in certain cancer types[50,107], and some transcription factors and cytokines are shared by these two proteins[106,108]. All the aforementioned reasons enlarge the population of $CD47^+PD\text{-}L1^+$ cancer cells. Thus, the enhanced antitumor effect was largely depending on the specific elimination of the $CD47^+PD\text{-}L1^+$ cancer cells by the CD47/PD-L1 bispecific agents[107,109−111]. Meanwhile, less binding of red blood cells (RBCs) or normal cells of these bispecific antibodies reduced the hematological toxicity and "antigen sink" effect caused by CD47-based monotherapy[107,110,111].

Combination strategies between agents targeting PD-L1 (or PD-1) and CD47 directly stimulate the innate and adaptive immune cells, thus enhancing the anti-cancer effect. Redoubled macrophage phagocytosis was shown after CD47 antagonist combined with anti-PD-L1 antibody[112] or a CD47/PD-L1 bispecific antibody treatment[111]. This finding could be partially explained by the identification of the PD1−PD-L1 axis as another anti-phagocytosis checkpoint[113]. Except for macrophage, the cancer cell elimination by T cells plays the central role in the combination context. Robust $CD8^+$ T cell response was dependent on the function of PD-1−PD-L1 and CD47−SIRPα blockade, the lack of each one abrogated the effect. An in-depth study further discovered that treatment with one CD47/PD-L1 bispecific antibody augmented the population of $Tcf7^+$ stem-like progenitor $CD8^+$ T cell in the TME and promoted its differentiation to an effector-like state[111]. In non-human primate experiment, the innate response in combination therapy was mostly relied on the blockade of the CD47−SIRPα axis, but T cells are still the main mediator in the anti-cancer process[111].

In clinical stage, the combination schemes of targeting PD-L1 (or PD-1) and CD47 could be divided into the bispecific antibody and co-treatment with anti-PD-L1 (or anti-PD-1) antibody and agents targeting CD47. To date, bispecific antibodies such as IBI322 (CD47/PD-L1 bispecific antibody), HX009 (PD-1/CD47 bispecific antibody) and PF-07257875 (CD47/PD-L1 bispecific antibody) were in clinical trials. The phase I result of HX009, which was used for treatment with advanced solid tumor, indicated that dosing up to 7.5 mg/kg of HX009 was well-tolerated without any dose limiting toxicities[114]. Moreover, antitumor activity was observed in 1 and 5 mg/kg cohorts with objective responses in multiple tumor types (NCT04097769). Preliminary phase I results of IBI322 in patients with advanced solid tumors was released. IBI322 was well tolerated and showed a favorable safety profile. Within evaluable 20 patients, 4 achieved partial response (PR) and 7 achieved SD after treatment with IBI322 at active doses[115]. In addition, treatment with anti-PD-L1 antibody

and anti-CD47 antibody also deserved exploration in clinic. A phase Ib clinical data about magrolimab combined with anti-PD-L1 antibody avelumab observed a 56% SD rate in enrolled platinum-resistant or refractory ovarian cancer patients[116].

5.2. *Anti-CTLA-4 therapy*

As a negative regulator of T cell activation, CTLA-4 is an immune checkpoint expressed in conventional T cells and regulatory T cells (Tregs)[117]. Accordingly, an antibody targeting CTLA-4 was reported to expand and prime T effector lymphocytes[118]. Preclinical evidence illustrated that the dual targeting therapy unlocked two inhibitory signals on T cells (CD47-TSP-1[55] and CTLA-4-CD80/CD86 interaction inhibited T effectors cells activation), subsequently exerting anti-cancer effect by fully activated T effectors lymphocytes. A triple combination strategy among anti-CTLA-4 antibody, anti-sense morpholino silencing CD47, and irradiation was partly mentioned in the "combination with radiotherapy" section. In particular, experiment identified that CD47 morpholino plus anti-CTLA-4 antibody ipilimumab enhanced antigen-dependent killing of irradiated cancer cells[48]. CD47 and CTLA-4 targeted therapy in the context of irradiation prolonged the survival and increased tumor necrosis by recruiting $CD8^+$ T cells, and intra-tumoral NK cells and expelling myeloid-derived suppressor cells (MDSCs).

The expression of CTLA-4 on Tregs is responsible for its immunosuppressive function. Utilization of anti-CTLA-4 antibody is able to deplete Tregs in the TME, partially *via* ADCP[119]. An enhanced phagocytosis of Tregs was presented by an inhibitor targeting CD47 plus anti-CTLA-4 antibody in a coculture system between Treg and macrophage[120]. However, sometimes, unselective depletion of Tregs in periphery by anti-CTLA-4 antibody contribute to immune-related adverse effects (irAEs)[121]. To reduce the binding of agents targeting CD47 to RBCs, anti-CTLA-4−SIRPα (hIgG1 with effector function) heterodimer was developed[122]. Anti-CTLA-4−SIRPα treatment demonstrated better tumor control than co-treatment with anti-CTLA-4 and SIRPα−Fc in different immunocompetent mouse models. Mechanism studies revealed that the precise ADCP of macrophage to tumor Tregs ($ICOS^{high}$ immunosuppressive Tregs) was essential for the heterodimer to exert the synergized effect[122].

5.3. *TLR agonists-based therapy*

Toll-like receptor (TLR) play a critical role in the immune system activation for their ability to recognize pathogen-associated molecular pattern signals expressed by pathogen and danger-associated molecular patterns signals released from stressed or dying cells[123,124]. TLRs are widely expressed in the immune cells, including macrophage, NK cells, DCs, neutrophils and T cells[125]. Activation of the TLR signaling pathway in different immune cell types leads to distinct immune response. TLR agonists were utilized by researchers to combine with other anti-cancer treatment for further therapeutic effect. Most preclinical studies about the combination between TLR agonists and agents targeting CD47 have focused on the function of TLR agonists in targeting macrophages. Previous researchers found that some TLR agonists enhanced macrophage phagocytosis when combined with CD47-targeted therapy[126]. Mechanism study revealed TLR signaling pathway activation in macrophage phosphorylated Bruton's tyrosine kinase, which contributed to CRT exposure of macrophage surface. The augment of "eat me" signal in macrophage and the blockade of CD47 explained for the higher phagocytosis rate[126].

Besides, some recent studies addressed the importance of pro-inflammatory TME (including the release of pro-inflammatory cytokines and the existence of pro-inflammatory immune cells) in prompting efficacy of the CD47-targeted therapy. Zhong et al.[127] discovered that the TLR3 activator Poly(I:C) was synergized with CD47 blockade *via* secretion of pro-inflammatory cytokines, mainly by IL-6. One recent study also presented that inflammatory stimulus, such as IFN-γ, GM-CSF treatment, and TLR agonists including the TLR4 agonist lipopolysaccharide (LPS), and Poly(I:C) stimulation bolstered macrophage phagocytotic function *via* pro-phagocytotic integrin CD11a/CD11c and the subsequent participation of CD18[128]. IFN-γ, LPS, and GM-CSF were canonical inducers that switched the macrophage phenotype to M1[29]. In the combination context, M1 macrophage was a better assistant to CD47-targeted therapy. One study found that repolarization of macrophage by pretreatment of IFN-γ and LPS combined with SIRPα−Fc protein promoted phagocytosis of AML cells[129]. Besides, pyrimido [5,4-*b*] indole (PBI1), a potential TLR4 activator, was identified to polarize macrophage to M1 type. After cooperating with anti-CD47 antibody, PBI1 promoted macrophage engulfment to B-cell lymphoma cells[130]. Furthermore, re-programming the macrophage metabolism by TLR agonists also increased the cancer cell elimination. *In vitro* phagocytosis assay indicated that the TLR9 agonist CpG oligonucleotide showed an increased phagocytotic ability to PDAC cells after co-treatment with anti-CD47 antibody[131].

TLRs signaling was related to APC activation[132], a study arm in one phase I/II clinical trial selected a small molecule agonist of TLR7 (SHR2150) to combine with chemotherapy drugs plus anti-CD47 antibody to treat patients with unresectable/metastatic solid tumor (NCT04588324). The study was designed to evaluate the safety and clinical efficacy of this regimen, and patients are currently being recruited.

5.4. *Others*

Some other immunotherapy-based combination strategies of CD47 should also be mentioned. This section is divided on the basis of the dominant immune cells of action for these combined strategies. Research progress has been made in related combined strategies that are based on T cells. Chimeric antigen receptor (CAR) T-cell therapy has attracted tremendous attention these years[133]. CD47 in CAR-T therapy was firstly utilized as a tumor-associated antigen to ensure the precision of cancer cell-targeting[134,135]. Further development on CAR-T therapy identified that targeting multiple antigens, including CD47, and localizing the designed CAR-T injection had enhanced therapeutic effect[136,137]. Huang et al.[138] recently designed a CAR-T therapy that simultaneously deliver SIRPα−Fc fusion protein to block CD47. The combination treatment increased the population of DCs and M1 type of macrophage in the tumor tissue, presenting a satisfied anti-cancer effect in solid tumor. Besides, a SIRPα−4-1BBL fusion protein (fusion between the extracellular domain of human SIRPα and human 4-1BBL, with no Fc portion) named DSP107 could not only block CD47 to enhance phagocytosis but also activate tumor-reactive T cell-mediated immune response. The co-stimulatory signal of T cells only occurred after the binding of 4-1BB to 4-1BBL expressed on the membrane, while soluble 4-1BBL has no activating function. Thus, the unique structure of DSP107 gained significant 4-1BB agonistic activity

when bound to tumor-overexpressed CD47, aiding in driving T cell immune responses in the TME[139]. Critically, two phase I clinical trials about DSP107 are ongoing for treating patients with solid tumor and hematological malignances (NCT04440735 and NCT04937166).

Other immune cells also played a vital role in CD47-based combination therapies. The activation of APCs initiates the cross-talk between innate and adaptive response, which is facilitated by STING activation and the release of type I interferon. STING activation by its ligand cGAMP repolarized TAMs to M1-like macrophages, intra-tumoral treatment with cGAMP and anti-CD47 antibody was recently identified to have a systemic anti-cancer immune response[140]. Additionally, a SIRPa−Fc−CD40L fusion protein induced the production of type I interferon in the TME, potentiating the ADCP after combining another antibody such as anti-CD20 antibody, and had an outstanding tumor-fighting activity due to the activation of the whole immune response[141]. The SIRPa−Fc−CD40L fusion protein is undergoing phase I clinical trial (NCT04502888). Moreover, the role of NK cells in the combination therapy could not be neglected. Two recent studies about the combination between anti-CD47 antibody and oncolytic virus illustrated that the cancer-fighting synergy was mainly through NK cell mediated ADCC and macrophage repolarization[142,143].

6. Discussion and future perspectives

More than 90% of CD47-related clinical trials are combination therapy. 30% and 70% of those combination therapy focuses on solid tumors and hematological malignancies, respectively. The current status of CD47-based combination therapy is that more exciting clinical trial results are obtained in hematological malignancies. AZA combined with CD47-targeted agents is the most common regimens in treating hematological malignancies including AML and MDS. In solid tumors, most clinical trials are centered around combination strategy between anti-PD-L1/PD-1 antibody plus CD47-targeted agents, and anti-HER2 antibody plus CD47-targeted agents (Table 2). Collectively, no matter in hematological or solid tumors, agent targeting CD47 plays an "indispensable" and "supportive" role in the combination strategy. The "indispensable" function was reflected in the clinical trial results of its combination strategy (NCT04435691) being superior to most other clinical combination regimens currently in the treatment for AML patients. Meanwhile, agent targeting CD47 in some combination schemes plays a strong supporting role, as its addition is able to enhance the efficacy of the already-existing treatment or overcome drug resistance. Various CD47-based dual-target agents are currently being tested in clinical trials (Table 2). Some dual-target agents such as IBI322 (CD47/PD-L1) and TG-1801 (CD47/CD19) hold various clinical trials in solid tumor and hematological malignancies. Compared with separated treatment, these bispecific agents maintain the combinational effect and simultaneously reduce side effects for their specificity to cancer cells. Hence, it is believed that they could have great therapeutic potential in the future.

Understanding the detailed mechanism sheds light on the subsequent development of combination strategies for CD47. Preclinical studies of combined strategies provide clues to answer the question (Table 1). First, combination therapies enhance macrophage phagocytosis. In-depth research on macrophage phagocytosis found that blocking the signal transduction of "don't eat me" alone sometimes could not trigger sufficient phagocytosis to eliminate cancer cells[12], while adding more "eat me" signals greatly improved the phagocytic efficiency of macrophages[144,145]. In that case, the combination therapies further enhance the phagocytosis by up-regulating the "eat me" signals such as increasing the CRT exposure or inducing more Fc−FcR interaction[144] (Fig. 1A). Another mechanism to increase phagocytosis is repolarizing TAMs to "M1-like" macrophage, which is a more pro-phagocytotic phenotype (Fig. 1B). Besides, the up-regulation of CD47 on cancer cells caused by other therapies provides an opportunity to combine agents targeting CD47 and consequently leads an increased phagocytosis (Fig. 1A).

Second, combination therapies enhance the tumor-targeting precision and reduced "off-target" toxicity. Of note, this advantage appears in most of CD47-based bispecific antibodies or fusion proteins[146]. The "on-target" rationales of these agents are the co-expression of CD47 and another TAA in cancer cell. Simultaneously blocking signal transduction of CD47 and another target stimulates two diverse functions. Compared with single target antibody, CD47-based bispecific agents could enhance macrophage ADCP or NK cells-mediated ADCC. Furthermore, the augmentation of cancer cells targeting by these agents circumvents the off-target toxicity, which is mainly the unnecessary "antigen sink" and anemia caused by the clearance of healthy RBCs (Fig. 2)[147,148].

Third, combination therapies regulate T cell-mediated immune response. Single agent targeting CD47 is identified to stimulate the innate immune response. In that case, adding another agent to stimulate the adaptive immune response further eliminates cancer cells. Direct activation of $CD8^+$ T cell by anti-PD-1/PD-L1 or 4-1BB ligand is helpful for further T cell activation and tumor regression (Fig. 3A). Furthermore, precise depletion to the tumor Tregs by macrophage and utilization of CD47-based CAR-T in the combination strategy perform a greater cancer fighting capacity, thus addressing the important role of T cells in the combination strategies (Fig. 3B, C).

In the future, the combination strategy of CD47 still needs to be deeply studied in terms of two aspects: enhancing the combined efficacy and reducing toxicity. These are also two major challenges that may halt the clinical translation of CD47-based therapy. Although some of them showed impressive outcomes, the efficacy of the current combination regimens could be further improved. Meanwhile, the design of some strategies focused more on enhancing the combinational effect but neglected to limit the toxicity. Therefore, some issues and insights need to be discussed after the data were integrated and the mechanism were summarized. 1) The idea of "precision medicine" is a reminder to explore the possibility of CD47 becoming a cancer biomarker. Retrospective analysis of several clinical studies speculated that the expression of CD47 in patients may predict the efficacy of CD47 antibodies and provide instruction into the need for further combination of CD47 antibodies in patients already receiving other treatments[149−153]. 2) Aside from targeting cancer cells, the utilization of macrophage phagocytosis to eliminate tumor resident immunosuppressive cells, such as MDSCs, Tregs, or CAFs could restore the immune activation of other immune cells. In recent years, the targeting of immunosuppressive cells in cancer has been approved to be a promising anti-cancer method[154,155]. The preliminary success of CD47-based dual target agents in preclinical study indicated that adding one more target largely extends their specificity to kill one certain type of cells[146]. Precise depletion of tumor Tregs by the anti-CTLA-4−SIRPα heterodimer has already set a good example[122]. 3) Beyond targeting CD47, targeting

SIRPα is worthy of attention. As the receptor of CD47, SIRPα is expressed only in some myeloid cells. This feature allowed agents that target it to avoid binding to erythrocytes[156,157]. Therefore, SIRPα-based combination strategies may have less blood toxicity, but their anti-cancer potential still needs more preclinical and clinical data for support. 4) Moreover, with the continuous improvement of understanding of the mechanism of phagocytosis, other phagocytosis checkpoints were discovered, such as major histocompatibility complex class I−leukocyte immunoglobulin-like receptor 1 axis, CD24−sialic-acid-binding Ig-like lectin 10 axis, and so on[144,158]. Even if CD47 remains the mainstay of anti-phagocytic functions currently, future in-depth study might reveal that other phagocytosis checkpoints are functionally equivalent to, or more important than CD47 in certain types of cancer. Comprehensive identification and utilization of these phagocytosis checkpoints is of great significance for the pursuit of better anti-cancer effects. Meanwhile, if some checkpoints act more like co-effectors with CD47 in the anti-phagocytosis process, targeting them could combine with CD47-based therapy to synergistically enhance phagocytosis and further fight cancer.

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Author contributions

Zi-Han Ye: Conceptualization, Data curation, Data collection, Writing - original draft, Writing - review & editing; Wei-Bang Yu: Data collection, Writing - original draft, Writing - review & editing; Mu-Yang Huang: Writing - review & editing; Jun Chen: Writing - review & editing; Jin-Jian Lu: Conceptualization, Data curation, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Conflicts of interest

The authors declare no conflicts of interest.

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