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Review Article

Anti-CD47 Antibodies: A Potential New Cancer Immunotherapy

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Abstract

Objective: Immune checkpoint inhibitors, particularly anti-programmed cell death protein 1 (PDCD1/PD-1), anti-CD274 molecule (CD274/PD-L1), or anti-cytotoxic T-lymphocyte-associated protein 4 inhibitors, have shown notable anticancer efficacy in 10%–40% of cancer patients and >10 different cancer types. However, they have also reached an impasse. Therefore, targeting tumor-associated myeloid immune cells has become another viable approach. The CD47 molecule (CD47) is a "do not eat me" signal to phagocytes widely expressed in various tumor cells as a potential immune surveillance evasion mechanism. CD47 receptor signal-regulating protein alpha (SIRPα) is expressed in myeloid immune cells and inhibiting the CD47-SIRPα signaling pathway leads to tumor cell phagocytosis. **Data Sources:** We organized and presented the preclinical research of anti-CD47 in the past 40 years, and we also reviewed the results of anti-CD47-based clinical trials from 2018 to 2022. **Study Selection:** All studies we cited focus on using anti-CD47 blocking agents for targeting CD47/SIRPα. **Results:** In preclinical studies, anti-CD47 showed effectiveness in inducing macrophage phagocytosis of both solid tumors and blood cancers. However, clinically, anti-CD47 can only produce effective therapeutic effects when combined with other drugs, especially in treating blood cancers. Anti-CD47 may depend on directly regulating macrophage phagocytosis, tumor antigen capture by dendritic cells, and open adaptive immunity through cross-priming. **Conclusion:** Ongoing studies are investigating anti-CD47 in combination with chemotherapy, radiotherapy, targeted therapy, and immunomodulatory agents. Their results are eagerly awaited and will help clinical practice.

Keywords: Anti-CD47, immune checkpoint inhibitors, SIRPα

Introduction

Immune checkpoint inhibitors in cancer

Recent cancer mechanism studies have proposed immune evasion as a key cancer hallmark in humans.[1] The introduction of immunotherapies has been a significant development in treating many advanced cancers, such as melanoma, Hodgkin lymphoma, colorectal (CRC), bladder, breast, small cell lung (SCLC), and non-SCLC (NSCLC) cancers; hepatocellular, skin squamous cell (SCC), esophageal SCC, and renal cell (RCC) carcinomas 10 cancer types.[2] While these

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options offer new hope to patients, comparing their efficacy/safety or determining their optimal treatment sequence based on preclinical and clinical data is challenging. Moreover, new emerging immunotherapies, particularly checkpoint inhibitor therapies, have completely transformed the new drug development landscape for virtually every cancer type.

Immune checkpoint inhibitor (ICI) therapy is one of the greatest successes in new oncology drug development.[3] The anti-cytotoxic T-lymphocyte-associated protein 4 ICI ipilimumab has been approved to treat advanced melanoma and as an adjuvant in postresection melanoma treatment.[4] ICI drugs targeting the programmed cell death 1 (PDCD1/PD-1) pathway, including anti-PD-1 (nivolumab and pembrolizumab) and anti-CD274 molecule (CD274/PD-L1; atezolizumab, durvalumab, and avelumab), have been approved to treat >10 cancer types.[2] Tumor cells are well-known to highly express PD-L1 to mediate a key immune evasion mechanism.[5] PD-L1's interaction with the PD-1 receptor on the activated cytotoxic T-lymphocytes' surface triggers an inhibitory signal suppressing their activation, enabling tumor cells to evade T-cell-mediated death. Therefore, the PD-1/PD-L1 interaction prevents an excessive adaptive immune response to antigens and autoimmunity. When blocked, tumor cells can be eliminated by activated cytotoxic T-cells.

Many recent clinical trials and preclinical studies have strongly suggested that restoring immune function by blocking PD-L1 or PD-1 in cancer microenvironments provides tumor-specific immunity and has great potential in new cancer therapies. The available clinical data show that these antibody-based drugs induce promising clinical responses. However, they are effective in only some cancer patients. Drugs such as nivolumab, pembrolizumab, atezolizumab, and durvalumab have shown overall response rates of 10%–40% in various solid tumors. The mechanism by which PD-1 inhibition induces tumor-specific immunity remains poorly understood.

Other immune checkpoints have also been identified, including hepatitis A virus cellular receptor 2 (HAVCR2/TIM3), lymphocyte activating 3 (LAG3), and T-cell immunoreceptor with Ig and ITIM domains.[6] As our understanding increases, so does the likelihood that these newly discovered immune checkpoints will be used clinically. For example, in March 2022, the US Food and Drug Administration approved a relatlimab (an anti-LAG3 antibody) and nivolumab combination to treat melanoma based on clinical studies.[7] Cancer immunotherapy targets inhibitory checkpoint molecules to disrupt key tumor immune evasion mechanisms. Indeed, multiple immune checkpoint co-stimulatory and inhibitory interactions regulate T-cell responses in tumor microenvironments, including TIM3 with GAL9, LAG3 with major histocompatibility complex-I and-II, and the CD40 molecule (CD40) with the CD40 ligand (CD40 LG/CD40 L). In addition to immune checkpoint inhibition of T-cell function, several molecular interactions act as negative myeloid cell regulators in the tumor microenvironment, such as "do not eat me" signals mediated by the CD47 molecule's (CD47) interaction with signal regulatory protein alpha (SIRPA/SIRPα).[8] These myeloid cell regulators might be important for the association between innate and adaptive immunity in cancer immunotherapy, which is discussed further in the next section.

CD47 in the physiological and immunological context

CD47 is an integrin-associated protein, firstly identified as an ovarian cancer antigen[9] and acts as a self-marker. It protects host cells from elimination by myeloid or natural-killer cells. *CD47* is expressed in red blood cells (RBCs) and platelets under normal physiological conditions. During cell senescence, rapid *CD47* downregulation in RBCs makes them prone to clearance by myeloid cells.[10,11] However, CD47's interaction with SIRPα inhibits the clearance mechanism, a so-called "do not eat me signal" that prevents target cell engulfment by antigen-presenting cells such as macrophage or dendritic cells (DCs).

SIRPα is an intracellular immune tyrosine-based inhibitory motif (ITIM) receptor. Once bound to CD47, SIRPα's ITIM domain is phosphorylated by Src tyrosine kinase and acts as a docking site for protein tyrosine phosphatase nonreceptor types 6 (PTPN6/SHP1) and 11 (PTNP11/SHP2).[12,13] SHP1/2 negatively regulate macrophage attachment by disrupting integrin subunit beta 2 (ITGB2/CD18/MAC-1)/integrin subunit alpha M (ITGAM/CD11b) signaling.[14,15] The CD18/CD11b interaction is essential for Fc receptor-mediated phagocytosis.[16] Macrophages deficient for SHP1 showed improved tumor cell phagocytosis.[17] Furthermore, CD47 ligation can localize SIRPα to phagocytosis synapses, which are enriched for CD18 and CD11b. Repositioning SIRPα directly inhibits the CD18/CD11b interaction to inhibit phagocytosis.[8,18,19] Initially, CD47 was identified as a "marker of self" on RBCs, preventing their clearance by splenic macrophages through the CD47/SIRPα interaction mechanism.

CD47 is also expressed in T, B, and other blood cells at relatively lower levels. During oncogenic transformation, malignant hematological tumors highly express *CD47*. In multiple myeloma (MM), *CD47* expression is remarkably higher in MM cells, with higher *CD47* expression in MM cells associated with tumor protein p53 (*TP53*) deletions.[20] Indeed, *CD47* is reportedly highly expressed in non-Hodgkin (NHL), B-cell (BCL), T-cell (TCL), and primary effusion lymphomas, and acute lymphoblastic (ALL) and myeloid (AML) leukemias. *CD47* expression levels in solid tumors vary widely compared to hematopoietic malignancies. Analysis of *CD47* expression in the Oncomine database (comprising 715 datasets with 86,733 cancer and normal tissue samples) showed high *CD47* expression in ovarian cancer, cervical SCC, head and neck SCC (HNSCC), and lung SCC. In contrast, *CD47* expression was low in adenoid cystic carcinoma, kidney chromophobe, kidney renal papillary cell carcinoma, and low-grade gliomas. Moreover, *CD47* expression was comparable to normal tissues in esophageal carcinoma, lung SCC, prostate cancer, and tenosynovial giant cell tumor, making it unsuitable as a

druggable target.[21] Anti-CD47-based immunotherapies have been developed for over a decade. We briefly summarize the procedure for identifying the CD47/SIRPα axis and its targeting in clinical trials in Figure 1.

Pre-clinical Anti-CD47 Studies

Macrophages

CD47 expression is necessary to prevent transfused RBCs, platelets, and lymphocytes from rapidly being eliminated by splenic macrophages. In addition, mobilized hematopoietic stem cells (HSCs) increase their *CD47* expression as they pass through phagocyte-lined sinusoids to protect them from phagocytosis, and then decrease it once in marrow niches. In 2009, Jaiswal *et al*. found that *CD47* was overexpressed in circulating HSCs and leukemia cells.[22] They used acute monocytic leukemia MOLM-13 cells as an experimental model, co-incubating them with bone marrow-derived macrophages. The phagocytosis index represented green-fluorescent-protein-positive MOLM-13 cells phagocytosed by macrophages. MOLM-13 cells with low *CD47* expression had a phagocytosis index of 20%, while MOLM-13 cells with high *CD47* expression had a phagocytotic index of only 2%. Blocking SIRPα, a CD47 receptor, restored macrophage phagocytosis capacity in MOLM-13 cells with high *CD47* expression. This study showed that AML acquires resistance to macrophage-mediated phagocytosis by *CD47* overexpression.[22]

Majeti *et al*. also used an anti-mouse CD47 monoclonal antibody (MIAP301) to block surface CD47 on AML cells. Blocking CD47 facilitated AML cell phagocytosis by macrophages but did not deplete normal HSCs *in vitro*.[23] Therefore, this was the first preclinical study to show that CD47 inhibition with an anti-CD47 monoclonal antibody could trigger macrophage phagocytosis to clear AML cells. In 2010, Chao *et al*. used anti-CD47 and anti-CD20 antibodies (Rituximab) to target B-cell NHL, showing an impressive synergetic effect. Anti-CD47 with anti-CD20 significantly increased macrophage phagocytosis and eliminated NHL cells through the Fc receptor-dependent (anti-CD20) and independent (anti-CD47) blockade of the "do not eat me" signal.[24] These two-antibody combinations with antibody-dependent cellular cytotoxicity and "do not eat me" signal-blocking ability will become mainstream in future clinical trials on anti-CD47-based therapies.

In macrophages, blocking the "do not eat me" signal was insufficient to induce phagocytosis; an "eat me" signal was also needed. Gardai *et al*. first discovered surface calreticulin (CALR/CRT) on target cells interacting with macrophage low-density related protein 1 (LRP1/LRP) to initiate the first "eat me" signal to induce phagocytosis. CD47/SIRPα was the second signal cooperating with the "eat me" signal to facilitate target cell clearance.[25] Ogden *et al*. demonstrated the interaction between CALR and LRP initiates macrophage engulfment of the apoptosis cell.[26] However, living cells expressing *CALR* were resistant to macrophage phagocytosis. Therefore, these results implied that a "do not eat me" signal must be present in living cells to prevent macrophage engulfment. This "do not eat me" signal was the CD47/SIRPα axis.

To confirm this "two signal" hypothesis, they used two *CRT*-expressing human cancer cells, Raji (Burkitt's lymphoma) and MOLM-13 (AML) as experimental models treated with or without anti-CD47. They also used CRT-blocking peptide to inhibit the "eat me" signal. Raji cells, which highly co-expressed *CD47* and *CRT*, were highly sensitive to anti-CD47-mediated macrophage phagocytosis. However, the CRT-blocking peptide completely abrogated the anti-CD47-mediated effect in treated tumor cells. Furthermore, they found that this "two signal theory" could be applied to anti-CD47 therapy of several human tumors, including AML, ALL, chronic myeloid leukemia, NHL, and bladder cancer.[27]

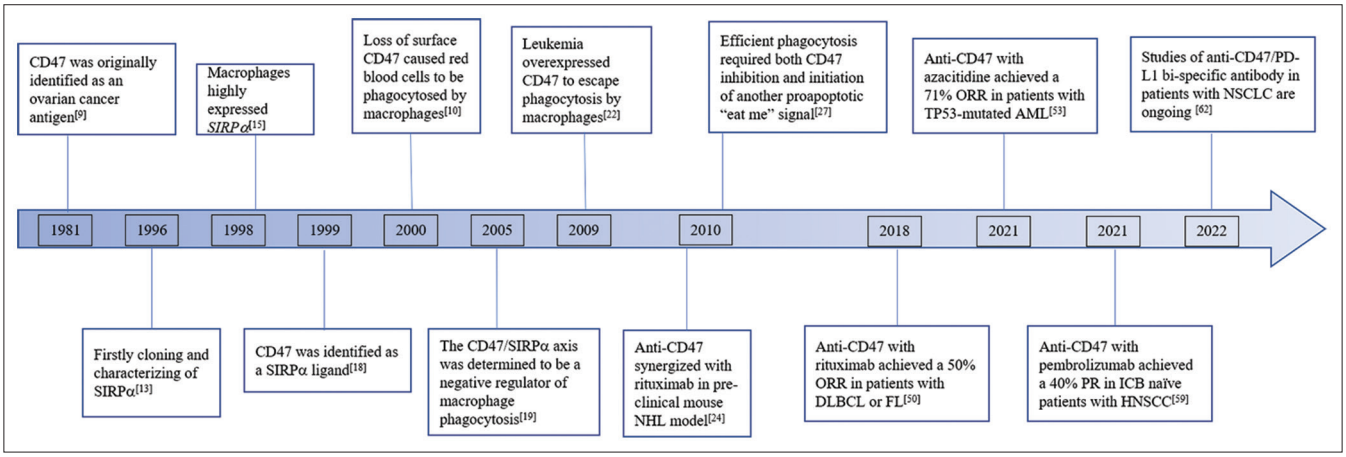

Figure 1: The timeline of key anti-CD47 discoveries. Milestones over the past 40 years in the development of anti-CD47 based immunotherapy pre-clinical and clinical. SIRPα: Signal-regulatory protein alpha, PD-L1: Programmed death-ligand 1, TP53: Tumor protein p53, ORR: Objective response rate, PR: Partial response, ICB: Immune checkpoint blockade, NHL: Non-Hodgkin's lymphoma, DLBCL: Diffuse large B-cell lymphoma, FL: Follicular lymphoma, AML: Acute myelogenous leukemia, NSCLC: Non-small cell lung cancer, HNSCC: Head-and-neck squamous cell carcinoma

Azacitidine is a pyrimidine analog first synthesized in 1963. After entering cells, it is converted into 5-aza-CTP and 5-aza-dCTP by several kinases, including uridine cytidine kinases and nucleoside monophosphate and nucleoside diphosphatase kinases. Then, 5-aza-CTP and 5-aza-dCTP are incorporated into RNA and DNA, respectively, forming an irreversible covalent bond with DNA methyltransferases 1 (DNMT1), 3 alpha (DNMT3A), and 3 beta (DNMT3B) to reduce their cellular levels.[28] Their loss causes hypomethylation and epigenetic modification loss, inducing cell cycle arrest and impairing DNA damage repair to trigger tumor cell apoptosis. Khan *et al*. studied the azacitidine-induced myelodysplastic syndromes (MDS) cancer cell death mechanism, finding a dose-dependent apoptosis induction with 0.5-2 μM azacitidine treatment. They also found azacitidine-induced activation of pro-apoptotic caspases 2 and 3.[29] Therefore, azacitidine-induced cancer cell apoptosis includes an "eat me" signal. Combining azacitidine with anti-CD47, which induces a "do not eat me" signal, would provide an ideal synergetic effect for triggering macrophage-mediated tumor cell phagocytosis. This concept was subsequently applied in several anti-CD47-based clinical trials that mainly used an anti-CD47 with azacytidine combination therapy.

Dendritic cells

Unlike hematological tumor cells that widely express SLAM family member 7 (*SLAMF7*), which is crucial for anti-CD47-dependent macrophage phagocytosis,[30] solid tumors express very low *SLAMF7* levels, potentially explaining why anti-CD47 only had a limited therapeutic effect on solid tumors in pre-clinical studies and clinical trials. In 2013, Tseng *et al*. applied the CD47/SIRPα "do not eat me" signal theory to solid tumor treatment. Surprisingly, the major anti-CD47 therapeutic effect on CRC appears to rely on the priming of $CD8^+$ T cells by antigen-presenting cells.[31] In addition to macrophages, DCs are another antigen-presenting cell that can acquire cytosolic antigens and cross-present to $CD8^+$ T-cells. DCs have superior cross-presentation efficiency compared to macrophages.[32] When given ovalbumin, granulocyte-macrophage colony-stimulating factor (GM-CSF)-derived DCs were 15-fold superior to GM-CSF-derived macrophages in inducing $CD8^+$ T cell expansion in OT-I mice,[32] potentially reflecting diverse endocytic pH in phagosome environments.

In macrophages, the endocytic pH (about pH 5.0) decreases rapidly during phagosome maturation when the vacuolar-type ATPase is active. They drive H + influx using energy derived from ATP hydrolysis.[33] In contrast, DCs maintain high pH (about pH 7.5) in their phagosome to prevent excessive degradation of internalized antigens. Since high phagosomal pH will limit protease activity, it is essential for DCs to cross-present tumor antigens to $CD8^+$ T cells. To achieve an alkaline environment, DCs recruit cytochrome b-245 beta chain (Cybb/Nox2) into the phagosomal compartment under the control of RAB27A member RAS oncogene family (Rab27a).[34] Phagosomal Nox2 catalyzes the transfer of electrons from nicotinamide adenine dinucleotide phosphate to oxygen through its catalytic subunit to produce superoxide ions. Superoxide ions then react with H + to neutralize the phagosome's acidic environment.[35] DCs deficient in Nox2 showed significantly decreased phagosomal pH, facilitating internalized antigen degradation and impairing their T cell priming capacity.[35]

Chowdhury *et al*. showed that Src-mediated tyrosine phosphorylation of neutrophil cytosolic factor 1 (NCF1/p47phox) is essential for NOX2 activity.[36] When recruited, the phosphatase SHP1 dephosphorylates p47phox, inhibiting NOX2 phagosomal activity.[37] As described above, the inhibitory signal induced by the CD47/SIRPα axis relies on recruiting SHP1/2 to the SIRPα ITIM receptor. Xu *et al*. used immunoprecipitation to show that antagonizing the CD47/SIRPα axis with an-ti-CD47 abrogated the SIRPα inhibitory signal by disrupting the SHP1/p47phox interaction, restimulating p47phox tyrosine phosphorylation and enhancing NOX2 activity in DC cells. NOX2 activation increased phagosomal pH and improved cross-priming capacity in DC cells.[38]

Another current perspective is that anti-CD47 positively regulates the DC's type 1 interferon pathway to elicit antitumor efficacy. In a murine colon tumor model, anti-CD47 treatment significantly induced interferon-alpha 1 (INFA/IFNα) in DCs but not macrophages. Furthermore, IFNα receptor deficient mice (Ifnar1fl/fl) lacked a therapeutic response to anti-CD47 therapy.[39] Hsieh *et al*. also found that anti-SIRP with anti-PD-1 and radiotherapy (RT) induced both interferon-alpha 1 (INFA/IFNα) and interferon-beta 1 (IFNB1/IFNβ) in bone marrow-derived DCs, potentially reflecting inhibition of the SIRPα-activated cyclic GMP-AMP synthase and stimulator of interferon genes (STING)-mediated type I interferon production. STING-deficient mice ($STING^{-/-}$) lost their response to anti-SIRPα, anti-PD-1, and RT triple therapy.[40]

The indispensable role of DCs in anti-PD-1 or anti-PD-L1 therapeutic efficacy has recently been gaining increasing attention. Using real-time imaging with single-cell RNA sequencing in a mouse anti-PD-1 model, Garris *et al*. showed that tumor-infiltrating DCs were required for anti-PD-1-mediated antitumor efficacy. Inhibition of PD-1-stimulated IFNγ secretion from T cells led DCs to produce interleukin 12 (IL12) in response to IFNγ. DC-derived IL12 induced T cells to mediate tumor cell killing. Mice with depleted DCs or IFNγ receptor deletion lacked an antitumor response to anti-PD-1 treatment.[41]

DCs largely dictate therapeutic efficacy in anti-PDL1 inhibitor therapy. In a phase 1 RCC study (NCT01375842) and a phase 2 NSCLC study (NCT01903993) on atezolizumab (anti-PD-L1) efficacy, a high DC signature was strongly associated with improved clinical benefits (hazard ratio [HR] = 0.38 for RCC and HR = 0.54 for NSCLC). Furthermore, $PD\text{-}L1^+$ patients with high DC signatures had further improved clinical benefits, with their HR reducing to 0.43.[42] These results indicate that durable antitumor immunity requires both innate (DC) and

adaptive (anti-PD-1/PD-L1) immunity. Combining CD47 antagonism with anti-PD-1/PD-L1 is a viable approach for reviving DCs and macrophages (innate immunity) and T cells (adaptive immunity). In a pre-clinical B16F10 melanoma mouse model, Sockolosky *et al*. showed that anti-CD47 or anti-PD-L1 alone was insufficient to induce antitumor immunity. However, when combined with PD-L1 inhibition, anti-CD47 with anti-PD-L1 elicited a strong synergetic antitumor effect, highlighting a potential role for anti-CD47 as an adjuvant therapy to enhance anti-PD-1/PD-L1 inhibitor therapeutic efficacy.[43] Anti-PD-1/anti-CD47 (IBI322) and anti-PD-L1/anti-CD47 (HX009) bispecific antibodies are currently being tested for antitumor efficacy in clinical trials.

While CD47/SIRPα signaling was first identified as an immune checkpoint in macrophages, increasing evidence shows that DCs and adaptive T-cell immunity also contribute to anti-CD47-based immunotherapy. The interactions among tumors, macrophages, DCs, and T cells in anti-CD47-based immunotherapy are briefly summarized in Figure 2.

Clinically use of anti-CD47 as a therapeutic target in cancer

Evaluation of magrolimab monotherapy in 62 patients with solid tumors or lymphoma found objective partial responses (PRs) in two patients with ovarian/fallopian tube cancers (5.2 and 9.2 months) and a mixed response in one patient with diffuse large BCL (DLBCL).[44]

CC-9002 is another monoclonal antibody disrupting the CD47/SIRPα interaction. Its monotherapy use was evaluated in 24 patients with relapsed or refractory (R/R) AML and four with high-risk R/R MDS, with the best overall response observed in two patients with stable MDS.[45]

TTI-621 (anti-CD47) monotherapy was evaluated in 56 patients with mycosis fungoides, Sézary syndrome, cutaneous TCL, or solid tumors. A rapid response occurred independent of disease stage or injection frequency, with 26 evaluable patients showing a decrease in Composite Assessment of Index Lesion Severity (CAILS) scores, which was ≥50% for 10. Reduced CAILS scores were found adjacent to noninjected lesions in 8/10 patients with paired assessments and distal to noninjected lesions in one patient.[46]

SRF231 (anti-CD47) monotherapy was evaluated in 37 patients with advanced solid and hematologic malignancies. However, no complete response (CR), PR, or prolonged stable disease (SD) was observed.[47]

IBI188 (anti-CD47) monotherapy was evaluated in 20 patients with advanced/refractory solid tumors or lymphoma. A 10 mg/kg maintenance dose resulted in >80% T cell receptor occupancy. With multiple doses (≥3 times, including the priming dose), RBC and T cell receptor occupancy tended to remain stable at ~90%.[48]

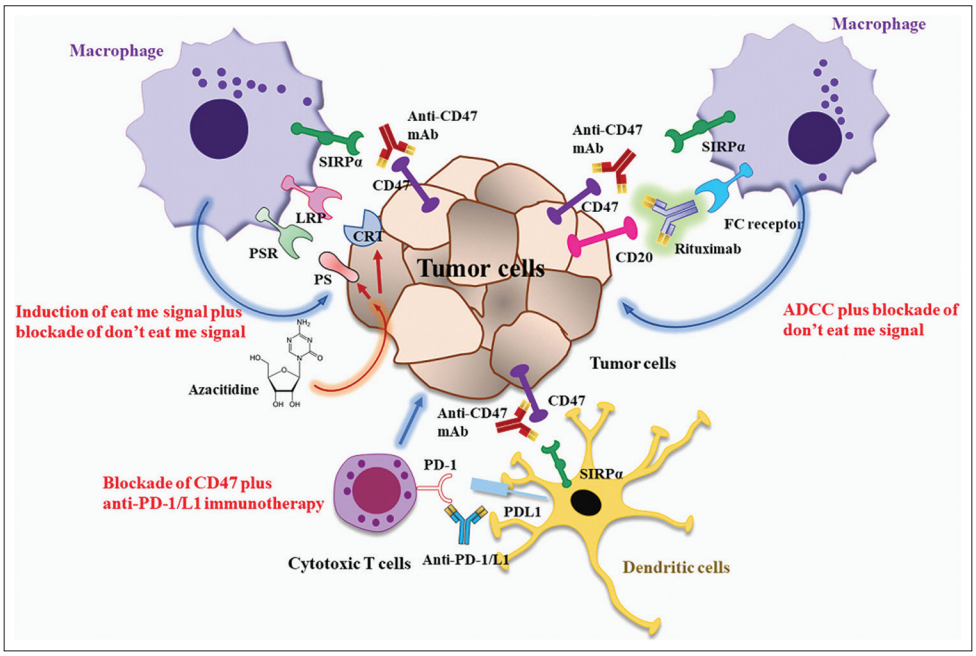

Figure 2: CD47/SIRPα inhibition leads to antitumor immunity through innate and adaptive immunity activation. We briefly summarize anti-CD47 therapeutic mechanisms into (1) ADCC plus blockade of "do not eat me" signal, (2) Induction of "eat me" signal plus blockade of "do not eat me" signal, and (3) Blockade of CD47 plus anti-PD-1/L1 immunotherapy. In macrophage, first, Fc receptor-dependent phagocytosis with rituximab (anti-CD20) combine with blockade of the "do not eat me" signal with anti-CD47 significantly eliminate NHL cells. Second, induction of "eat me" signal with azacitidine in combination with blockade of "do not eat me" signal (with anti-CD47) effectively triggers macrophage-mediated AML and MDS phagocytosis. In DCs, some populations of DCs such as type 2 conventional DCs or plasmacytoid DC expresses SIRPα. Inhibition of CD47/SIRPα with anti-CD47 unleash DCs, improve cross-priming to activate cytotoxic CD8 T-cells. SIRPα: Signal-regulatory protein alpha, AML: Acute myelogenous leukemia, NHL: Non-Hodgkin's lymphoma, ADCC: Antibody-dependent cell-mediated cytotoxicity, MDS: Myelodysplastic syndromes, DCs: Dendritic cells

AO-176 (anti-CD47) monotherapy was evaluated in 27 patients with MM. One patient with endometrial carcinoma who did not respond to four prior systemic regimens had a confirmed PR and remained in the study for >1 year. In addition, seven patients had SD as their best response, of which two (with endometrial carcinoma or gastric cancer) remained in the study for >6 months.[49]

Anti-CD47 in Combination Regimens

Humanized anti-CD47 antibody

Anti-CD47 combined with anti-CD20 (rituximab)

NCT02953509 is a phase Ib clinical trial on Hu5F9-G4 (anti-CD47) with rituximab (anti-CD20) in DLBCL and follicular lymphoma (FL). Among 22 patients (15 with DLBCL and 7 with FL), the odds ratio was 40% (6/15) in DLBCL, with five (33%) achieving CR, and 71% (5/7) in FL, with three (43%) achieving CR.[50]

CC-90002 is a humanized immunoglobulin-G4 anti-CD47 antibody evaluated in combination with rituximab in a phase I study (NCT02367196) on 28 patients with NHL, of which 24 were treated. Their objective response rate (ORR) was 13%, and their SD rate was 25%, with a median response duration of 3.9 months.[51]

Anti-CD47 combined with azacitidine

NCT03248479 is a phase Ib trial study on magrolimab (anti-CD47) with azacitidine in 43 untreated high-risk MDS (n = 18) and AML (n = 25) patients, of which 29 (13 with MDS and 16 with AML) were evaluable for efficacy.[52] The patients with MDS had an ORR of 100% (13/13), with 54% (7/13) achieving a CR and 39% (5/13) a marrow CR. The patients with AML had an ORR of 69% (11/16), with 50% (8/16) achieving a CR. In this trial, patients with *TP53*-mutant AML had an encouraging ORR of 71% (15/21), with 48% (10/21) achieving a CR. Median overall survival (OS) was 18.9 months for patients with *TP53*-mutant AML, compared to 12.9 months for patients with *TP53*-wildtype AML.[53]

Anti-CD47 combined with cetuximab

NCT02953782 is a phase Ib/II clinical trial on magrolimab (anti-CD47) with cetuximab (C) in refractory KRAS proto-oncogene GTPase (*KRAS*)-wildtype and-mutant CRC tumors. The 30 patients with *KRAS*-wildtype CRC had an ORR of 6.7%, a median OS of 10.1 months, and a median progression-free survival (PFS) of 3.6 months. While no response was reported for the 40 patients with *KRAS*-mutant CRC, 45% had SD; their median OS was 10.4 months, and their median PFS was 1.9 months. Of the 40 patients with *KRAS*-mutant CRC, 28 were TAS-102 or regorafenib naïve, and their median OS was 12.4 months, longer than historical reports.[54]

Engineered CD47 binding protein ALX148

ALX148 is a fusion protein of SIRPα binding domain linked to an inactive Fc domain, efficiently blocks CD47/SIRPα interaction and avoids inappropriate Fc domain-induced macrophage phagocytosis.

ALX148 combined with anti-CD20 (rituximab)

A phase I trial (NCT05025800) evaluated the efficacy of ALX148 (anti-CD47) with rituximab (anti-CD20) in 33 patients with NHL, of which 22 were given 10 mg/kg ALX148 with rituximab (ALX10), and 11 were given 15 mg/kg ALX148 with rituximab (ALX15). The objective response rate (ORR) was 40.9% for ALX10 patients (four with CR, five with PR, and six with SD) and 63.6% for ALX15 patients (three with CR, four with PR, and one with SD).[55]

ALX148 combined with anti-HER2 (trastuzumab)

NCT03013218 is a phase I clinical trial of evorpacept (ALX148) with trastuzumab on patients with erb-b2 receptor tyrosine kinase 2 (*ERBB2*/*HER2*)-positive gastric or gastroesophageal junction (G/GEJ) cancer. Among the 19 patients who received ALX148 with trastuzumab, four (21.1%) achieved a PR, and five (26.3%) achieved SD.[56]

ALX148 combined with trastuzumab, ramucirumab, and paclitaxel

NCT05002127 is a phase I clinical trial on ALX148 with trastuzumab, ramucirumab, and paclitaxel (ATRP) in *HER2*-positive G/GEJ cancers, showing impressive therapeutic efficacy. The 18 patients with *HER2*-positive G/GEJ cancers treated with ATRP had an ORR of 73.3%, an OS at 1 year of 70%, and a median PFS of 9.8 months;[57] their median OS was not reported. In contrast, in phase 3 clinical trial NCT01170663, patients with *HER2*-positive G/GEJ cancers treated only with ramucirumab and paclitaxel had an ORR of 28% and a median OS of 9.6 months.[57] Moreover, in phase 2 clinical trial NCT03329690, patients with HER2-Positive Gastric Cancer treated with anti-HER2 antibody-drug conjugate trastuzumab deruxtecan (DS-8201) had an ORR of 51% and a median OS of 12.5 months.[58] Therefore, ALX148 has shown promising antitumor efficacy when combined with trastuzumab, ramucirumab, and paclitaxel in *HER2*-positive tumors.

ALX148 combined with pembrolizumab

NCT03013218 is a phase I dose-escalation and dose-expansion clinical trial on evorpacept (ALX148) with pembrolizumab in 110 patients with HNSCC and NSCLC, of which 28 received evorpacept alone, 52 received evorpacept with pembrolizumab, and 30 received evorpacept with trastuzumab. The overall response rate (ORR) was 20% (4/20) in patients with HNSCC and 5% (1/20) in patients with NSCLC who received evorpacept with pembrolizumab. Patients with HNSCC who received evorpacept with pembrolizumab had a median OS of 15.5 months and a PFS of 2.1 months. Patients with NSCLC who received evorpacept with pembrolizumab had a median OS of 9.1 months and a PFS of 2.0 months.[59] Notably, the 10 patients with HNSCC who had never previously received checkpoint inhibitor treatment had an ORR of 40%, a median OS >17.9 months, and a PFS of 4.61 months.[56] Due to the relatively few patients (n = 10), further trials are needed to confirm the therapeutic efficacy of anti-CD47 with pembrolizumab in checkpoint inhibitor naïve HNSCC patients.

Bispecific Antibodies

HX009 (CD47/PD-1 bispecific antibody)

Currently, only one phase 1 clinical trial has examined TTI-621 with nivolumab in patients with Hodgkin lymphoma (NCT02663518). Several phase 2 clinical trials have investigated CD47 with pembrolizumab, including monoclonal antibodies or fusion proteins. Most have explored evorpacept, sometimes combined with other treatments, such as chemotherapy or targeting specificity antibodies. The two monoclonal antibody clinical trials on AO-176 (NCT03834948) and Hu5F9-G4 (NCT04788043) focus on patients with advanced solid tumors and Hodgkin lymphoma.

Several studies have evaluated using a PD-1/vascular endothelial growth factor bispecific antibody (AK112) with an anti-CD47 antibody (AK117) or chemotherapy for advanced malignant tumors (NCT05235542, NCT05229497, and NCT05214482). In addition, one active clinical trial (NCT04886271) is evaluating a CD47/PD-1 bispecific antibody (HX009).

In pre-clinical models, combining anti-PD-L1 with anti-CD47 has shown positive results with monoclonal[60] and bispecific[61] antibodies. This combination enhanced antitumor activity, potentially through the cross-priming mechanism, which eventually upregulates the antitumor activity of CD8 + T cells. Clinical trials favor bispecific antibodies, such as PF-07257876 and IBI322, of which most are phase-one and focus on several advanced malignant tumor types.

IBI322 (CD47/PD-L1 bispecific antibody)

Two phase 1 clinical trials (NCT04328831 and NCT04912466) have evaluated a recombinant anti-CD47/PD-L1 bispecific antibody (IBI322) in 58 patients with advanced tumors, of which 16 had prior received checkpoint inhibitor therapy. Among 20 patients treated with an active IBI-322 dose, four (20%) achieved a PR. Among nine patients with advanced NSCLC, three (33%) achieved a PR, and five (55.6%) achieved SD.[62] Several CD47-based therapeutic antibodies or combined drugs have been developed and tested in clinical trials. They are divided into CD47 monoclonal or bi-specific antibodies in Table 1.

Conclusion and Perspectives

Anti-CD47-based combination therapies have shown impressive clinical outcomes for AML, MDS, and DLBCL in completed clinical trials. However, there remains limited rationale about how such combinations work. For example, there is a lack of appropriate pre-clinical models to interpret

Table 1: List of clinically used CD47 monoclonal antibodies

Compound	Target	Phase of clinical trial development	Responsible party
Hu5F9-G4 (GS-4721) (ONO 7913) Magrolimab	CD47	I/II/III	Gilead Sciences, Inc.
CC-90002 (INBRX-103)	CD47	I	Celgene Co.
TTI-621 Ontorpacept	CD47	I/II	Pfizer, Inc.
ALX148 Evorpacept	CD47	I/II/III	ALX Oncology, Inc.
TTI-622 (PF-07901801)	CD47	I/II	Pfizer, Inc.
IBI188 Letaplimab	CD47	I/II	Innovent Biologics (Suzhou) Co. Ltd.
AO-176	CD47	I/II	Arch Oncology, Inc
ZL-1201	CD47	I	Zai Lab (Shanghai) Co. Ltd.
TJ011133 Lemzoparlimab	CD47	I/II	AbbVie, Inc
IMC-002	CD47	I	muneOncia Therapeutics, Inc.
AK117 Ligufalimab	CD47	I/II	Akeso, Inc
STI-6643	CD47	I	Sorrento Therapeutics, Inc.
IMM01	CD47	I/II	ImmuneOnco Biopharmaceuticals (Shanghai), Inc.
HMPL-A83	CD47	I	Hutchison Medipharma Ltd.
AUR103	CD47	I	Aurigene Discovery Technologies Ltd.
List of clinically used CD47 bispecific antibodies			
TG-1801 (NI-1701)	CD47/CD19	I	TG Therapeutics, Inc.
HX009	CD47/PD-1	I/II	Waterstone Hanxbio Pty Ltd.
IBI322	CD47/PD-L1	I	Innovent Biologics (Suzhou) Co. Ltd.
DSP107 (KAHR-107)	CD47/4-1BB	I/II	Kahr Medical Ltd.
CPO107 (JMT-601)	CD47/CD20	I/II	Conjupro Biotherapeutics, Inc.
TQB2928	CD47/SIRPα	I	Chia Tai Tianqing Pharmaceutical Group Nanjing Shunxin Pharmaceutical Co., Ltd.
PF-07257876	CD47/PD-L1	I	Pfizer, Inc
IBC0966	CD47/PD-L1	I/II	SUNHO (China) BioPharmaceutical CO., Ltd.
IMM2902	CD47/HER2	I	ImmuneOnco Biopharmaceuticals (Shanghai), Inc.
BAT7104	CD47/PD-L1	I	Bio-Thera Solutions Ltd.

SIRPα: signal regulatory protein alpha

the complex mechanisms behind the effects of anti-CD47 with azacitidine in AML or MDS. Therefore, the key biomarkers responsible for anti-CD47-based therapies may remain unidentified. Notably, azacitidine is a demethylating agent that targets several genes and pathways. Therefore, multiplex gene profiling, single-cell RNA profiling, and bioinformatics are needed to identify which gene clusters and signaling pathways underlie anti-CD47-based therapies.

The effectiveness of anti-CD47-induced phagocytosis is relatively limited in solid tumors. Nevertheless, anti-CD47-based combined therapies have shown promising clinical outcomes in HNSCC and *HER2*-positive G/GEJ cancers. Anti-CD47 has also shown its antitumor effects in several mouse solid tumor models. Surprisingly, in some solid tumor animal models, macrophage depletion does not completely abrogate anti-CD47's therapeutic effect. Instead, depleting T cells or DCs impaired anti-CD47 effectiveness. Therefore, T cells or DCs may have an essential role in anti-CD47-based antitumor immunity, which may be dependent or independent of cell phagocytosis. In pre-clinical studies, a key enzyme regulating antigen cross-priming, NOX2, was identified as the downstream target of CD47/SIRPα in DCs. CD47/SIRPα inhibition improved DC cross-presenting tumor-associated antigens and activated T cell immunity. Observing some clinical patients with elevated *NOX2* expression in their DCs when receiving anti-CD47 may facilitate the development of an immune adjuvant to enhance or prolong the existing ICI antitumor response beyond the originally proposed phagocytosis induction. These studies may help to develop additional combination strategies for anti-CD47-based immunotherapies.

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Conflicts of interest

There are no conflicts of interest.

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