Review

Cancer immunotherapy targeting the CD47/SIRPα axis

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Abstract The success of cancer immunotherapy has generated tremendous interest in identifying new immunotherapeutic targets. To date, the majority of therapies have focussed on stimulating the adaptive immune system to attack cancer, including agents targeting CTLA-4 and the PD-1/PD-L1 axis. However, macrophages and other myeloid immune cells offer much promise as effectors of cancer immunotherapy. The CD47/signal regulatory protein alpha (SIRPα) axis is a critical regulator of myeloid cell activation and serves a broader role as a myeloid-specific immune checkpoint. CD47 is highly expressed on many different types of cancer, and it transduces inhibitory signals through SIRPα on macrophages and other myeloid cells. In a diverse range of preclinical models, therapies that block the CD47/SIRPα axis stimulate phagocytosis of cancer cells in vitro and anti-tumour immune responses in vivo.

A number of therapeutics that target the CD47/SIRPα axis are under preclinical and clinical investigation. These include anti-CD47 antibodies, engineered receptor decoys, anti-SIRPα antibodies and bispecific agents. These therapeutics differ in their pharmacodynamic, pharmacokinetic and toxicological properties. Clinical trials are underway for both solid and haematologic malignancies using anti-CD47 antibodies and recombinant SIRPα proteins. Since the CD47/SIRPα axis also limits the efficacy of tumour-opsonising antibodies, additional trials will examine their potential synergy with agents such as rituximab, cetuximab and trastuzumab. Phagocytosis in response to CD47/SIRPα-blocking agents results in antigen uptake and presentation, thereby linking the innate and adaptive immune systems. CD47/SIRPα blocking therapies may therefore synergise with immune checkpoint inhibitors that target the adaptive immune system. As a critical regulator of macrophage phagocytosis and activation, the potential applications of CD47/SIRPα blocking therapies extend beyond human cancer. They may be useful for the treatment of infectious disease, conditioning for stem cell transplant, and many other clinical indications.

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1. Introduction

The field of immuno-oncology has rapidly translated hypothetical concepts into clinical strategies, yielding a new era of cancer investigation and treatment. A large emphasis has been placed on therapies that stimulate the adaptive immune system to attack cancer, in particular T cells. This is rightfully due to the success of immune checkpoint inhibitors targeting CTLA-4 and the PD-1/PD-L1 axis, which disable inhibitory signals to T cells and generate anti-tumour responses. However, both adaptive and innate immune cells are endowed with specialised functions to eliminate pathogens, and many of these functions can be redirected against tumours. Cells of the myeloid lineage are the most abundant immune cells in the body, yet few immunotherapeutic approaches have been aimed at stimulating them to attack cancer. Macrophages in particular have remarkable potential as mediators of anti-cancer therapies based on their robust ability to perform phagocytosis. However, macrophages have a complex relationship with tumours, and in many cases they may promote tumour growth or metastasis. The CD47/signal regulatory protein alpha (SIRPα) axis is a critical molecular interaction that inhibits the activation of macrophages and other myeloid cells against tumours and thereby acts as a myeloid-specific immune checkpoint (Fig. 1). Therapies targeting the CD47/SIRPα axis have demonstrated success in a wide range of preclinical models and are now under investigation in clinical trials for both solid and haematologic malignancies. The CD47/SIRPα axis has emerged as one of the most promising new targets for immuno-oncology.

2. Discovery of CD47 as a ‘marker of self’

CD47 is a 50 kDa multipass transmembrane protein with an extracellular region composed of a single immunoglobulin superfamily (IgSF) domain. Early studies described CD47 as a molecule expressed by a variety of ovarian cancers [1], a cell-surface protein that interacted with integrins on haematopoietic cells [2] and a glycoprotein on the surface of red blood cells [3]. A unifying theme to these studies and subsequent investigations was widespread expression of CD47 on both normal and malignant tissues.

The development of CD47−/− knockout mice permitted further functional evaluation of CD47, thereby establishing its role as an immunoregulatory molecule. Oldenborg et al. found that upon transfusing CD47−/− red blood cells into wild-type mice, the mutant red blood cells were rapidly eliminated from circulation [4]. Removal of CD47−/− cells was impaired in spleenectomised mice and those treated with liposomal clodronate, indicating a requirement for macrophages of the reticuloendothelial system for the removal process.

Histological analysis confirmed the mutant red blood cells were engulfed by macrophages in the spleen, and in vitro phagocytosis assays identified SIRPα as the inhibitory receptor that mediated this process [4]. Further studies using cells from CD47−/− mice revealed a similar effect on platelet removal [5]. Moreover, transplanted bone marrow cells from CD47−/− mice failed to engraft when transplanted into wild-type mice, suggesting a role for CD47 in protecting stem and progenitor cells from removal by macrophages [6]. A number of studies have now demonstrated CD47 acts as a ‘marker of self,’ and it plays particular importance in permitting solid and haematologic transplant across xenogeneic barriers [7–9].

3. Molecular characterisation of the CD47/SIRPα interaction

Extensive biophysical characterisation of the CD47/SIRPα interaction has been performed, including crystallographic analysis of the extracellular domain of SIRPα alone an in complex with CD47 [10,11]. These studies have demonstrated that the distal, N-terminal domain of SIRPα is responsible for contacting CD47 [11,12]. SIRPα was first characterised as a receptor tyrosine kinase that associates with the inhibitory phosphatases SHP-1 and...

![Fig. 1. The CD47/SIRPα myeloid-specific immune checkpoint.](image-url)
SHP-2 [13]. SIRPα has three extracellular IgSF domains, with the most distal domain being an immunoglobulin variable-type domain [11,12,14]. The intracellular domain of SIRPα contains immunoreceptor tyrosine-based inhibition motifs (ITIMs), which confer its properties as an inhibitory receptor. Within the immune system, SIRPα is highly expressed on the surface of cells throughout the myeloid lineage, including macrophages, granulocytes, monocytes and myeloid dendritic cells [13–19]. Ligation of CD47 to SIRPα promotes phosphorylation of the intracellular ITIMs and activates the inhibitory phosphatases SHP-1 and SHP-2 [20]. The SHP phosphatases have a multifactorial role in negatively regulating immune cell activation, including dephosphorylation of proteins containing immunoreceptor tyrosine-based activation motifs [20,21]. Within macrophages, the dephosphorylation cascade initiated by CD47 also disrupts myosin elements to prevent contractile engulfment [22].

4. The CD47/SIRPα axis in cancer

Expanding on knowledge of CD47 in bone marrow engraftment, the functional role of CD47 in protecting haematopoietic stem cells (HSCs) was further examined. HSCs intermittently enter into a migratory phase during which they exit the bone marrow and circulate systemically through the blood stream [23]. These migratory HSCs upregulate CD47 on their surface to avoid removal by macrophages of the reticuloendothelial system [23]. A similar mechanism is employed by acute myelogenous leukaemia (AML) stem cells to evade immune detection [23,24]. CD47 is more highly expressed on the surface of AML stem cells and bulk AML cells relative to normal bone marrow or peripheral blood cells [24]. CD47 expression also correlates with adverse prognosis in AML [24]. These findings prompted investigations of the CD47/SIRPα axis as a therapeutic target on AML and other malignancies. Treatment with CD47-blocking antibodies stimulated macrophage phagocytosis of AML cells in vitro [24]. Additionally, CD47-blocking antibodies produced anti-tumour effects in xenograft mouse models using human cancer cells and in an immunocompetent model of murine AML [24]. Collectively, these studies validated the CD47/SIRPα axis as a therapeutic target for leukaemia. Additional studies extended these findings to a diverse range of haematologic malignancies, including non-Hodgkin lymphoma, acute lymphocytic leukaemia and multiple myeloma [25–27].

When solid tumours were examined, a moderate increase in CD47 surface expression was detected relative to surrounding normal tissues [28]. As with AML, CD47 transcript expression correlated with adverse prognosis in solid tumours such as ovarian cancer and gliomas [28]. In a variety of human xenograft models—including ovarian, colon, breast and bladder cancer, leiomyosarcoma, pancreatic neuroendocrine tumours and small cell lung cancer—treatment with anti-CD47 antibodies stimulated macrophage phagocytosis in vitro and suppressed tumour growth in vivo [28–31]. In many cases, treatment with CD47-blocking therapies exhibited efficacy in patient-derived xenograft models, which better models the heterogeneity of tumours [28–31]. In some models, anti-CD47 treatment also suppressed metastasis, including bladder cancer and melanoma [28,32].

5. Mechanisms of action

Throughout these studies, the predominant mechanism of action investigated has been macrophage activation. In vitro, CD47 blockade stimulates macrophage phagocytosis of cancer cells, a finding that has been extensively validated by microscopy and flow cytometry [24,25,28,31,33]. In vivo, treatment of mice with liposomal clodronate—which depletes macrophages—abrogates the tumouricidal effects of CD47-blocking therapies, indicating macrophage are required for robust anti-tumour responses [24,25]. Furthermore, evidence of in situ phagocytosis has been demonstrated in models of leukaemia, breast cancer and colon cancer [24,28,33]. Undoubtedly, macrophage activation plays a key role in the response to CD47-blocking therapies.

Additional studies suggest blocking CD47 may further recruit macrophages to tumours. Phagocytosis in response to CD47-blocking therapies causes cytokine secretion by both mouse and human macrophages. Secreted factors include cytokines and chemokines that recruit additional immune cells to tumours, and may provide a positive feedback mechanism that amplifies the therapeutic response to CD47 blockade [31,34]. Monocyte chemotactic protein 3 (MCP-3) was identified as one cytokine secreted in response to CD47-blocking therapies and may participate in this effect in mice [31]. Further investigations are needed to determine if these factors contribute to the efficacy of CD47-blocking therapies or predict a response to treatment in patients.

Therapies targeting the CD47/SIRPα axis may also alter the polarisation state of macrophages in tumours. A simplified paradigm of macrophage activation has been proposed, in which pro-inflammatory ‘M1’ macrophages are those that attack tumours and immune-suppressive ‘M2’ macrophages are those that promote tumour growth. These states are promoted by cytokines such as interferon gamma, interleukin-4 (IL-4), and IL-10, as well as other inflammatory mediators of the tumour microenvironment. Further research has shed more light on polarisation, and it is now recognised that macrophages and their precursors can exist in multiple activation states and these states can be fluid, transient, or overlapping [35,36]. Regardless, macrophages within
the tumour microenvironment have the capacity to act as effectors of CD47 blockade. A recent study using models of glioblastoma confirmed that CD47-blocking therapies convert tumour-associated macrophages to a pro-inflammatory, anti-tumour state [37].

Although the effects on macrophages are the best-studied mechanisms of action, other immune cells can also respond to CD47/SIRPα-blocking therapies. SIRPα is highly expressed on myeloid immune cells [14,15,17], therefore it may be a critical regulator across the myeloid lineage. Both monocytes and granulocytes express Fc receptors, and one study found that CD47/SIRPα blockade augmented neutrophil antibody dependent cell-mediated cytotoxicity [38]. In mice, CD47 regulates antigen uptake by SIRPα⁺ dendritic cells [39], and dendritic cells contributed to the in vivo efficacy of CD47-blocking therapies in immunocompetent tumour models [40]. By stimulating antigen presentation by either macrophages or dendritic cells, therapies targeting the CD47/SIRPα axis may promote adaptive immune responses against tumours (see below).

There is some evidence that non-myeloid immune subsets, such as T cells and natural killer (NK) cells, may also respond to therapies targeting CD47 [41]. In one example, anti-CD47 antibodies directly stimulated T cell activation in a mouse model of melanoma. Another study found that NK cells exhibited greater cytotoxicity against CD47⁻/− cells in vitro [42]. However, subsequent investigations found no enhanced NK cell activity when tested in response to CD47-blocking therapies [25]. T cells and NK cells express low or absent levels of SIRPα [18], therefore, these findings may be independent of SIRPα and could alternatively be due to direct alterations in CD47 signalling.

CD47 has intracellular components that are capable of signalling [43]. In some cell types, such as endothelial cells, CD47 signalling may mediate nitric oxide synthesis and release [44]. Direct signalling by CD47 can also induce caspase-independent cell death [45,46]. Some studies have identified direct cytotoxicity to CD47⁺ tumour cells as a mechanism of action of CD47-targeting therapies [47,48]. The ability of an anti-CD47 agent to cause direct cytotoxicity may vary based on the epitope targeted and the ability to cross-link or cluster CD47 on the cell surface. The process may also depend on disruption of other proteins that interact with CD47 outside of the CD47/SIRPα binding interface. A number of CD47-blocking therapies that specifically target the SIRPα binding epitope do not exert direct cytotoxicity on tumour cells [31,49].

6. Approaches to targeting the CD47/SIRPα axis

A variety of therapeutics targeting the CD47/SIRPα axis are under preclinical and clinical investigation, including conventional antibodies, recombinant polypeptides, and bispecific molecules (Table 1). For each of these agents, there exist benefits and tradeoffs for successful translation to the clinic. Special considerations pertain to each agent’s efficacy, toxicity, and pharmacokinetic and pharmacodynamic properties.

To date, anti-CD47 antibodies are the best characterised therapies targeting the CD47/SIRPα axis. They have demonstrated efficacy in vitro and in vivo using mouse xenograft models and immunocompetent mouse tumour models. Anti-CD47 antibodies have been investigated against a plethora of haematologic and solid malignancies, and they have now entered clinical trials (Table 2). These include Hu5F9-G4, a humanised anti-CD47 antibody of the human IgG4 subclass [50]. The crystal structure of the variable region of Hu5F9-G4 has been solved in complex with CD47, which reveals its mechanisms of antagonism is due to the direct overlap with the binding epitope of SIRPα [31]. Another agent, CC-90002 has been developed that is likewise under phase I investigation in both solid tumours and haematologic malignancies. A fully human anti-CD47 antibody, SRF231, has recently been described that was generated by means of phage display and is under evaluation in preclinical models of leukaemia.

Recombinant polypeptides derived from SIRPα have also been developed as CD47-blocking reagents. They act as decoy receptors by binding to CD47 and competing with endogenous SIRPα on immune cells. One variant was created by fusing wild-type SIRPα to a human Fc domain [51]. In vitro, it was able to stimulate phagocytosis of

Table 1
CD47/SIRPα-targeting therapeutics under clinical development.

<table>
<thead>
<tr>
<th>Company</th>
<th>Drug</th>
<th>Stage</th>
<th>Description</th>
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<tbody>
<tr>
<td>Forty Seven Inc.</td>
<td>Hu5F9-G4</td>
<td>Phase I</td>
<td>Humanised anti-CD47 antibody, human IgG4 subclass</td>
</tr>
<tr>
<td>Celgene</td>
<td>CC-90002</td>
<td>Phase I</td>
<td>Humanised CD47-blocking antibody</td>
</tr>
<tr>
<td>Trillium Therapeutics Inc.</td>
<td>TTI-621</td>
<td>Phase I</td>
<td>SIRPα-Fc fusion protein, human IgG1 subclass</td>
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<tr>
<td>Alexo Therapeutics</td>
<td>ALX148</td>
<td>Phase I</td>
<td>High-affinity SIRPα variant</td>
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<tr>
<td>Novimmune</td>
<td>NI-1701</td>
<td>Preclinical</td>
<td>Fully human CD47/CD19 bispecific κλ antibody</td>
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<td></td>
<td>NI-1801</td>
<td>Preclinical</td>
<td>Fully human CD47/mesothelin bispecific κλ antibody</td>
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<tr>
<td>Tioma Therapeutics</td>
<td>Undisclosed</td>
<td>Preclinical</td>
<td>Anti-CD47 antibodies</td>
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<tr>
<td>Surface Oncology</td>
<td>SRF231</td>
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<td>Fully human anti-CD47 antibody</td>
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<td>OSE Immunotherapeutics</td>
<td>Effi-DEM</td>
<td>Preclinical</td>
<td>Anti-SIRPα antibody</td>
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human AML cells when macrophages were pre-activated with interferon gamma and lipopolysaccharide [51]. The first of such agents to enter clinical trials is TTI-621, which is composed of the N-terminal domain of SIRPα fused to human IgG1. This agent is under investigation in a phase I trial for AML and myelodysplastic syndrome. Additional studies are planned for treatment of solid tumours and mycosis fungoides with intratumoural injections rather than intravenous treatment. This strategy has been effective in mouse models [40], and it may reduce potential systemic toxicity.

Next-generation variants of SIRPα-Fc fusion proteins have been engineered for enhanced binding to CD47. Compared to wild-type SIRPα, which binds to CD47 with $\sim 1 \text{ uM}$ affinity, these high-affinity SIRPα variants bind to CD47 with extraordinary potency ($\sim 10 \text{ pM}$) to act as competitive antagonists to CD47 [33]. High-affinity SIRPα-Fc fusion proteins have been evaluated as fusions to human IgG4. Due to their enhanced potency, they are able to induce phagocytosis of cancer cells without any additional stimulation [30,31,33,49]. They are effective as single agents in vitro against haematologic and solid tumours, and exhibit efficacy in xenograft models of solid tumours. High-affinity SIRPα-Fc fusion proteins have not yet entered clinical trials.

7. Combining CD47/SIRPα blockade with tumour-opsonizing antibodies

Although CD47-blocking antibodies are sufficient to induce phagocytosis as single agents, macrophage phagocytosis is dependent on the integration of pro-phagocytic (‘eat me’) and anti-phagocytic (‘don’t eat me’) signals. CD47 acts as a predominant inhibitory signal that prevents macrophage phagocytosis of cancer cells. A number of pro-phagocytic signals have also been described, including intrinsic molecules that contribute to immunological cell death such as phosphatidylserine and calreticulin [52,53]. Extrinsic signals such as antibodies, complement, or pathogen-associated molecular patterns can also activate macrophages [54–56]. Antibody Fc chains provide a robust stimulus for macrophage activation, and inhibitory signalling from the CD47/SIRPα axis is balanced by positive signals from Fc receptors [34]. As demonstration of this principle, opsonised red blood cells from CD47–/– mice underwent nearly four-fold greater phagocytosis relative to opsonised red blood cells from wild-type mice [57]. A similar effect can be achieved by combining CD47-blocking agents with therapeutic anti-cancer antibodies that opsonise tumour cells for destruction. Thus, in models of non-Hodgkin lymphoma, CD47 antibodies synergised with the anti-CD20 antibody rituximab to augment phagocytosis and eliminate disease in xenograft mouse models [25]. Similarly, SIRPα knockout mice exhibited a greater response to trastuzumab when used in an immunocompetent Her2+ murine tumour model [38].

These findings indicate the efficacy of CD47-blocking antibodies depends on both disruption of the CD47/SIRPα axis and an Fc fragment that engages Fc receptors on immune cells. These principles were further...
established by the use of high-affinity SIRP\(\alpha\) variants. When employed as single-domain ‘monomers’ that lack an Fc fragment, the high-affinity SIRP\(\alpha\) proteins block CD47 but do not stimulate phagocytosis [33]. However, when they were combined with tumour-opsonising antibodies—such as rituximab, trastuzumab and cetuximab—they augmented phagocytosis and produced robust anti-tumour effects in vivo [33]. In theory, any therapeutic antibody that opsonises tumour cells for destruction could be enhanced by combination with CD47-blocking therapies [58]. For this reason, CD47-blocking therapies are now being studied in combination with other investigational tumour-opsonising antibodies. The combination of high-affinity SIRP\(\alpha\) monomers with lorvotuzumab, an anti-CD56 antibody in clinical trials for small cell lung cancer (SCLC), augmented phagocytosis of SCLC in vitro [31]. In studies of melanoma, CD47-blocking therapies enhanced the response to anti-CD271 antibodies that mark a subset of melanoma-initiating cells [32]. These studies form the rationale for combination of CD47-blocking therapies with tumour-opsonising antibodies. Clinical trials are now planned to evaluate CD47-blocking agents in combination with rituximab, cetuximab and trastuzumab (Table 2).

Direct blockade of SIRP\(\alpha\) is an alternative approach to targeting the CD47/SIRP\(\alpha\) axis. Since these agents target the axis on the side of the immune cell and thus do not opsonise tumour cells directly, their efficacy is dependent on combination with tumour-opsonising antibodies. As one example, anti-SIRP\(\alpha\) antibodies exhibited limited efficacy by themselves, but augmented myeloid cell activation when combined with trastuzumab against Her2\(^+\) breast cancer cells in vitro [38]. In a second example, variants of the CD47 extracellular domain were engineered that bind and antagonise SIRP\(\alpha\) [18]. These molecules act similarly to anti-SIRP\(\alpha\) antagonistic antibodies and high-affinity SIRP\(\alpha\) monomers. When tested as single agents, they exhibit no appreciable effect. However, when combined with tumour-opsonising antibodies they augmented phagocytosis in vitro [18]. In vivo, an anti-SIRP\(\alpha\) antibody demonstrated no significant effect against SIRP\(\alpha\)\(^+\) tumours when used as a single agent [59]. However, the combination of anti-SIRP\(\alpha\) with rituximab produced augmented responses in a xenograft model of lymphoma [59]. Anti-SIRP\(\alpha\) antibodies were able to produce anti-tumour effects as single agents against SIRP\(\alpha\)\(^+\) tumours, such as melanoma and renal cell carcinoma [59]. The observed single-agent efficacy is likely due to the ability of the anti-SIRP\(\alpha\) antibody to opsonise the tumour cells and disrupt the CD47/SIRP\(\alpha\) axis.

As another approach to achieving greater specificity towards tumours and avoiding on-target toxicity to healthy cells expressing CD47, bispecific agents have been generated. The first of these agents were directed against B-cell malignancies based on the preclinical success of combined targeting of CD20 and CD47. A bispecific antibody targeting CD19 and CD47 demonstrated efficacy in preclinical studies [60]. Additionally, bispecific antibodies targeting CD20 and CD47 [61] and CD47 and mesothelin are under investigation. Other bispecific agents have been generated by fusing the binding domain of SIRP\(\alpha\) to a tumour-targeting antibody. These have been engineered for dual-targeting of CD47 and either CD20 or CD33 [62,63]. In the case of bispecific agents, having a CD47-blocking component with lower affinity than the tumour-specific component may help limit toxicity.

8. Considerations for clinical development of CD47/SIRP\(\alpha\)-blocking therapies

As described above, one of the greatest distinctions between agents targeting the CD47/SIRP\(\alpha\) interaction is whether they act as a ‘monotherapy’ against cancer or an ‘adjuvant’ to tumour-binding antibodies. Any agent that contains an Fc region that engages activating Fc receptors on macrophages has the potential to act as a monotherapy for cancer. These therapies include anti-CD47 antibodies and SIRP\(\alpha\)-Fc fusion proteins. As a class, they pose a greater risk for on-target toxicity to normal cells expressing CD47. On the other hand, pure CD47/SIRP\(\alpha\) antagonists, such as high-affinity SIRP\(\alpha\) monomers, are not sufficient to induce macrophage phagocytosis but instead act as adjuvant therapies to lower the threshold for phagocytosis in the presence of a separate, tumour-binding antibody. Agents that block SIRP\(\alpha\) directly, such as anti-SIRP\(\alpha\) antibodies, also act as adjuvants to tumour-binding antibodies [18,38]. These agents are more restricted in their potential application since they must be combined with a tumour-binding antibody for a therapeutic effect, and therapeutic antibodies do not currently exist for the majority of cancers. However, they exhibit less toxicity to normal cells expressing CD47 [33], and therefore may exhibit a greater therapeutic window.

Although CD47 is ubiquitously expressed on all cells throughout the body, preclinical studies with anti-CD47 antibodies in mice and cynomolgus macaques suggest these therapeutics are well tolerated overall with anaemia as the predominant toxicity observed [28,33,50]. This toxicity seems to be Fc-dependent since anti-CD47 antibodies and SIRP\(\alpha\)-Fc fusion proteins produce this toxicity whereas high-affinity SIRP\(\alpha\) monomers do not [33,50]. The effect may be avoided if a low ‘priming’ dose is first administered and then followed by higher doses [50]. Similarly, repeated administration of high-affinity SIRP\(\alpha\)-Fc fusion proteins produced a moderate anaemia in mice but did not lead to further reductions in red blood cell indices over time with repeated administration [33]. CD47 serves as an age marker on red blood cells, and older red blood cells
may be more susceptible to phagocytosis [64,65]. Red blood cells may also express ‘eat me signals’ or lack redundant ‘don’t eat me signals’ that could make them more susceptible to elimination by macrophages in the liver and spleen, thereby explaining the observed anaemia.

Due to ubiquitous expression of CD47 throughout the body, a large ‘antigen sink’ may exist that could require large loading doses or frequent drug administration to achieve therapeutic blockade of CD47. Nonetheless, studies in cynomolgus macaques have demonstrated therapeutic serum concentrations of Hu5F9-G4 using dosing strategies that were comparable to those of other therapeutic antibodies [50]. Agents such as high-affinity SIRPα monomers may be challenged by their lower molecular weight (∼14 kDa), which could result in rapid renal clearance. However, a smaller size leads to increased diffusion of macromolecules into tumours [66], and it enables a larger stoichiometric dose to be administered on a weight per volume basis. Furthermore, the extraordinary potency of the high-affinity SIRPα monomers may enable prolonged receptor occupancy and duration of action to achieve efficacy. Development of an ideal CD47-blocking reagent may depend on careful considerations of these pharmacokinetic properties.

SIRPα is more restricted in its histological distribution compared to CD47, offering the possibility for less toxicity and greater blockade when targeted therapeutically. However, in addition to myeloid immune cells, SIRPα is highly expressed on cells of the central and peripheral nervous systems [15]. Although large protein-based therapeutic have difficulty penetrating the blood–brain barrier [67], the potential for neurologic side-effects should be considered when using agents targeting SIRPα directly. Due to their sequence similarity, possible cross-reactivity between other SIRP family members (SIRPβ and SIRPγ) may also occur, and the consequences of targeting these receptors is not yet fully understood [14]. Given that at least ten polymorphisms of human SIRPα have been identified [8], the optimal therapeutic agent may be one that is able to antagonise all SIRPα alleles.

9. The CD47/SIRPα axis and the adaptive immune system

Beyond activation of innate immune cells, mounting evidence suggests CD47 blockade can also initiate or augment adaptive immune responses. In a mouse ovalbumin (OVA) tumour model, treatment with anti-CD47 antibodies resulted in greater phagocytosis and antigen presentation [34]. Transfer of macrophages that had engulfed tumour cells into immunocompetent mice was able to protect the mice from a subsequent challenge with B16 cells expressing OVA [34]. These results suggested CD47 blockade could mediate a link between innate and adaptive immunity. In a subsequent study, anti-CD47 treatment was found to stimulate dendritic cell antigen uptake from tumours [40]. In immunocompetent murine models of lymphoma and lung cancer, robust anti-tumour responses seemed to be partially dependent on the presence of an intact immune system [40]. Direct intratumoural injections were able to produce systemic anti-tumour responses using anti-CD47 antibodies and high-affinity SIRPα-Fc fusion proteins [40]. This process was mediated by the STING pathway.

The link between innate and adaptive immunity suggests that agents targeting CTLA-4, PD-1/PD-L1, or other adaptive immune checkpoints could be enhanced by combination with CD47/SIRPα-blocking agents. In support of this hypothesis, a recent study engineered a nanobody that antagonises murine CD47 [68]. This agent exhibited no substantial anti-tumour efficacy on its own, but was able to produce robust anti-tumour responses when combined with a tumour-opsonising antibody and an anti-PD-L1 antibody [68]. Additionally, the combination of an anti-SIRPα antibody with an anti-PD-1 antibody produced augmented responses relative to single agent controls in a syngeneic mouse model of colon cancer [59]. Further exploration of these immunotherapeutic combinations is warranted in preclinical and clinical investigations.

10. Additional applications of CD47/SIRPα-blocking therapies

The principles of targeting the CD47/SIRPα axis can also be applied to other non-malignant states of disease. In theory, diseased cells may be more susceptible to macrophage phagocytosis, and any therapeutic antibody that depletes cells by engaging immune effector functions could be enhanced by combination with CD47-blocking agents. Possible combinations include those with antibodies targeting persistently infected cells, such as broadly neutralising HIV antibodies [69,70]. Therapeutic antibodies can also be used to deplete immune subsets in inflammatory or autoimmune disorders. For example, rituximab is used to deplete non-malignant B-cells in rheumatoid arthritis [71]. As additional antibodies become available for clinical use, they should be evaluated for synergy with agents targeting the CD47/SIRPα axis.

A recent study examined the use of CD47 blockade as a conditioning regimen for haematopoietic cell transplantation [72]. In order to achieve successful transplant, host HSCs must be cleared from their niches within the bone marrow microenvironment to allow engraftment of donor HSCs. Conventional methods of niche clearance depend on myeloablative chemotherapy and radiation regimens to non-specifically eliminate cells from the bone marrow. A plethora of diseases could benefit
from haematopoietic stem cell transplant, but a major barrier to more widespread application lies in the burden of morbidity and mortality from conditioning. As a method to limit these side-effects and therefore enable more widespread use of haematopoietic cell transplantation, HSC niche clearance could be achieved using antibodies to c-Kit, a marker of haematopoietic stem cells [73]. By combining a CD47-blocking agent with anti-c-Kit antibodies, complete niche clearance can be achieved [72]. Consequently, the combination of CD47 blockade with anti-c-Kit antibodies permits successful engraftment of donor HSCs in the absence of cytotoxic chemotherapy or radiation [72].

The application of CD47-blocking therapies may also extend beyond disease in humans. Cancer is the leading cause of disease-related mortality in dogs [74]. Canine lymphoma bears strong similarity to human lymphoma, including expression of CD20 [75]. Therapeutic antibodies targeting canine lymphoma are now under development, including a speciated anti-canine CD20 antibody [76]. The CD47/SIRPα axis is biochemically and functionally conserved in dogs, and the high-affinity SIRPα variants cross-react with canine CD47 [49]. As Fc fusion proteins, they exhibited single-agent efficacy against canine lymphoma in mouse xenograft models [49]. Moreover, when combined with anti-canine CD20 antibody 1E4-cIgGb, they eradicated all evidence of lymphoma in the mice [49]. These studies suggest the potential of anti-CD47 therapies for the benefit of companion animals, and may further inform studies in humans.

11. Conclusions

Myeloid cells hold much promise as effectors of cancer immunotherapy, and therapeutic targeting of the CD47/SIRPα axis may unlock their potential. Preclinical studies indicate CD47/SIRPα-blocking therapies are effective against a broad range of cancers, and ongoing clinical trials will determine their efficacy as single agents and as combination therapies. The application of these therapeutics extends beyond human cancer, and additional preclinical and clinical studies will reveal their benefit to patients.

Conflict of interest statement

K.W. is an inventor of US patent applications pertaining to CD47-blocking assigned to Stanford University. K.W. declares consulting and/or equity ownership in Alexo Therapeutics, Inc. and Forty Seven Inc.

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