Review

Structural analysis and binding sites of inhibitors targeting the CD47/SIRPα interaction in anticancer therapy

Bo Huang a, Zhaoshi Bai c,*, Xinyue Ye b, Chenyu Zhou b, Xiaolin Xie a,b, Yuejiao Zhong c, Kejiang Lin a,*, Lingman Ma a,b,*

*Department of Medicinal Chemistry, School of Pharmacy, China Pharmaceutical University, 639 Longmian Road, Nanjing, Jiangsu 211198, China
b School of Life Science and Technology, China Pharmaceutical University, 639 Longmian Road, Nanjing, Jiangsu 211198, China
* Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & the Affiliated Cancer Hospital of Nanjing Medical University, Baiziting 42, Nanjing, Jiangsu 210009, China

E-mail addresses: baizhaoshi23@126.com (Z. Bai), link@cpu.edu.cn (K. Lin), 1620174416@cpu.edu.cn (L. Ma).

© 2021 The Author(s). Published by Elsevier B.V. on behalf of Research Network of Computational and Structural Biotechnology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ABSTRACT

Cluster of differentiation 47 (CD47)/signal regulatory protein alpha (SIRPα) is a negative innate immune checkpoint signaling pathway that restrains immunosurveillance and immune clearance, and thus has aroused wide interest in cancer immunotherapy. Blockade of the CD47/SIRPα signaling pathway shows remarkable antitumor effects in clinical trials. Currently, all inhibitors targeting CD47/SIRPα in clinical trials are biomacromolecules. The poor permeability and undesirable oral bioavailability of biomacromolecules have caused researchers to develop small-molecule CD47/SIRPα pathway inhibitors. This review will summarize the recent advances in CD47/SIRPα interactions, including crystal structures, peptides and small molecule inhibitors. In particular, we have employed computer-aided drug discovery (CADD) approaches to analyze all the published crystal structures and docking results of small molecule inhibitors of CD47/SIRPα, providing insight into the key interaction information to facilitate future development of small molecule CD47/SIRPα inhibitors.

Contents

1. Introduction ..................................................................................................... 5495
2. Structures of CD47/SIRPα complexes and cocrystal structures of monoclonal antibodies ................................................................................................................................. 5496
3. Bioactive peptide inhibitors blocking the CD47/SIRPα interaction ................. 5497
   3.1. CD47-targeted peptides .................................................................................. 5498
      3.1.1. Pep-20 and its derivatives ....................................................................... 5498
      3.1.2. Rs-17 .................................................................................................. 5498
   3.2. SIRPα-targeted peptides .............................................................................. 5498
      3.2.1. d4-2 .................................................................................................. 5498
      3.2.2. Sp5 .................................................................................................. 5498
   3.3. CADD guides the design of peptide inhibitors ............................................ 5498
4. Small molecule inhibitors blocking the CD47/SIRPα interaction ..................... 5498
   4.1. NCC00138783 and its derivatives ................................................................. 5498
   4.2. 1,2,4-oxadiazole compounds ..................................................................... 5499
   4.3. CADD guides the design of small molecule inhibitors ............................... 5501
5. Summary and outlook .................................................................................... 5501
CRediT authorship contribution statement .......................................................... 5501
Declaration of Competing Interest ....................................................................... 5502

* Corresponding authors at: Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & the Affiliated Cancer Hospital of Nanjing Medical University, Baiziting 42, Nanjing, Jiangsu 210009, China (Z. Bai); Department of Medicinal Chemistry, School of Pharmacy, China Pharmaceutical University, 639 Longmian Road, Nanjing, Jiangsu 211198, China (K. Lin); School of Life Science and Technology, China Pharmaceutical University, 639 Longmian Road, Nanjing, Jiangsu 211198, China (L. Ma).

https://doi.org/10.1016/j.csbj.2021.09.036
2001-0370/© 2021 The Author(s). Published by Elsevier B.V. on behalf of Research Network of Computational and Structural Biotechnology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

Currently, the high morbidity and mortality of tumors remain a major challenge to the effectiveness of cancer treatment even though a series of anticancer drugs have been developed based on different treatment strategies [1]. Cancer immunotherapy, which aims to improve antitumor immune responses with fewer off-target effects than chemotherapy and other agents that directly kill cancer cells, provides an alternative strategy to treat cancer through the immune system rather than the tumor itself [2] and is an exciting area in current cancer research [3,4]. This novel therapy, including immune checkpoint blockade, adoptive cellular therapy and cancer vaccinology [5], has led to a growing number of immunotherapy drug approvals, with numerous treatments in clinical and preclinical development [3].

Herein, the immune checkpoint plays a central role in tissue homeostasis self-reactivity and autoimmunity, which targets the innate and adaptive immune systems [6], and is the signal recognition factor for immunosurveillance in immune cells [7]. However, some tumor cells evade immune clearance by modulating signal recognition between immune cells and tumor cells; thus, immune checkpoint blockade is the main promising system in immunotherapy [8,9]. To date, the blockade of two well-known adaptive immune checkpoint regulators because of its inhibitory effect on the activation of macrophages and other myeloid cells, such as monocytes, macrophages, neutrophils, and dendritic cells (DCs) [10,24,25], CD47 interacts with SIRPα, tagging it with a “self” or “do not eat” signal, to trigger an inhibitory signaling cascade through the ITIM and ITSM motifs of SIRPα, inhibiting macrophage phagocytosis (Fig. 1) [26–28]. Unfortunately, when overexpressed CD47 on the surface of solid malignancies binds to SIRPα on macrophages, this can suppress the phagocytic responses of macrophages [29]. Therefore, as a dominant macrophage checkpoint, disruption of the CD47/SIRPα pathway can induce macrophage-mediated phagocytosis of tumor cells and thus is employed when generating next-generation immunoregulatory drugs [30–32]. Currently, the gradual increase in patents with treatments targeting the CD47/SIRPα pathway clearly demonstrates the great effort being made to discover new inhibitors of this target. Consequently, some CD47/SIRPα pathway-targeting antibodies against various cancers (Table 1), including acute myeloid leukemia (AML) [33,34], anaplastic thyroid carcinoma (ATC) [35], lymphoma [36–38], lung cancer [39,40] and breast cancer [41], have showed attractive results. For example, magrolimab (Hu5F9-G4) is an anti-CD47 monoclonal antibody that is currently undergoing phase III clinical trials, with an objective response rate of 75% in phase Ib clinical results during the treatment of myelodysplastic syndromes. ALX-148 is also a CD47 targeting antibody in phase II clinical trials whose objective responses could be observed in phase I clinical results for the treatment of patients with head and neck squamous cell carcinoma. It is undeniable that some adverse effects occur when using these antibodies therapeutically, such as the rapid target-mediated clearance, transient anemia, erythrocyte toxicity and infusion-related reactions [42], which limit their rapid development. In addition, there are certain limitations of using these antibodies, including a long half-life, poor permeability and lacking oral availability. Thus, the development of low molecular-weight inhibitors with superb pharmacokinetics and druggability is an effective strategy.
and research focus to overcome the limitations of therapeutic antibodies [43]. Because the development of small molecule inhibitors of the CD47/SIRPα interaction has been slower than the development of antibody treatments, research on the CD47/SIRPα pathway is crucial. Currently, high-throughput screening and computer-aided drug design (CADD) are common approaches in the discovery of small molecule inhibitors [44–48]. CADD is a molecular design method based on computational chemistry [49,50] and can help to produce valuable information on target proteins, lead compounds and protein–ligand interactions for rational drug design. Thus, CADD is used both to analyze hot spots of target proteins and protein–ligand interactions model for further drug discovery. In this review, we have employed the “View Interactions” tool in CADD to analyze the hot spots of CD47 and SIRPα based on their cocrystal structures. Subsequently, “LibDock” in CADD was further applied to predict the key interacting amino acids of CD47 and SIRPα from a small molecule inhibitor docking experiment. In addition, some of the peptides and small molecule inhibitors that have been reported to block the CD47/SIRPα interaction in fundamental research studies have been summarized, and their structure–activity relationships have been analyzed and compared to guide the discovery and design of new inhibitors blocking the CD47/SIRPα interaction with superb pharmacokinetics and druggability.

2. Structures of CD47/SIRPα complexes and cocrystal structures of monoclonal antibodies

The first high-resolution crystallographic structure of the CD47/SIRPα d1 complex (the ligand-binding domain) was published by

| Code Name (Generic Name) | Target | Organization | Therapeutic Groups | Highest Phase |
|-------------------------|--------|--------------|--------------------|--------------|
| Hu5F9-G4 (Magrolimab)   | CD47   | Gilead       | Bladder Cancer, Breast Cancer, Colorectal Cancer, Hematological Cancer, Lymphoma, Myeloid Leukemia, Non-Hodgkin’s Lymphoma, Ovarian Cancer. | III |
| ALX-148                 | CD47   | Alexo Therapeutics | Gastric Cancer, Head and Neck Cancer, Non-Hodgkin’s Lymphoma. | II |
| TJC-4 (Lemzoparlimab)   | CD47   | AbbVie       | Lymphoma, Myeloid Leukemia. | II |
| DSP-107                 | CD47   | KAHR Medical | Non-Small Cell Lung Cancer. | II |
| IBI-188 (Letaplilimab)  | CD47   | Innoven Biologics | Myeloid Leukemia, Non-Hodgkin’s Lymphoma, Ovarian Cancer. | II |
| AO-176                  | CD47   | Arch Oncology | Lymphoma, Multiple Myeloma. | II |
| TTI-622                 | CD47   | Trilium Therapeutics | Lymphoma, Multiple Myeloma, Myeloid Leukemia, Non-Hodgkin’s Lymphoma, Ovarian Cancer. | II |
| ZL-1201                 | CD47   | ZAI Lab      | Lymphoma Therapy. | I |
| AK-117                  | CD47   | Akeso Biopharma | Non-Hodgkin’s Lymphoma Therapy. | I |
| IMC-002                 | CD47   | ImmuneOncia Therapeutics | Lymphoma. | I |
| SRF-231                 | CD47   | Surface Oncology | Hematological Cancer Therapy, Lymphocytic Leukemia Therapy, Lymphoma Therapy, Multiple Myeloma Therapy. | I |
| CC-90002                | CD47   | Celgene      | Myeloid Leukemia Therapy, Non-Hodgkin’s Lymphoma Therapy. | I |
| TTI-621                 | CD47   | Trilium Therapeutics | Hematological Cancer Therapy, Lymphocytic Leukemia Therapy, Myeloid Leukemia Therapy, Non-Hodgkin’s Lymphoma Therapy. | I |
| GS-0189                 | SIRPα  | Gilead       | Oncolytic Drug. | I |
| CC-95251                | SIRPα  | Celgene      | Solid Tumors. | I |
| FSI-189                 | SIRPα  | Gilead       | Non-Hodgkin’s Lymphoma | I |
| BI-765063               | SIRPα  | Boehringer Ingelheim | Solid Tumors Therapy | I |

| Table 1 | CD47/SIRPα targeting mAbs in clinical trial. |
|---------|-----------------------------------------------|
| Code Name (Generic Name) | Target | Organization | Therapeutic Groups | Highest Phase |
| Hu5F9-G4 (Magrolimab)   | CD47   | Gilead       | Bladder Cancer, Breast Cancer, Colorectal Cancer, Hematological Cancer, Lymphoma, Myeloid Leukemia, Non-Hodgkin’s Lymphoma, Ovarian Cancer. | III |
| ALX-148                 | CD47   | Alexo Therapeutics | Gastric Cancer, Head and Neck Cancer, Non-Hodgkin’s Lymphoma. | II |
| TJC-4 (Lemzoparlimab)   | CD47   | AbbVie       | Lymphoma, Myeloid Leukemia. | II |
| DSP-107                 | CD47   | KAHR Medical | Non-Small Cell Lung Cancer. | II |
| IBI-188 (Letaplilimab)  | CD47   | Innoven Biologics | Myeloid Leukemia, Non-Hodgkin’s Lymphoma, Ovarian Cancer. | II |
| AO-176                  | CD47   | Arch Oncology | Lymphoma, Multiple Myeloma. | II |
| TTI-622                 | CD47   | Trilium Therapeutics | Lymphoma, Multiple Myeloma, Myeloid Leukemia, Non-Hodgkin’s Lymphoma, Ovarian Cancer. | II |
| ZL-1201                 | CD47   | ZAI Lab      | Lymphoma Therapy. | I |
| AK-117                  | CD47   | Akeso Biopharma | Non-Hodgkin’s Lymphoma Therapy. | I |
| IMC-002                 | CD47   | ImmuneOncia Therapeutics | Lymphoma. | I |
| SRF-231                 | CD47   | Surface Oncology | Hematological Cancer Therapy, Lymphocytic Leukemia Therapy, Lymphoma Therapy, Multiple Myeloma Therapy. | I |
| CC-90002                | CD47   | Celgene      | Myeloid Leukemia Therapy, Non-Hodgkin’s Lymphoma Therapy. | I |
| TTI-621                 | CD47   | Trilium Therapeutics | Hematological Cancer Therapy, Lymphocytic Leukemia Therapy, Myeloid Leukemia Therapy, Non-Hodgkin’s Lymphoma Therapy. | I |
| GS-0189                 | SIRPα  | Gilead       | Oncolytic Drug. | I |
| CC-95251                | SIRPα  | Celgene      | Solid Tumors. | I |
| FSI-189                 | SIRPα  | Gilead       | Non-Hodgkin’s Lymphoma | I |
| BI-765063               | SIRPα  | Boehringer Ingelheim | Solid Tumors Therapy | I |
Hatherley et al. in 2008 and the identifier of this complex in Protein Data Bank is 2JJT (PDB ID: 2JJT) [51]. Within the crystal, CD47 and SIRPα d1 form a 1:1 stoichiometry complex. The CD47 and SIRPα d1 molecules are interdigitated to each other so that their interaction is mainly mediated by loops at the intracellular side, which is consistent with what had been proposed by other authors based on their analyses [52]. The CD47/SIRPα d1 interaction interface is mainly formed of four N-terminal loops of the SIRPα d1 domain and the FG loop of CD47, which embeds into the cavity on the surface of SIRPα d1. Thr102 of the FG loop inserts deep into SIRPα d1 (Fig. 2, produced by Discovery Studio2019). The hot spot residue-mediated polar interactions on CD47 comprise Glu97, Thr99, Glu100, Arg103, Glu104, and Glu106. Among them, Glu104 and Glu106 of CD47 form hydrogen bonds with SIRPα (Table 2), and the BC loop surrounding the FG loop interacts with the wide edge of the SIRPα groove. Comparative analysis of the CD47/SIRPα d1 complex structures with isolated structures of CD47 and SIRPα demonstrates that complex formation slightly impacts the backbone of CD47. In contrast, the complex rearranges the CD47-interacting loops in SIRPα d1. In addition, Weiskopf et al. reported the high-affinity SIRPα variant F(D6/CD47 complex in 2013 [53]. The root mean square deviation (RMSD) between the CD47/C47B161 complexes demonstrate that the interaction interface of all three antibodies overlaps with the SIRPα binding epitope regions on the FG loop of CD47. Thus, these complex structures demonstrate that the FG loop of CD47 is a key component of the interaction, indicating that the FG loop may become a potential target for further structure-based drug design.

Magrolimab mainly binds to N-terminal pyroglutamate of CD47 which is critical for CD47/SIRPα interaction and magrolimab binds to the BC and FG loops, which are highly overlapping epitopes with SIRPα [55,56]. Analogously, the common binding area of B6H12.2, C47B161 and C47B222 is the C’ and FG loops on CD47. According to these findings, the N-terminal pyroglutamate and the BC, C’ and FG loops can be viewed as potential binding areas for subsequent structure-based drug design, and Tyr37, Asp46, Glu97, Glu100 and Glu106 may be developed into binding sites for inhibitors targeting CD47. Additionally, the loops of SIRPα undergo structural changes in the interaction with CD47, which indicates that CD, DE, and FG loops may be possible targets. Among these loops, Glu54, Gly55, Ser66 and Ser98 undergo considerable movement, implying that these residues play crucial roles in the interaction and may act as binding sites for designing future SIRPα inhibitors.

### Table 3

| Code Name (Generic Name) | Target  | Organization | Sequence |
|--------------------------|---------|--------------|----------|
| RS-17 [58]               | CD47    | China Pharmaceutical University | RRYKQDGWSSHPWSS-NH2 |
| Pep-20 [55]              | CD47    | Sun Yat-sen University and Zhengzhou University | AWSATWSNYWRH |
| D4-2 [59,60]             | SIRPα   | Kobe University | Ac-yRYSAYSHPSWCG-NH2 |
| SPS [62]                 | SIRPα   | Zhengzhou University | CTQDAWHIC |

3. Bioactive peptide inhibitors blocking the CD47/SIRPα interaction

The development of non-antibodies with succinct synthesis and lower modification cost has led to the discovery of RS-17, Pep-20, D4-2, and SPS.
D4-2, and SP5. All four peptides, whose sequences are listed in Table 3, can directly block the CD47/SIRPα interaction. RS-17 and Pep-20 block the CD47/SIRPα interaction by binding to CD47, while D4-2 and SP5 can bind to SIRPα to disrupt the CD47/SIRPα interaction.

3.1. CD47-targeted peptides

3.1.1. Pep-20 and its derivatives

In 2020, Pep-20 and its derivatives that have comparable affinity to the CD47/SIRPα interaction were identified by Wang et al. using a subtractive phage biopanning strategy [57]. The K<sub>d</sub> values of pep-20 binding to human and mouse CD47 are 2.91 ± 1.04 μM and 3.63 ± 1.71 μM, respectively, which are close to that of cognate SIRPα [58,59]. In addition, a human CD47/SIRPα blocking assay also revealed that pep-20 exhibited an IC<sub>50</sub> of 24.56 μM with the anti-CD47 antibody (B6H12), which served as a positive control. Pep-20 remarkably enhances the phagocytosis of MCF7 (human breast tumor cell lines), HT29 (human colon tumor cell lines) and Jurkat (human leukemia cell lines) and Jurkat (human leukemia cell lines) and exhibits an enhancement of phagocytosis similar to that of the positive control (B6H12). Excitingly, the injection of pep-20 at a dose of 2 mg/kg daily in mice had no obvious influence on the reduction in the number of red blood cells, which is a common toxicity effect of CD47/SIRPα blockade [57]. Furthermore, after replacing three terminal residues of pep-20 with D-amino acids, the obtained peptide pep-20-D12 significantly improved stability without a functional decrease compared with pep-20, accompanied by an intravenous elimination T1/2 that increased by tenfold compared with pep-20. Pep-20-D12 remarkably slows tumor progression, and the combination treatment of pep-20-D12 and IR shows tumor growth regression in colon tumor (MC38 cells)-bearing mice [57]. A subsequent docking model and aline substitution experiment of pep-20/CD47 revealed that Phe<sub>56</sub>, Glu<sub>104</sub> and Gln<sub>106</sub> of CD47 are key positions for inhibitors targeting CD47. These findings provide valuable information of CD47 binding sites and the key structure of pep-20 for small-molecule inhibitor design.

3.1.2. Rs-17

Additionally, Xu et al. from China Pharmaceutical University discovered RS-17 in 2020 [60]. The K<sub>d</sub> value of RS-17 binding to the CD47 protein was 3.85 ± 0.79 nM. At a concentration of 20 μg/ml, RS-17 effectively binds to CD47 of SCC-13 (human epidermal squamous tumor cells) and HepG2 (human liver tumor cells) with corresponding binding rates of 55.5% and 71.2%, respectively; thus, the phagocytic efficiency of macrophages against cells) with corresponding binding rates of 55.5% and 71.2%, respectively, which are close to that of cognate SIRPα [58,59]. Moreover, an in vivo assay demonstrated that the weight loss and tumor volume increase in liver tumor-bearing mice were similar between RS-17 and B6H12 and that RS-17 effectively inhibited tumor growth in liver tumor-bearing mice.

3.2. SIRPα-targeted peptides

3.2.1. d4-2

Hazarma et al. utilized random nonstandard peptides integrated discovery (RaPID) system, which combines flexzyme-assisted generic code reprogramming and mRNA display to obtain macrocyclic peptides of interest, to design and gain anti-SIRPα peptides L4-4, D4-1, D4-2 and D4-4 [61,62]. Among these peptides, D4-2 shows comprehensive high binding affinity to SIRPα of C57BL/6 and NOD mouse strains with corresponding K<sub>d</sub> values of 10 nM and 8.22 nM, respectively. D4-2 evidently blocked the mCD47-Fc/NOD SIRPα interaction in a dose-dependent manner in HEK293A (human embryonic kidney cells) cells with an IC<sub>50</sub> value of 0.180 mM. The crystal structure of the D4-2/NOD SIRPα complex shows that the interaction area of D4-2/NOD SIRPα occupies 976.5 Å<sup>2</sup>, Arg<sub>2</sub>, Ser<sub>6</sub>, Ala<sub>7</sub>, Val<sub>6</sub>, Ile<sub>7</sub>, His<sub>10</sub>, Pro<sub>11</sub>, Ser<sub>12</sub>, Trp<sub>13</sub> and Gly<sub>15</sub> of D4-2 form hydrogen bonds with IgV-NOD SIRPα, and Arg<sub>2</sub> of D4-2 forms a salt bridge with Asp<sub>84</sub> of IgV-NOD SIRPα. Ala<sub>9</sub> and Pro<sub>11</sub> of D4-2 form hydrophobic interactions with Phe<sub>51</sub> and Phe<sub>56</sub> of IgV-NOD SIRPα. All these residues stabilize the cyclic structure of D4-2 and mediate the binding of D4-2 to IgV-NOD SIRPα. Further crystal structure comparison shows that the binding of D4-2 to Phe<sub>56</sub> and Ala<sub>55</sub> in the C′E loop of IgV-NOD SIRPα, which are key residues controlling the interaction with CD47, changes the conformation and induces the inhibition of the CD47/IgV-NOD SIRPα interaction [63].

3.2.2. Sp5

In addition to D4-2, a series of macrocyclic peptides binding to SIRPα, including Sp1 to Sp6, were also developed in 2020 by Xu et al from Zhengzhou University [64]. Among these, Sp4 and Sp5 display higher affinity to SIRPα with K<sub>d</sub> values of 0.85 μM and 0.38 μM and block the SIRPα/CD47 interaction in a dose-dependent manner in CHO-K1-hSIRPα cells. Sp5 (200 μM) not only effectively promotes the phagocytosis of HT29 (human colon tumor cells) by macrophages but also exhibits desirable in vivo efficacy by inhibiting tumor growth in colon tumor MC38 mouse model and melanoma B16-OVA mouse model.

3.3. CADD guides the design of peptide inhibitors

Analysis of the previously described peptides can lead to conclusions that these peptides share similar interaction areas that overlap with the epitopes in the CD47/SIRPα interaction area. For example, Pep-20 occupies Phe<sub>4</sub>, Gln<sub>104</sub> and Gln<sub>106</sub> of CD47 to block the D47-SIRPα interaction, and D4-2 binds to Phe<sub>56</sub> and Ala<sub>55</sub> of SIRPα to block the D47-SIRPα interaction. Moreover, these peptides mainly form hydrogen bonds with their receptor. Collectively, residues Phe<sub>4</sub>, Gln<sub>104</sub> and Glu<sub>106</sub> of CD47 and residues Phe<sub>56</sub> and Ala<sub>55</sub> of SIRPα may be developed into binding sites for structure-based CD47/SIRPα small molecule inhibitor design.

4. Small molecule inhibitors blocking the CD47/SIRPα interaction

4.1. NCCG00138783 and its derivatives

Miller et al. utilized quantitative high-throughput screening (qHTS) assays to screen NCATS chemical libraries based on time-resolved Förster resonance energy transfer (TR-FRET) and bead-based luminescent oxygen channeling assay formats (AlphaScreen), resulting in the discovery of the parent compound NCCG00138783 [65,66], whose scaffold is 2-((2-(2-(3,5-dimethyl-1H-pyrazol-4-yl)ethyl)-5,6-dihydro-[1,2,4] triazolo [1,5-c] quinazolin-5-yl)thio) butanil. This compound selectively blocks the CD47/SIRPα interaction without disrupting its binding to other receptors [67–69]. A novel laser scanning cytometry assay (LSC) was established to measure the cell surface binding of these compounds, and the results showed that NCCG00138783 has an IC<sub>50</sub> value of 40 μM. Further medicinal chemistry work attempting to optimize the potency and drug-like properties of NCCG00138783 led to the discovery of its derivatives (Fig. 4, produced by ChemDraw). The acyl group of NCCG00138783 is linked with a monocyclic substituted amino group and hydroxyl group to obtain a range of compounds displaying great inhibitory activity toward the CD47/SIRPα interaction. Among these small molecule compounds, NCCG00138783, NCC00538430 and NCC00538419
showed antagonistic activity in both the ALPHA screening assay and LSC assay.

To facilitate the understanding of NCGC00138783 binding to CD47/SIRPα, we conducted docking experiments of NCGC00138783 docking to CD47 and SIRPα and employed the CD47/SIRPα complex (PDB ID: 2JJT) as a receptor. Consequently, we found that NCGC00138783 is more prone to bind to SIRPα than CD47 with the corresponding highest LibDock Score of 134 and 85. Furthermore, the 3,5-dimethyl-1H-pyrazolyl group, central [1,2,4] triazolo[1,5-c] quinazoline group and amide group of NCGC00138783 are predicted to form hydrogen bonds and T-stacking interactions with SIRPα, including Leu10, Gly34, Pro35, Gln52, Lys53 and Lys93, which are key residues in the CD47/SIRPα interaction. The central [1,2,4] triazolo[1,5-c] quinazoline scaffold is predicted to form pi-pi stacking with Phe74 and hydrogen bonding with Gly93, which makes it insert into the hydrophobic cavity. The amide group of 3,5-dimethyl-1H-pyrazolyl is predicted to form hydrogen bonding with Gln52, which lies in the high polarity area (Fig. 5, produced by Discovery Studio2019). Above all, NCGC00138783 binds to SIRPα and occupies the key binding positions of the CD47/SIRPα interaction which is essential information for future small molecule inhibitor design and helpful for the discovery of novel inhibitors blocking the CD47/SIRPα interaction.

4.2. 1,2,4-oxadiazole compounds

In a patent application, inventors from Aurigene Discovery Technologies Limited reported a series of small molecules blocking the CD47/SIRPα interaction [70]. The scaffold of these compounds is oxadiazole which can enhance macrophage-mediated phagocytosis of human lymphoma and myeloma cells, with corresponding normalized phagocytosis rates in the range of 20%–66% and 17%–77% at 10 μM. Among these small molecules, compounds 1 to 14 display comprehensive effects on both luciferase-based and
FACS-based phagocytosis assays, and compound 6 has normalized phagocytosis rates of 66% and 74%, respectively (Fig. 6, produced by ChemDraw). Moreover, compound 6 inhibited tumor growth in a dose-dependent manner with inhibition rates of 53%, 64% and 67% at doses of 3, 10 and 30 mg/kg, respectively, in an A20 mouse model without body weight loss.

Further docking analyses of compound 6 to CD47 (PDB ID: 2JJT) by us revealed that the core 1,2,4-oxadiazol and butyramide groups insert into a hydrophobic pocket containing Trp, Thr and Lys of CD47, which is the key residue in the CD47/SIRPα interaction. The carbonyl group of butyramide is predicted to form hydrogen bonds with Thr7 and Thr107, which is near the core.
CD47/SIRPα interaction area. Two carbonyl groups of carbamoyl proline are predicted to interact with Asn⁵, which is close to Lys⁶ of CD47 (Fig. 7, produced by Discovery Studio2019). Overall, promising compound 6 could inspire the design of follow-up lead compound scaffolds, and the binding model of compound 6 to CD47 may provide information for further discovery of small molecules blocking the CD47/SIRPα interaction.

4.3. CADD guides the design of small molecule inhibitors

Analysis of our above docking results and the published phagocytosis assays revealed the common structural characteristics of anti-CD47 compounds: (i) hydrogen bond interactions are crucial for anti-CD47 compound activity; (ii) the 2-position side chain of oxadiazol inserted into the pocket consisting of Asn⁵, Thr⁷, Pro²², Phe²⁴ and Thr¹⁰⁷ has a crucial effect on phagocytic activity; (iii) the terminal group of the oxadiazol 2-position side chain, including the amino group, carbonyl group, amide group and guanidine group, which can form hydrogen bonds with Thr⁷ and Thr¹⁰⁷ in the pocket, exhibits higher phagocytic activity; and (iv) the oxadiazol 5-position side chain contains a terminal carbonyl group and ureido. Oxadiazol, ureido and carbonyl groups are separated by one carbon atom, which makes two carbonyl groups in suitable positions to form hydrogen bonds with Asn⁵; (v) the substituted short carbon chain and substituted ring alpha carbon of the terminal carbonyl group in the oxadiazol 5-position side chain can achieve better activity; (vi) central oxadiazole is needed for p-lone pair conjugation with Thr⁷.

In addition, analysis of anti-SIRPα compounds also indicates several features: (i) hydrophilic interactions are essential for SIRPα binding; (ii) central quinazoline reaches a hydrophobic pocket containing Val²⁷, Leu¹⁰¹, Ile¹⁰⁶, Phe²⁴ and Lys⁹³ to form two T-stacking interactions with Phe²⁴; and (iii) amide groups forming hydrogen bonds with Gln⁵², benzene, and four-membered ring- or three-membered ring- substituted amide groups show favorable antagonistic activity.

5. Summary and outlook

Recently, CD47/SIRPα inhibitors have aroused enormous interest among researchers and have been remarkably affective in cancer treatment. This field is progressing rapidly, and some mAbs targeting the CD47/SIRPα pathway have reached clinical phase II and phase III [71]. Notably, despite the excellent clinical performance shown by CD47/SIRPα antibodies, the limitations of antibody drugs including poor tumor permeability, undesirable oral bioavailability and poor stability, hinder their clinical application [72–75]. Small-molecule inhibitors can eliminate the problems caused by antibody drugs and thus have attracted the attention of researchers and have become a promising research area.

Currently, several crystal structures of CD47/SIRPα and the structures of antibodies together with receptors have been published which provides guidance for the rational design of small molecule inhibitors blocking the CD47/SIRPα interaction. Moreover, there are two published small molecule compound categories: small molecules containing 3,5-dimethyl-1H-pyrazolyl and [1,2,4] triazolo[1,5-c] quinazoline scaffolds and small molecules containing oxadiazole scaffolds. Unfortunately, no small molecules blocking the CD47/SIRPα interaction have reached clinical research yet. The shortage of target structure information limits the development of small molecule inhibitors. Excitingly, using CADD to analyze the CD47/SIRPα interaction will provide some crucial information for the design of small molecule inhibitors. First, CADD allows researchers to analyze the interaction between inhibitors and their receptors based on their crystal structures, which improves the understanding of the interaction process and helps to determine key information, including pocket atoms and hot spot residues. Next, CADD helps researchers to explore the interaction between inhibitors and receptors without crystal structure through docking. Here, based on the previous reports and our docking research, we conclude that Glu¹⁰⁴ and Glu¹⁰⁶ are hot spots on CD47, while Gln⁵², Lys⁵³ and Phe⁵⁶ are hot spots on SIRPα.

There is a limited number of small molecule inhibitors targeting the CD47/SIRPα pathway, which indicates the early stage of this research, but the favorable clinical results are promising. The CADD technologies will accelerate the discovery of novel inhibitors. These peptides and small molecule inhibitors will be the foundation for the design of new compounds. As it relates to drug design, the interaction area of CD47/SIRPα is broad. Therefore, it is crucial to identify the best binding positions for small molecules, and drug design based on these structural data will lead to the successful development of CD47/SIRPα inhibitors.

CRediT authorship contribution statement

Bo Huang: Conceptualization, Writing – original draft, Writing – review & editing, Visualization. Zhaoshi Bai: Supervision, Writ-
ing – review & editing. Xinyue Ye: Visualization. Chenyu Zhou: Resources. Xiaolin Xie: Visualization. Yuejiao Zhong: Resources. Kejiang Lin: Supervision, Conceptualization. Lingman Ma: Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation (81903642), China Postdoctoral Science Foundation (2020M681528), Postdoctoral Science Foundation of Jiangsu Province (2021K369C) and Jiangsu Cancer Hospital Postdoctoral Science Foundation (SZL202015).

References

[1] Wang H, Yin Y, Wang P, Xiong C, Huang L, Li S, et al. Current situation and future usage of anticancer drug databases. Apostasis 2016;21:778–94.
[2] Couzin-Frankel J. Cancer immunotherapy. Science 2013;343:1432–3.
[3] Riley RS, June CH, Langer R, Mitchell MJ. Delivery technologies for cancer immunotherapy. Nat Rev Drug Discov 2019;18:175–96.
[4] Rosenberg SA. IL-2: the first effective immunotherapy for human cancer. J Immunol 2014;192:5451–8.
[5] Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol 2020;20:651–68.
[6] Khari DO, Bax HJ, Mele S, Crescioli S, Pellizzari G, Khambay A, et al. Combining immune checkpoint inhibitors: established and emerging targets and strategies to improve outcomes in melanoma. Front Immunol 2019;10.
[7] Zitvogel L, Tesniere A, Kroemer G. Cancer despite immunosurveillance: selection-immune selection and immunosurveillance. Nat Rev Immunol 2006;6:715–7.
[8] Fardell DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252–64.
[9] Sadreddini S, Baradaran B, Aghebati-Maleki A, Sadreddini S, Shanehbandi D, Fotouhi A, et al. Immune checkpoint blockade opens a new way to cancer immunotherapy. J Cell Physiol 2019;234:8451–8.
[10] Veillette A, Chen J. SIRP-α CD47 immune checkpoint blockade in anticancer therapy. Trends Immunol 2018;39:173–84.
[11] Lipson EJ, Drake CC. Iplimuminab: an anti-CTLA-4 antibody for metastatic melanoma. Clin Cancer Res 2011;17:6958–62.
[12] Postow MA, Callaham MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. J Clin Oncol 2015;33:1974–82.
[13] Alsaab HO, Sau S, Alzhrani R, Tatiparti K, Bhise K, Kashaw SK, et al. PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanisms, combinations, and clinical outcome. Front Pharmacol 2017;8:561.
[14] Qin SJ, Xu Y, Yi M, Yu S, Wu K, Luo S. Novel immune checkpoint targets: moving beyond PD-1 and CTLA-4. Mol Cancer 2019;18.
[15] Ratter J, Waldmann TA, Janakiram M, Brammer JE. Rapid progression of adult T-cell leukemia-lymphoma after PD-1 inhibitor therapy. N Engl J Med 2018;378:1947–8.
[16] Limagne E, Richard C, Thibaultin M, Fument J-D, Truntzer C, Lagrange A, et al. Tim-3/galectin-9 pathway and mMDSC control primary and secondary immune responses. Front Immunol 2019;10.
[17] Zhang X, Wang Y, Fan J, Chen W, Luan J, Mei X, et al. Blocking CD47 efficiently potentiates therapeutic effects of anti-angiogenic therapy in non-small cell lung cancer. J Immunother Cancer 2019;7.
[18] Puro RJ, Bouchalaka MN, Hiebsch RR, Capobbia BC, Donio MJ, Manning PT, et al. Development of AO-176, a next-generation humanized anti-CD47 antibody with novel anticancer properties and negligible red blood cell binding. Mol Cancer Ther 2020;19:857–68.
[19] Kaur S, Elkhourih AG, Singh SP, Chen Q-R, Meerzaman DM, Song T, et al. A function-blocking CD47 antibody suppresses stem cell and EGF signaling in triple-negative breast cancer. Oncotarget 2017;8:10133–32.
[20] Sikic BI, Lakhani N, Patnaik A, Shah SA, Rasco D, et al. First-in-human, first-in-class phase I trial of the anti-CD47 antibody Hu5F9-G4 in patients with advanced cancers. J Clin Oncol 2019;37:946.
[21] Li K, Tian H. Development of small-molecule immune checkpoint inhibitors of PD-1/PD-L1 as a new therapeutic strategy for tumour immunotherapy. J Drug Target 2019;27:244–56.
[22] Xu T, Zheng W, Huang R. High-throughput screening assays for SARS-CoV-2 bioinformatics and cell-based approaches. Drug Discovery Today 2020;25:1807–21.
[23] Clare RH, Bardelle C, Harper P, Hong WD, Borjesson U, Johnston KL, et al. Industrial scale high-throughput screening delivers multiple fast acting macrofilaricides. Nat Commun 2019;10.
[24] Li JS, Neves BJ, Casares GC, Gomes MN, Tomaz KCP, Ferreira LT, et al. Calreticulin as a basis for computer-aided drug design: innovative approaches to tackle. Fut Med Chem 2019;11:2835–46.
[25] Efe G, Kumar R, Parete S, Yoon S, Lee G, Kim D, et al. Identification of ACK1 inhibitors as anticancer agents by using computer-aided drug designing. J Mol Struct 2021;1235.
[26] Clarke DF. What has computer-aided molecular design ever done for drug discovery? Expert Opin Drug Discov 2006;1:103–10.
[27] Macalino SJ, Gosu V, Hong S, Choi S. Role of computer-aided drug design in modern drug discovery. Arch Pharm Res 2015;38:1686–701.
[28] Hatherley D, Graham SC, Turner J, Harlos K, Stuart DI, Barclay AN. Paired receptor specificity explained by structures of signal regulatory proteins alone and complexed with CD47. Mol Cell 2008;31:266–77.
Hatherley D, Harlos K, Dunlop DC, Stuart DI, Barclay AN. The structure of the macrophage signal regulatory protein alpha (SIRP alpha) inhibitory receptor reveals a binding face reminiscent of that used by T cell receptors. J Biol Chem 2007;282:14567–75.

Weiskopf K, Ring AM, Ho CCM, Volkmer J-P, Levin AM, Volkmer AK, et al. Engineered SIRP alpha variants as immunotherapeutic adjuvants to anticancer antibodies. Science 2013;341:88–91.

Pietzsch EC, Dong J, Cardoso R, Zhang X, Chin D, Hawkins R, et al. Anti-leukemic activity and tolerability of anti-human CD47 monoclonal antibodies. Blood Cancer J 2017;7.

Wu Z, Weng L, Zhang T, Tian H, Fang L, Teng H, et al. Identification of Glutaminy Cyclase isoenzyme isoQC as a regulator of SIRPalpha-CD47 axis. Cell Res 2019;29:502–5.

Logtenberg MEW, Jansen JHM, Raaben M, Toebes M, Franke K, Brandsma AM, et al. Glutaminyl cyclase is an enzymatic modifier of the CD47- SIRPalpha axis and a target for cancer immunotherapy. Nat Med 2019;25:612–9.

Wang H, Sun Y, Zhou X, Chen C, Jiao L, Li W, et al. CD47/SIRP alpha blocking peptide identification and synergistic effect with irradiation for cancer immunotherapy. J Immunother Cancer 2020;8.

Hatherley D, Lea SM, Johnson S, Barclay AN. Polymorphisms in the human inhibitory signal-regulatory protein alpha do not affect binding to its ligand CD47*. J Biol Chem 2014;289:10024–8.

Rodriguez PL, Harada T, Christian DA, Pantano DA, Tsai RK, Discher DE. Minimal "Self" peptides that inhibit phagocytic clearance and enhance delivery of nanoparticles. Science 2013;339:971–5.

Xu H, Wang X. Polypeptide RS-17 with anti-CD47 immune checkpoint antagonistic activity and application thereof. 2020.

Yamagishi Y, Shoji I, Miyagawa S, Kawakami T, Katoh T, Goto Y, et al. Natural product-like macrocyclic N-methyl-peptide inhibitors against a ubiquitin ligase uncovered from a ribosome-expressed de novo library. Chem Biol 2013;18:1562–70.

Hazama D, Yin Y, Murata Y, Matsuda M, Okamoto T, Tanaka D, et al. Macrocyclic-peptide-mediated blockade of the CD47-SIRP alpha Interaction as a potential cancer immunotherapy. Cell Chem Biol 2020;27:1181.

Nakashia A, Hirose M, Yoshimura M, Onyama C, Saito K, Kuki N, et al. Structural insight into the specific interaction between murine SHPS-1/SIRP alpha and its ligand CD47. J Mol Biol 2008;375:650–60.

Gao Y, Li Y, Zhai W, Qi Y, Wang H. Sirp alpha protein affinity cyclic peptide and application thereof. China: Zhengzhou University; 2020.

Burgess TL, Amason JD, Rubin JS, Duveau DY, Lamy L, Roberts DD, et al. A homogeneous SIRP alpha-CD47 cell-based, ligand-binding assay: Utility for small molecule drug development in immuno-oncology. PLoS ONE 2020;15.

Miller TW, Amason JD, Garcia ED, Lamy L, Dranchak PK, Macarthur R, et al. Quantitative high-throughput screening assays for the discovery and development of SIRP alpha-CD47 interaction inhibitors. PLoS ONE 2019;14.

Courageot M-P, Duca L, Martiny L, Devarenne-Charpentier E, Morjani H, El Btaouri H. Thrombospondin-1 receptor CD47 overexpression contributes to P-glycoprotein-mediated multidrug resistance against doxorubicin in thyroid carcinoma FTC-133 cells. Front Oncol 2020;10.

Bisinger R, Petkova-Kirova P, Mykhailova O, Oldenborg P-A, Novikova E, Donkor DA, et al. Thrombospondin-1/CD47 signaling modules transmembrane cation conductance, survival, and deformability of human red blood cells. Cell Commun Signal 2020;18.

Wang Q, Onuma K, Liu C, Wong H, Bloom MS, Elliott EE, et al. Dysregulated integrin alpha(v)beta(3) and CD47 signaling promotes joint inflammation, cartilage breakdown, and progression of osteoarthritis. JCI Insight 2019;4.

Sasikumar Pottayil Govindan Nair RM, Naremaddepalli Seetharamaiah Setty Sudarshan, Chennakrishnareddy Gundala, 1,2,4-Oxadiazole Compounds as Inhibitors of CD47 Signalling. India2019.

Bewersdorf JP, Risk-Adapted ZAM. Individualized Treatment Strategies of Myelodysplastic Syndromes (MDS) and Chronic Myelomonocytic Leukemia (CMMI). Cancers (Basel) 2021;13.

Sifniotis V, Cruz E, Eroglu B, Kayser V. Current Advancements in Addressing Key Challenges of Therapeutic Antibody Design, Manufacture, and Formulation. Antibodies (Basel, Switzerland). 2019;8.

Ren T, Tan Z, Ehampanaranath V, Lewandowski A, Chose S, Li ZJ. Antibody disulfide bond reduction and recovery during biopharmaceutical process development – A review. Biotechnol Bioeng 2021.

Kitten O, Martineau P. Antibody alternative formats: antibody fragments and new frameworks. M S-Med Sci 2020;35:1092–7.

Ma H, O’Fagian C, O’Kennedy R. Antibody stability: A key to performance - Analysis, influences and improvement. Biochimie 2020;177:213–25.