Review

Insights into CD47/SIRPα axis-targeting tumor immunotherapy

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ABSTRACT

During the last decade, inhibitors targeting immune checkpoint programmed death ligand 1/PD-1 and cytotoxic T-lymphocyte-associated protein 4 have been one of the most significant advances for cancer therapy in clinic. However, most of these therapies focused on stimulating the adaptive immune system-mediated elimination of tumor. Recent studies indicated that CD47/Signal-regulatory protein alpha (SIRPα), an innate anti-phagocytic axis between cancer cells and macrophages, could be a promising therapeutic target. Here, we review the current knowledge about developing CD47/SIRPα checkpoint inhibitors, avoiding potential side effect and designing optimal combination therapies, and highlight the key points for future clinical applications of CD47/SIRPα axis-targeted tumor immunotherapy.

Statement of Significance: Tumor immunotherapy targeting CD47/SIRPα axis has been one hotspot in cancer therapy. Here, we summarize the preclinical evidence and emerging data from clinical trials to support the development of CD47/SIRPα inhibitors, designing combination therapies and further application of CD47/SIRPα-based immunotherapy.

KEYWORDS: CD47/SIRPα axis; immune checkpoint inhibitors; adaptive immunity; immunotherapy; combination immunotherapy

RISE OF IMMUNE CHECKPOINT INHIBITORS

In the last decade, cancer immunotherapy has rapidly translated into clinical strategy, yielding a new era of cancer therapy, alongside surgery, radiation and chemotherapy [1–3]. Inhibitors targeting immune checkpoint programmed death ligand 1 (PD-1) and its receptor PD-1 and cytotoxic T-lymphocyte-associated protein 4, such as nivolumab, pembrolizumab and ipilimumab, are now moving from second-line treatment to first-line therapy of a broad range of malignancies [4–6]. These monoclonal antibodies work on the general premise that functions of immune cells are suppressed in tumor microenvironment and relief of these suppression stimulates anti-tumor immune response [7]. To date, most of the therapies are focused on stimulating the adaptive immunity, in particular T cells, to eliminate cancer cells. However, recent studies indicated that not only T cells associated with adaptive immunity but also innate immune responses mediated by myeloid cells, for example, macrophages are endued with specialized function to clear solid and hematopoietic cancers.

CD47/SIRPα, AN IMMUNE CHECKPOINT FOR INNATE IMMUNE SYSTEM

Among cells of the myeloid lineage, macrophage has prominent potentials as the mediator of anti-cancer therapeutics based on its robust phagocytosis ability [8,9]. CD47, known as an integrin-associated protein, was first identified as a transmembrane protein from red blood cells (RBC) [10]. Signal-regulatory protein alpha (SIRPα), a transmembrane protein on macrophage, is the main receptor of CD47 [11]. CD47 binding to SIRPα triggers the coupling of SIRPα to these phosphatases, thereby delivering the ‘don’t eat me’ signals to macrophage then preventing their activation [8,12,13]. CD47 expression is regarded as a self-protective mechanism of normal cells, including transfused...
RBC, lymphocytes and platelets, to resist the elimination of macrophage phagocytosis [14]. It is well documented that kinetic hematopoietic stem cells (HSC) protect themselves from macrophage phagocytosis by upregulating the expression of CD47 as they pass across sinusoids then they decrease CD47 expression after relocating to the narrow [15]. In addition, the level of CD47 expression predicts the probability whether HSC could be phagocytized by macrophage while circulating [16]. While, the absence of CD47–SIRPα interaction could activate pro-phagocytic receptors to trigger macrophage phagocytosis of RBC [17].

In the process of carcinogenesis, overexpression of CD47 has been identified across most tumors (Fig. 1). In hematological cancer, CD47 expression on acute myeloid/lymphoblastic leukemia, non-Hodgkin’s lymphoma cells and bone marrow of multiple myeloma samples was detected as several folds increase compared with normal tissues and CD47 level was predictive of the overall survival to primary treatment [16,18]. Furthermore, studies also found that solid tumors, including breast, ovarian, bladder, colon, glioblastoma, prostate tumor and hepatocellular carcinoma, expressed about three- to five-fold more CD47 than the corresponding normal tissues [19]. When patients were assigned into ‘CD47 low’ and ‘CD47 high’ groups based on a univariate analysis, high CD47 mRNA expression level was shown to be associated with the decreased progression-free survival and overall survival [19]. Therefore, those evidences indicated that CD47/SIRPα axis was exploited by malignant cells to transmit ‘don’t eat me’ signal to evade macrophage surveillance and phagocytosis, which highlighted that blocking CD47/SIRPα axis could be used to promote the ability of macrophages to phagocytose and eliminate tumor cells.

**CURRENT STATUS ABOUT CD47/SIRPα-TARGETED TUMOR IMMUNOTHERAPY**

Similar to other immune checkpoint molecules, there are also two targets to choose from when disrupting the CD47/SIRPα axis. Different inhibitors targeting CD47/SIRPα axis have been generated to investigate their therapeutic effects on a variety of cancer types (Table 1). According to their design concepts, these agents fall into the following three main categories: anti-CD47 monoclonal antibody (mAb), anti-SIRPα mAb and SIRPα-Fc fusion protein (Fig. 1).

**Anti-CD47 antibodies**

Preclinical studies employed the well-known anti-CD47 monoclonal antibodies, such as B6H12, Bric126 and Hu5F9-G4, to block CD47-SIRPα interactions, and these antibodies have shown to effectively facilitate the destruction of extensive solid and hematopoietic tumors [20–25]. There are three main mechanisms underlying the potent anti-tumor effect of these anti-CD47 antibodies. First, anti-CD47 antibodies targeting CD47-SIRPα could eliminate the ‘don’t eat me’ signaling and activate phagocytosis of malignant cells by macrophage, which was the primary task for the development of CD47/SIRPα inhibitors. Second, using of a complete antibody of IgG1 type to target CD47 not only disrupt CD47/SIRPα axis, but also simultaneously induce antibody-dependent cellular cytotoxicity (ADCC) against cancer cells, thereby creating a dual signal to obliterate cancer cells [26–28]. Using no opsonizing F(ab′)2 fragment, the opsonization of B6H12, an intact antibody, was demonstrated to be essential for at least anti-tumor effect against both solid and hematological cancer cells *in vitro* [28]. Efforts have been devoted to minimize FcγR-mediated ADCC of anti-CD47 antibody, which might cause obvious side effects of the blood system. Hu5F9-G4, a novel CD47-blocking antibody with IgG4 Fc fragment, has been generated [29]. This specific design significantly reduced the anemia and improved the biosafety of anti-CD47 antibody. To determine the role of Fc function, EC Pietsch and colleagues generated anti-CD47 antibodies based on the Fc fragment of IgG1 and the effector function silent IgG2α. The data showed that the therapeutic effect of anti-CD47 antibody was dependent on Fc effector function [30]. Third, adaptive T cell immunity is significantly activated in tumor-bearing mice after CD47 antibody-based immunotherapy. While xenograft models are widely employed for preclinical testing of antibodies targeting CD47/SIRPα, there are also some limitations. These mice lack T cells and therefore have oversimplified immune systems [9]. Using syngeneic immunocompetent models of B cell lymphoma and colon carcinoma, the therapeutic effects of MIAP301, an anti-mouse-CD47 antibody, depended on dendritic cell cross-priming of T cell responses and the effects could be abrogated in T cell-deficient mice. Underlying mechanism investigation showed that cytosolic sensing of DNA from the targeted
Table 1. A summary of CD47/SIRPα-targeting immune checkpoint inhibitors under clinical development

| Agent      | Description                                      | Identifier          | Strategy                                      | Phase  | Condition                                      |
|------------|--------------------------------------------------|---------------------|-----------------------------------------------|--------|-----------------------------------------------|
| Hu5F9-G4   | Humanized anti-CD47 antibody, human IgG4 subclass | NCT02216409         | Single agent                                  | Phase I| Solid tumor [29,48]                           |
|            |                                                  | NCT02678338         | Single agent                                  | Phase I| Acute myeloid leukemia, Myelodysplastic syndrome [29,48] |
|            |                                                  | NCT02953509         | Single agent; combination with rituximab      | Phase I| Lymphosarcoma, Non-Hodgkin lymphoma, Diffuse large B-cell lymphoma |
|            |                                                  | NCT02953782         | Single agent, combination with cetuximab      | Phase I| Colorectal neoplasms, Solid tumors           |
|            |                                                  | NCT03248479         | Single agent; combination with azacitidine    | Phase I| Acute myeloid leukemia, Myelodysplastic syndromes |
|            |                                                  | NCT03558139         | Single agent; combination with avelumab       | Phase I| Ovarian cancer                                |
| CC-90002   | Humanized CD47-blocking antibody, human IgG4 subclass | NCT02367196         | Single agent; combination with rituximab      | Phase I| Hematologic neoplasms [48]                   |
| TTI-621    | SIRPα-Fc fusion protein, human IgG1 subclass     | NCT02663518         | Single agent; combination with rituximab; combination with nivolumab |
|            |                                                  | NCT02890368         | Single agent; combination with PD-1/PD-L1 inhibitor; combination with pegylated interferon-α2a |
| ALX148     | High-affinity SIRPα variant, inactive Fc domain  | NCT03013218         | Single agent; combination with atezolizumab; combination with trastuzumab; combination with rituximab |
| SRF231     | High-affinity anti-CD47 antibody                 | NCT03512340         | Single agent                                  | Phase I| Advanced solid cancers, Hematologic cancers |
| TTI-622    | SIRPα-Fc fusion protein, human IgG4 subclass     | NCT03530683         | Single agent; combination with rituximab; combination with PD-1/PD-L1 inhibitor; combination with proteasome-inhibitor regimen |

Cancer cells were increased by anti-CD47 antibody, bridging the innate and adaptive immunity [31]. In view of the excellent preclinical anti-tumor effects, therapeutics based on anti-CD47 antibodies, such as Hu5F9-G4, CC-90002 and SRF231, are now in clinical trials for solid and hematologic malignancies (Table 1). However, given the ubiquitous expression of CD47 on RBC and platelets, the general toxicity of anti-CD47 therapies, such as anemia and reduced platelets, should cause some concerns.

**Anti-SIRPα antibodies**

As the receptor of CD47, SIRPα has also been targeted to block the CD47–SIRPα interaction. KWAR23, an anti-SIRPα mAb, was generated and proved to increase macrophage-mediated phagocytosis of patient-derived tumor cells. While, administration of KWAR23 alone did not show obvious anti-tumor effects in lymphoma xenograft models. But when in combination with rituximab,
a tumor-opsonizing antibody, KWAR23 augmented myeloid cell-mediated elimination of tumor cells in vitro and in vivo, indicating that anti-SIRPα antibody could be a promising agent for combination therapy [32]. High expression of SIRPα was observed in melanoma and renal cell carcinoma for the first time, and MY-1, an anti-SIRPα antibody of IgG2a type was generated and found to suppress the tumor formation. MY-1 showed potent effect via two mechanisms: induction of antibody-mediated macrophage phagocytosis and disruption of CD47-SIRPα axis [33]. Effi-DEM (OSE-172), an anti-SIRPα mAb, targeting tumor-associated macrophage and myeloid-derived suppressor cell, could modify the tumor microenvironment and facilitate cytotoxic immune cells infiltration. CD47 is ubiquitously expressed on cell surface, especially RBC and platelets. Some agents targeting CD47 have recently shown blood toxicity such as anemia or reduced platelets. Researches have showed that anti-SIRPα antibodies do not bind to RBC or platelets, which might be a hematological safety advantage compared with anti-CD47 antibody. These novel finding showed that anti-SIRPα antibodies could also be potential regents for malignant cancer immunotherapy.

**SIRPα-Fc fusion proteins**

Recently, several effective decoy receptors were engineered to target CD47: TT1-621, TT1-622, ALX148 and IMM01. TT1-621, a fusion protein consisting of IgG1 Fc fragment and the extracellular domain of SIRPα, enhanced macrophage phagocytosis against a broad spectrum of solid and hematologic tumor cells in vitro, and elicited potent effects in leukemia and lymphoma xenograft models. Importantly, different from anti-CD47 antibody, TT1-621 binds minimally to erythrocytes. Data from the clinical trial of TT1-621 (NCT02663518) showed that repeat dosing of TT1-621 overcame the ‘antigen sink’ (elimination of antibody that binding to membrane antigen is faster at low dose due to the fact that the unbound target will serve as a sink to ‘sop up’ antibody) and maintained acceptable levels of platelet in clinic [34,35]. TT1-622, Trillium’s second SIRPα-Fc fusion protein, has an IgG4 Fc region instead of the IgG1 Fc fragment and thus TT1-622 delivers a modest ‘eat me’ signal to macrophage than TT1-621. To minimize the potential hematologic toxicity, ALX148, a fusion protein containing high affinity CD47-binding domains of SIRPα and an inactive Fc domain, was generated. The preclinical data of ALX148 showed that it safely activated multiple immune cell types against non-Hodgkin lymphoma and advanced solid tumors. Another fusion protein for this came from our team that IMM01 consisting of IgG1 Fc fragment and the first domain extracellular domain of SIRPα was generated to target CD47 and triggered macrophage-mediated phagocytosis against non-small cell lung cancer and glioblastoma cells, eliciting significant efficacy in xenograft models. Using T-cell depleting antibody, further experiment demonstrated that adaptive immune response, especially CD8+ T cells, played a critical role in murine IMM01-triggered tumor rejection [36,37]. Thus, it appeared that blocking CD47 by fusion protein as a trap for CD47 in immunocompetent models has a beneficial effect and more work was still needed to confirm this observation in other types of tumors.

**RATIONAL COMBINATION IMMUNOTHERAPY: TARGETING CD47/SIRPα AND AUTOPHAGY**

Although increasing evidence showed that anti-CD47 therapies have potent anti-tumor effect in several hematologic and solid malignancies, additional investigation to increase the anti-tumor efficacy is ongoing [21]. Autophagy, a crucial player in microenvironment maintenance for tumor cells, could be activated by some condition, including nutrient deprivation and drug administration [38–41]. The first evidence is from our own studies in which we detected autophagosome formation and accumulation, autophagosome fusion with lysosome and autophagosome degradation in lysosome, the three main stages of complete autophagy, in the CD47-targeted cancer cells. Results showed that targeting CD47 by SIRPα-D1-Fc, a CD47-blocking fusion protein, activated autophagy and complete autophagic flux in glioblastoma and non-small cell lung cancer cells [36,37]. Importantly, inhibition of autophagy by inhibitors or knockdown of autophagy-related genes significantly increased CD47 blockade-induced macrophage phagocytosis of non-small cell lung cancer and glioblastoma cells and potentiated the in vivo anti-tumor effects of CD47/SIRPα blockade. These data revealed that autophagy played a cyto-protective role in CD47-targeting tumor immunotherapy and highlighted the synergistic anti-tumor effects of blocking CD47 and autophagy, providing novel approach to further enhance the anti-tumor effect of CD47-SIRPα checkpoint inhibitors.

**BISPECIFIC IMMUNOTHERAPY: TARGETING CD47 AND CD20/CD19, TARGETING CD47 AND MESOTHELIN AND TARGETING CD47 AND PD-L1**

Based on the various evidence listed above, blocking CD47-SIRPα axis is certainly a very effective approach, but specificity in the response to the cancer cells was still a challenge. In order to find an effective solution, combining a CD47/SIRPα inhibitor with a tumor cell-specific opsonizing antibody was intensively studied. In non-Hodgkin lymphoma, anti-CD47 antibody (BRIC126 or B6H12) in combination with rituximab, the clinically used anti-CD20 antibody, resulted in synergetic elimination of human lymphoma cells in both disseminated and localized xenograft models. Subsequently, a bispecific antibody targeting CD20 and CD47 was engineered and the bispecific antibody has a relatively low affinity to CD47, decreasing its binding to normal cells that are expressing CD47, but maintaining the CD20 binding capacity at the same time. In comparison with the anti-CD47 antibody alone, the bispecific antibody reduced lymphoma burden and overcame the ‘antigen sink’ via selective binding to the lymphoma cells [42]. CD19, a transmembrane protein on B cells, has been proven to be a potential therapeutic
target for the anti-CD20 resistant malignance. NI-1701, a novel bispecific antibody, was designed to co-engage CD47 and CD19, offering an alternative or adjunct therapeutic option to patients with B cell lymphoma and leukemia refractory/resistant to mAb therapy alone [43]. Meanwhile, NI-1801, another bispecific antibody based on human IgG molecular with bispecific antigen-binding regions, was generated by Novimmune (Geneva, Switzerland) to utilize the innate immunity to eliminate mesothelin-positive tumors. Due to the co-expression of CD47 and PD-L1 on some tumor cells, Yajun Guo and colleagues constructed IAB, a CD47 and PD-L1 bispecific fusion protein. The data of IAB indicated that dual-targeting CD47 and PD-L1 could induce synergistic therapeutic effect by activating innate and adaptive immunity simultaneously [44]. Combination therapy with multiple monoclonal antibodies has several advantages compared with monotherapy in non-Hodgkin lymphoma or other cancer. Primarily, treatment solely with antibody targeting cancer antigen will decrease off-target toxicity compared with the current approaches that are utilizing chemotherapy. Then, synergistic effect between two different antibody-mediated effector mechanisms could elicit potent therapeutic effect. Thirdly, antibodies that are targeting two cell-surface antigens would be more likely to clear tumor cells with epitope loss or pre-existing epitope variants, such as rituximab-resistant cancer patients [45–47]. Finally, a bispecific antibody with one arm binding to CD47 and the other arm binding to a validated target could retain the synergistic effect and reduce potential toxicity [18].

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