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Targeting CD47 as a cancer therapeutic strategy – the cutaneous T cell lymphoma experience

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Abstract

Purpose of review—To describe the relevance of CD47 in the tumor microenvironment and summarize data on anti-CD47 therapies, including its role in cutaneous T-cell lymphoma (CTCL).

Recent findings—CD47 is expressed on all normal cells and targets SIRPα on the surface of myeloid cells. However, CD47 is found to be overexpressed on cancer cells. CD47-SIRPα interaction inhibits macrophage phagocytosis, allowing cancer cells to escape immune surveillance. Current focus in immunotherapy has been targeted towards inhibiting CD47-SIRPα interaction via anti-CD47 antibodies. This activates innate immunity, promoting cancer cell destruction by macrophages. It also activates adaptive immunity resulting in antigen-presentation, mostly by dendritic cells, leading to anti-tumor cytotoxic reactions. Current CD47 antagonists undergoing clinical trials include Hu5F9 (an anti-CD47 antibody that directly inhibits the CD47-SIRPα interaction) and TTI-621, (a fusion protein composed of CD47 binding domain of human SIRPα and linked to the Fc region of IgG1). These agents have continued to show strong efficacy against solid and hematological tumors.

Summary—In the CTCL tumor microenvironment, increased immune checkpoint inhibition expression via CD47 bound to SIRPα correlates with a more advanced disease state. Continued success in treating these patients require further studies on CD47 antagonists, specifically when combined with other antibodies.

Keywords

cutaneous T-cell lymphoma; innate immune checkpoint inhibitor; anti-CD47; human SIRPα

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Introduction

Primary cutaneous lymphomas consist of T-cell, B-cell, and natural killer (NK) cell neoplasms [1**]. Cutaneous T-cell lymphomas (CTCL) include many types of T-cell lymphomas. Mycosis fungoides (MF) and Sézary Syndrome (SS) are the most common types and often synonymously named CTCL. They are characterized by malignant T-cells homing to the skin. The neoplastic T-cells usually contain a CD4+ and CD8- phenotype with frequent loss of CD5 and/or CD7 [1**]. While initial skin lesions in early disease present with a pro-inflammatory background, advanced lesions are characterized by an accumulation of malignant T-cells, which promote a hostile inflammatory environment that further enhances tumor growth and expansion [2**]. Development of this tumor microenvironment creates a shift from partly anti-tumorigenic to pro-tumorigenic, signifying disease progression [2**]. Currently there is no cure for CTCL and chronic, relapsing disease requires repeat treatment regimens. Targeted immunotherapy has been a growing field and more research is being done on its effect on the tumor microenvironment.

Review of CD47

CD47 is a glycoprotein composed of a single extracellular V-set immunoglobulin superfamily (IgSF) domain attached to a 5-time transmembrane spanning domain and a short cytoplasmic region for alternative splicing [2**]. It is the principal ligand for signal regulatory protein alpha (SIRPα) in humans, pigs, and rodents and is expressed on all normal cells in the body [2**]. SIRPα is a surface glycoprotein and an immunoreceptor expressed on all myeloid cells (including monocytes, macrophages, granulocytes, and dendritic cells) and on neuronal cells in the central nervous system [2**]. CD47 targets and binds to the N-terminus of SIRPα on the surface of myeloid cells, which promotes phosphorylation of tyrosine residues found within two immunoreceptor tyrosine-based inhibitory motifs (ITIM) [2**, 3, 4**]. These motifs are located within the SIRPα cytoplasmic tail [2**]. Phosphorylated ITIM recruits and activates protein tyrosine phosphatases (PTPase), particularly of the Src homology region 2 (SH2) domains [2**, 3]. Once phosphorylated ITIM of SIRPα interacts with phosphatase SH2-domains, enzymatic activity is triggered [2**, 3]. This counterbalances an activating signaling pathway creating a “do not eat me” signal elicited by CD47, suppressing phagocytosis by macrophages [2**, 4**, 5*].

CD47-SIRPα interactions play a role in both healthy and pathological conditions, such as red blood cell (RBC) homeostasis, autoimmunity, infection, and inflammation [2**]. CD47 is ubiquitously expressed; it functions as a “marker of self” on erythrocytes and other cells in order for the innate immune system (e.g. macrophages, dendritic cells) to recognize a host cell from foreign (e.g. microorganisms) [6]. Down-regulation of CD47 on erythrocytes can induce phagocyte activation and destruction of aged or damaged RBC by SIRPα-expressing leukocytes and clearance via splenic macrophages [2**, 6]. Phagocytosis is elicited by macrophage actin-myosin contraction [2**]. Upregulation of CD47 inhibits macrophage phagocytosis of normal, healthy hematopoietic stem cells (HSC) while allowing these cells to migrate to the periphery in response to inflammatory insults [4**, 7]. Upregulation of CD47 is a particularly important mechanism used by cancer stem cells to bypass the

immune system, which plays a pivotal role in cancer recurrence [4**]. Earlier studies show an increase in the anti-phagocytic CD47 protein expression on dysplastic human hematopoietic cells, particularly in acute myelogenous leukemia (AML) and in chronic myeloid leukemia (CML), and inhibits phagocytosis of these cells through CD47-SIRPα interactions allowing evasion of immunosurveillance [7, 8, 9]. It was found that there is a direct correlation with immune evasion and poor prognosis in patients with leukemia, non-Hodgkin lymphoma (NHL), bladder, and breast carcinomas [4**, 10**]. Increased CD47 mRNA expression in multiple NHL subtypes independently predicted adverse clinical outcomes, particularly in diffuse large B-cell lymphoma, B-cell chronic lymphocytic leukemia, and mantle cell lymphoma [11]. Increased expression of CD47 is also a poor prognostic factor when found on solid tumors, such as ovarian, breast, colon, bladder, prostate, glioblastoma, and hepatocellular carcinomas [12]. In breast cancer studies, specific breast cancer subtypes have CD47 associated super-enhancers (long stretches of DNA) that induce overexpression of CD47, enabling its growth and spread [13*]. Recent research found that high levels of CD47 are not only expressed in CTCL, but also on fibroblasts following activation of transcription factor c-Jun contributing to development of diseases such as idiopathic pulmonary fibrosis and scleroderma [14*]. Atherosclerosis is another disease process involving upregulation of CD47, compared to non-atherosclerotic vascular tissue, suggesting a pathological commonality in both cancer and cardiovascular disease [15].

Immune checkpoints

Inhibiting the binding of CD47 to SIRPα promotes presentation of tumor antigens to CD8 T-lymphocytes, activating both innate and adaptive anti-tumor responses [2**, 16].

Innate immune checkpoints

Blockade of the CD47-SIRPα interaction activates innate immune surveillance, allowing macrophages and neutrophils to promote killing of malignant cells [2**]. The primary mechanisms involved are antibody-dependent cellular phagocytosis (ADCP) and cytotoxic reactions targeting multiple tumor types [2**, 4**, 5*]. Macrophages are able to target cancer cells through calreticulin (CRT), an endoplasmic reticulum protein [16]. CRT activates Toll-like receptor pathways within macrophages causing phosphorylation of Bruton's tyrosine kinase [16]. This results in expression of CRT on the surface of macrophages, which activates an anti-CD47 mediated process called programmed cell removal (PrCR) [16]. PrCR is the mechanism by which macrophages kill malignant cells via phagocytosis and intracellular degradation [2**, 16]. Macrophage phagocytosis is further promoted by antibody opsonization of the cancer cells via ADCP [2**]. Neutrophils destroy tumor cells by antibody-dependent cellular cytotoxicity (ADCC) in the presence of tumor-opsonizing therapeutic antibodies [2**].

Adaptive Immune Checkpoints

Macrophages and dendritic cells are antigen presenting cells (APC), or cells of innate immunity that present foreign material to T-cells, initiating an adaptive immune response [17]. Blockade of CD47-SIRPα checkpoint also activates the adaptive immune system,

enhancing tumor cell antigen uptake and presentation by APC [2**]. Macrophages were assumed to be the major APC for cytotoxic T-lymphocyte (CTL) induction [10**]. However, syngeneic mouse models have recently shown that dendritic cells (DC) play a more important role in activating the CD8+ T-cell population and therefore anti-tumor activity [2**, 10**]. DC interact with the T-cell receptor (TCR) on these T-cells resulting in release of cytokines that initiate effective T-cell responses [18**]. The exact mechanism in which inhibition of CD47-SIRPα interactions induce tumor-specific cytotoxic T-cell responses is not known [2**]. It is proposed that anti-CD47-mediated phagocytosis results in negative signals to CD4+ T-cells and positive signals to CD8+ T-cells [17]. Also, enhanced tumor material uptake by DC followed by improved antigen presentation and/or opsonization of tumor cells by anti-CD47 antibody likely contributes to this process [2**].

CTCL and the tumor microenvironment

Primary cutaneous T-cell lymphoma (CTCL) make up about 70% of primary cutaneous lymphomas [1**]. There are many variants of CTCL, however mycosis fungoides (MF) comprises majority of cases at around 54–72% [2**]. Classic MF presents as erythematous patches and/or plaques on photoprotected areas such as the breasts, lower trunk, groin, and buttock [1**]. These lesions often exhibit indolent behavior [2**]. Histological findings of patch/plaque lesions can show various degrees of epidermotropism consisting of clusters of atypical lymphocytes (Pautrier microabscess) and/or superficial band-like infiltrate in the basal layer and superficial dermis [1**]. In one third of patients, lesions can evolve into erythroderma and/or tumors, indicating a more advanced disease [1**, 2**]. The malignant T-cells in these advanced stages can leave the skin and spread to the blood, lymph nodes, visceral organs, and bone marrow [2**]. Histological features of these tumor lesions show deep dermal infiltrates, usually lacking epidermotropism [1**].

Skin lesions in CTCL are composed of malignant T-cells, non-malignant T-cells, and other immune cells depending on the type of lesion [19]. For example, MF and SS typically consists of the mature CD4+ memory T-cell phenotype [19]. In addition to malignant T-cells are the presence of nonmalignant immune cells, including CD8+ tumor-infiltrating T-cells, Tregs, DC, macrophages, and mast cells [19]. DC subsets are found in cutaneous and mucosal surfaces in both inflammatory and non-inflammatory states [18**]. In typical CTCL tumor lesions, the number of mast cells are increased and particularly prominent at the lymphoma margins [19]. Higher mast cells were found in those with progressing disease or advanced/tumor stages [19]. In early stage mycosis fungoides, the infiltrate consists of few malignant T-cells, nonmalignant Th1 cells, and CD8+ tumor-infiltrating T-cells [19]. Higher densities of CD8+ tumor-infiltrating T-cells, FOXP3+, and Tregs have been correlated with an improved survival rate in CTCL [19].

T-cells express TCRs for antigens presented by DCs and other costimulatory receptors involved in initiation of effective T-cell anti-tumor responses [18**]. The type and duration of signals released by DC at the time of antigen presentation is what determines subsequent T-cell activation and therefore immune responsiveness [18**]. Studies have shown that malignant CTCL cells grew in long-term co-cultures with immature DC, through an interaction between the major histocompatibility complex (MHC) class II peptides on

dendritic cells and the TCR on neoplastic T-cells [19]. It is suggested that tumor growth occurs because of continuous antigen presentation overload by the immature DC to functionally over-activated T-cells, subsequently causing T-cell exhaustion leading to impaired immune surveillance and overexpression of inhibitory checkpoint receptors [18**, 19]. Increased checkpoint expression, including PD-1, LAG-3, TIM-3, and CTLA-4, correlates with a more advanced disease stage [18**].

Tumor-associated macrophages (TAMs) can express either the M1 or M2 phenotype and plays a major role in many murine and human tumors [20**]. TAMs have been shown to play a role in creating a tumor microenvironment by remodeling the extracellular matrix and releasing factors that promote cell proliferation, angiogenesis, and cellular migration [12]. It was shown that a high abundance of TAMs are associated with metastasis and therefore a poor prognosis [21**]. However, recent studies show that high TAM density can also correlate with increased survival in certain malignancies, such as pancreatic and colorectal carcinomas, depending on their phenotype [20**]. Activated M1 cells produce pro-inflammatory cytokines and are involved in the killing of intracellular pathogens and have an anti-tumorigenic role [5*]. Whereas, activated M2 macrophages play a role in mycosis fungoides pathogenesis, encouraging tumor growth [21**]. CD163 and IL-10 are characteristic of M2 cells and both lead to immunosuppression [5*]. TAMs can either be anti-tumorigenic or pro-tumorigenic [20**]. There are phenotypic changes from M1 to M2 cells during tumor progression and can be highlighted by an increase in CD163 and macrophages [22*]. A study by Sugaya et al. observed that the number of CD163 (+) cells in skin lesions of CTCL, atopic dermatitis, or psoriasis were significantly larger than in normal controls [19]. Another study demonstrated that the numbers of CD163 (+) or CD68 (+) cells in patients with CTCL increased as more neoplastic T-cells were found in the infiltrate, and their number decreased after treatment with topical steroid and ultraviolet light [19]. TAMs can also express programmed cell death protein 1 (PD-1), an immune checkpoint receptor [23**]. TAMs expressing a M2 phenotype tend to express PD-1, whereas the M1 population is generally PD-1 negative [23**]. M2 PD-1+ TAMs may accumulate over time in the tumor microenvironment contributing to progression [23**].

Treatment options

In various xenograft tumor models, human CD47-blocking monoclonal antibodies were proven to have excellent efficacy against solid and hematological malignancies [10**]. Anti-CD47 antibody therapies not only inhibit the growth of primary site tumors, but can also prevent tumor metastasis [12]. A humanized anti-CD47 antibody has been shown to penetrate the blood brain barrier and is deemed effective against brain tumors such as medulloblastoma and pediatric glioblastoma while not displaying toxicity toward nonmalignant neural cells [5*]. These antibodies can target human tumors without being absorbed by other normal cells such as red blood cells [10**]. There are currently several CD47 antagonists in active phase I clinical trials against hematological malignancies and solid tumors, in particular Hu5F9 and TTI-621 [4**].

Hu5F9 is a humanized anti-CD47 antibody of the immunoglobulin (IgG)4 subclass [24**]. It binds human CD47 with tight affinity and blocks the CD47- SIRPα interaction, allowing

phagocytosis of primary AML and NHL cells [25]. In the report by Liu et al., Hu5F9 was found to synergize with rituximab in the elimination of NHL in xenograft models [25]. It is proposed that Hu5F9 will be able to synergize with any antibody capable of engaging Fc receptors in order to elicit a pro-phagocytic signal to eliminate the tumor cells [25]. Hu5F9 was not found to induce ADCC, CDC, or direct apoptosis on target cells [25]. However, the induction phagocytosis in an Fc-dependent manner could not be ruled out [25].

TTI-621 is a recombinant fusion protein composed of human SIRPα N-terminal domain fused to the Fc receptor of IgG1 [4**, 24**]. Containing the binding domain of CD47 allows this protein to not only block CD47's "do not eat me signal" but also enhances phagocytosis and antitumor activity via engagement of macrophage Fcγ receptors with IgG1 Fc receptor [26**]. In a study by Querfeld et al., nine adult patients with mycosis fungoides and/or Sézary Syndrome were treated with intratumoral injections of TTI-621 [26**]. The lesions injected were frequently biopsied in order to assess the effects of TTI-621 on the tumor microenvironment [26**]. It was found that the patients treated experienced not only a decrease in tumor size rapidly clinically, but also had decreased circulating Sézary cells (in those with Sézary Syndrome) after a single injection [26**]. NanoString analysis of peripheral blood and tumor tissue before and after injections revealed upregulation of IFN-associated genes and alterations in innate immunity activation genes, confirming the role of TTI-621 as an innate immune checkpoint inhibitor [26**]. Therefore, direct antitumoral injection of TTI-621 elicits both local and systemic antitumoral activity [26**]. Of note, TTI-621 was well tolerated in all 9 patients with fatigue, chills, and decreased appetite being the main treatment related adverse effects [26**].

An important difference between TTI-621 and Hu5F9-G4 is the incidence of anemia and thrombocytopenia, which has been proven to be higher with Hu5F9-G4 administration [4**]. Despite the lower impact TTI-621 has on RBCs compared to Hu5F9-G4, there is an overall higher toxicity to other cells due to strong ADCC from IgG1 [4**].

More research needs to be done on the therapeutic benefits of CD47-SIRPα inhibitors in combination with others agents such as atezolizumab (an anti-PD-L1 antibody), rituximab, trastuzumab, and cetuximab [2**, 4**, 24**]. Rituximab, an anti-CD20 antibody, is a standard therapy for many CD20-positive B-cell lymphomas [11, 27]. It alone is not curative in majority of B-cell NHL patients, however, it has significantly improved long term survival when combined with conventional chemotherapy [11, 27]. Synergistic effects were observed between anti-CD47 antibody and rituximab through both Fc receptor-dependent and -independent mechanisms, both leading to stimulation of macrophage mediated phagocytosis [11]. Trastuzumab and cetuximab (antibodies that target molecules Her2/Neu and EGFR on the cancer cell respectively) can interact with the Fc receptor on macrophages as well to induce ADCC and further enhance anti-CD47 antibody phagocytosis. Anti-CD47 in combination with interferon-α can also provide therapeutic effects to NHL patients. A recent study by Akilov et al. showed some promising results after administration of PEGylated interferon-α2a was found to work synergistically with TTI-621 [28**]. In the described patient with Sézary syndrome, PEGylated interferon-α2a was given 7 days after TTI-621, which demonstrated a decrease in symptoms and leukemic burden [28**]. SIRPα variants should also be considered for universal immunotherapeutic adjuvants, as these have

proved to increase phagocytosis in vitro and enhance anti-tumor responses against cancer cells in vivo [29].

Conclusion

CD47 antagonists are a relatively new focus in cancer immunotherapy with benefits targeted toward solid and hematological malignancies, atherosclerosis, and fibrotic diseases. More specifically, anti-CD47 antibodies have been proven, thus far, to have therapeutic effects against the tumor microenvironment in CTCL. Intralesional TTI-621, for example, has shown a good response in patients with tumor lesions. Anti-CD47 therapies are generally well tolerated with low side effects. In patients with MF, consideration should be made in finding the best modality and/or combinations with synergistic antibodies in order to favorably treat this CTCL subtype.

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Abbreviations

ADCC	Antibody-dependent cellular cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
AML	Acute myelogenous leukemia
APC	Antigen presenting cell
CML	Chronic myeloid leukemia
CRT	Calreticulin
CTCL	Primary cutaneous T-cell lymphomas
CTL	Cytotoxic T lymphocyte
DC	Dendritic cells
EGFR	Epidermal growth factor receptor
Her2/Neu	Human epidermal growth factor receptor
HSC	Hematopoietic stem cells
IFN	Interferon
IgSF	Immunoglobulin superfamily
ITIM	Immunoreceptor tyrosine-based inhibitory motifs
MF	Mycosis fungoides
MHC	Major Histocompatibility Complex

NHL	Non-Hodgkin lymphoma
NK	Natural killer cells
PD-1	Programmed cell death protein 1
PrCR	Programmed cell removal
PTPase	Protein tyrosine phosphatases
RBC	Red blood cell
SIRPα	Signal regulatory protein alpha
SS	Sézary Syndrome
TAMs	Tumor-associated macrophages
TCR	T-cell receptor
Tregs	Regulatory T-cells

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Key Points

- Activated M2 macrophages play a role in the pathogenesis of mycosis fungoides and have a pro-tumorigenic effect
- CD47-SIRP α interaction allows cancer cells to evade both innate and adaptive anti-tumor responses
- CD47 targets and binds to SIRP α on the surface of myeloid cells and elicits a “do not eat me” signal; blocking this interaction allows phagocytosis via macrophages
- Human CD47-blocking monoclonal antibodies were proven to be efficacious against solid and hematological malignancies
- More research needs to be done on combination therapy with CD47-SIRP α blockade agents