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Targeting "Do Not Eat Me" Signal CD47 in Cancer Immunotherapy

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Cells of the innate and adaptive arm of the immune system including macrophages, natural killer (NK) cells, neutrophils, T cells, and B cells, etc. are crucial for the maintenance of the body's homeostatic balance and prevention of multiple diseases including cancer. The quest for eradicating the devastating disease cancer by harnessing the body's immune system has delivered remarkable outcomes in the clinic and offered new hope for cancer cure. Developments of check-point inhibitors, anti-programmed death-ligand 1 (anti-PDL1) and/or PD-1, anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA4) and generation of chimeric antigen receptor (CAR) T-cells have given a dramatic shift in how we fight cancer. Macrophage, neutrophils and NK cells work as the first line of defense against infection and recognize transformed or infected cells before any other cells of the immune system. Massive phagocytosis potential and antigen-presenting capabilities of macrophages make them a crucial component of the innate immune system. Also, macrophages are one of the immune cell populations that are present in the tumor microenvironment in large quantities and significantly influence the disease pathobiology. Healthy and normal cells of body evade macrophages mediated phagocytosis by expressing anti-phagocytic surface proteins called 'do not eat me' signals including the cluster of differentiation (CD) 47, PD-L1, beta-2 microglobulin subunit of the major histocompatibility class I complex (B2M) and the cluster of differentiation (CD) 24. Loss of 'do not eat me' signals expression in the apoptotic or senescent cells and elevated expression of other 'eat me signals' results in phagocytosis. Cancer cells exploit the similar strategy and escape macrophage-mediated clearance by overexpressing 'do not eat me' signals on their surface. B2M and CD24 are recently identified anti-phagocytosis signal and their therapeutic potential is still being explored. CD47, an innate immune checkpoint inhibitor is suggested to be the most prominent 'do not eat me' signal expressed on the cancer cells surface.

CD47 is expressed ubiquitously but significantly upregulated in several human malignancies including breast cancer, colon cancer, prostate cancer, ovarian cancer and hepatocellular carcinoma [1,2]. CD47 expressing tumor cells interacts with signal regulatory protein alpha (SIRPα) of macrophages resulting in the phosphorylation of SIRPα and the inhibitory tyrosine phosphatases SHP-1 and SHP-2 leading to inhibition of the phagocytic engulfment machinery of macrophages [3]. Because normal cells also express CD47 and anti-CD47 antibodies may also induce phagocytosis in these cells by macrophages, however, this has not been observed in practice. This could be due to the significantly higher level of CD47 on cancer cell surfaces in comparison to a normal cell, and thus anti-CD47 antibodies tend to target cancer cells more efficiently than normal cells. Also, "eat me" signals are selectively expressed on the surface of cells going for apoptosis and tumor cells, thereby creating a favorable environment for macrophages phagocytosis. The CD47-SIRPα signaling axis is known as a major innate immune checkpoint pathway for immune evasion by tumor cells and is being intensively explored as a cancer immunotherapy target both preclinically and clinically.

Inhibiting or blocking of CD47-SIRPα interaction to promote the immune cells mediated phagocytosis can be achieved by either targeting CD47 or SIRPα alone, or by targeting both. Antibodies generated against CD47

and SIRPα antibodies and, 4N1K cell-binding domain adhesive peptides resulted in elevated macrophage phagocytic and elimination of tumors in various murine models [4,5]. Anti-CD47 antibodies also promote antibody-dependent cellular phagocytosis (ADCP) and/or antibody-dependent cellular cytotoxicity (ADCC) by opsonizing and eliminating cancer cells by macrophages or neutrophils. Moreover, cells of the adaptive immune system also contribute to removing the cancer cells following macrophage-mediated phagocytosis and antigen presentation to the T cells. An anti-CD47 humanized monoclonal antibody (mAb), Hu5F9-G4 (5F9) that binds to monomeric human CD47 demonstrated inhibition of CD47-SIRPα axis leading to enhanced phagocytosis of human cancer cells in multiple myeloma, breast and colon cancer cells [6]. Indirect inhibition of CD47 by glutaminyl peptide cyclotransferase-like protein is shown to increased antibody-dependent cellular phagocytosis and cellular cytotoxicity of tumor cells [7]. Further, bispecific antibodies that co-target endothelial growth factor receptor (EGFR) and CD47, vascular endothelial growth factor (VEGF) and CD47 are being explored in the research setting to get better results [8,9]. Another anti-CD47 antibody CC-90002 is in a Phase-I clinical trial (NCT02367196) for the treatment of hematologic and solid malignancies [10]. SRF231 and B6H12.2 are recently developed anti-CD47 antibodies that have shown promising results in preclinical studies [11,12].

The efficacy of monoclonal antibodies TI-061 and SRF231 developed against anti-CD47 in patients with advanced solid and hematologic cancers are also being explored in a clinical trial (NCT03512340) [13]. The SIRPα fusion protein TTI-621 that binds to CD47 has demonstrated growth inhibitory potential in AML and lymphoma xenografts and is in the Phase 1 trial (NCT02663518, NCT02890368) [14,15]. Also, the efficacy of TTI-621 in combination with PD-1 checkpoint inhibitors, and radiation therapy is under investigation (NCT02890368) [15]. Another SIRPα-Fc fusion protein, TTI-622, is being tested in patients with advanced relapsed lymphoma or myeloma (NCT03530683) [16]. CD47-blocking molecules, ALX148, generated by fusing the inactivated IgG1 Fc with a modified SIRPα D1 domain shown to activate the dendritic cell, increased T cell effector function, induce macrophage phagocytosis leading to anti-tumor effects [17]. The use of miRNAs and siRNAs based approached to downregulate CD47 expression has also been considered to gain better clinical outcomes. Transfection of esophageal squamous cell carcinoma tumor cells with miR-133a that target CD47 that led to the attenuation of tumor progression in a mouse xenograft model [18]. Further, delivery of liposome loaded anti-CD47 siRNAs inhibited the melanoma growth in mice [19]. Interestingly, CD47-targeting siRNAs showed decreased accumulation of M2 macrophages leading to reduced migration of colon cancer cells [20]. However, the poor uptake, low bioavailability, and off-target effects limit the efficacy of gene silencing strategies and pose additional challenges including toxicity.

Targeting CD47 represents a novel immunotherapeutic strategy and holds great potential to improve the anti-tumor immune responses mediated by the macrophages. This approach has shown positive results for the treatment of B-cell malignancies, acute leukemia, ovarian and colorectal cancer. Also, the results obtained by blocking the 'do not eat me' signaling pathway are very encouraging in several other malignancies. However, there are several questions that we as a research community need to address including the most effective method to block the CD47-SIRPα axis, application of CD47 inhibition alone or in a combination with chemotherapy, radiotherapy or immunotherapy. A combination of anti-CD47 antibodies with macrophage-triggering cytokines may promote macrophage recruitment in the tumor microenvironment and same time phagocytosis of cancer cells. Also, administration of chemotherapeutic agents or checkpoint inhibitors such as anti-PD-L1 antibodies in combination with anti-CD47 antibodies may improve the anti-tumor responses. Enhancing the efficacy of macrophages against tumor cell by targeting CD47 has the potential to translate into the clinic that can benefit patients with advanced malignancies and improve outcomes.

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