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Review

Translating CD47-targeted therapy in gastrointestinal cancers: Insights from preclinical to clinical studies

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SUMMARY

This review presents a thorough investigation of the role of CD47 in gastrointestinal cancers. We performed a comprehensive, in-depth review of over 100 preclinical and clinical studies focused on inhibiting CD47. The research highlights the potential of targeted CD47 to enhance existing treatments by boosting the immune response to cancer cells. Considering the essential need to balance the toxicity and efficacy of CD47 inhibition, our review emphasizes the need to optimize CD47 inhibitors. We also demonstrate the necessity of combining CD47 antibodies with conventional chemotherapy, radiotherapy, or other targeted therapies to enhance treatment effectiveness. Finally, we propose the integration of CD47-targeted therapies into treatment plans as a promising approach to reshape the therapeutic landscape of gastrointestinal cancers. Continued research in this field holds great potential for improving the outcomes of gastrointestinal cancer patients and overcoming the challenges associated with this formidable spectrum of diseases.

INTRODUCTION

Cancer continues to be a leading cause of mortality worldwide, with gastrointestinal cancers being particularly devastating due to their high incidence and mortality rates.[1] Major gastrointestinal cancers, classified under the 11th edition of the International Classification of Diseases, include esophagus, stomach, colorectum, liver, gallbladder, and pancreas.[2] According to GLOBOCAN 2022, gastrointestinal cancers accounted for approximately 2.97 million new cases and 2.05 million deaths globally.[3]

Traditional treatments like chemotherapy, radiation therapy, and surgery often fall short, especially in advanced stages of gastrointestinal cancers. This is starkly evident in pancreatic cancer, which has become the third leading cause of cancer-related deaths in regions like the European Union and the United States.[4,5] These trends underscore the urgent need for more effective therapies. In response to these challenges, immunotherapy, particularly using immune checkpoint inhibitors targeting PD-1, has emerged as a promising approach.[6] However, only a limited subset of patients, especially those exhibiting a high mutational burden or immune-related genetic abnormalities such as elevated microsatellite instability, demonstrate favorable responses to PD-1 inhibitors.[7]

CD47, which serves as a "don't eat me" signal on cancer cells, thereby protecting them from macrophage-mediated phagocytosis, a mechanism distinctly different from the T cell modulation observed with PD-1 inhibitors, has emerged as a compelling new target in cancer immunotherapy.[8] While CD47 inhibitors have demonstrated significant potential in hematological malignancies, their application in solid tumors, particularly gastrointestinal cancers, has been limited.[9] The modest efficacy and unresolved toxicity issues of early CD47 inhibitors in these cancers suggest substantial room for improvement. This introduction aims to explore the landscape of CD47 as a novel therapeutic target within the broader context of gastrointestinal cancers, assessing both the achievements and limitations of current strategies.[10] By providing a comprehensive overview of the existing clinical and preclinical data, this review will discuss the potential of CD47-targeted therapies for gastrointestinal cancers, paving the way for more effective and less toxic treatment options.

CD47 AND THE TUMOR MICROENVIRONMENT OF GASTROINTESTINAL CANCERS

CD47 and SIRPα in immune regulation

CD47, also known as the integrin associated protein, is a member of the immunoglobulin superfamily.[11] CD47 is ubiquitously expressed on the surface of human cells, and interacts with signal regulatory protein α (SIRP α), thrombospondin-1, and integrins[12,13] to mediate apoptosis, proliferation, and immune response.[14] The CD47 and SIRPα interaction exerts significant regulatory effects on macrophages and dendritic cells (Figure 1).[15] SIRPα, a member of the SIRP family, possesses the longest intracellular region compared to other SIRP family members. The intracellular region of SIRPα contains two

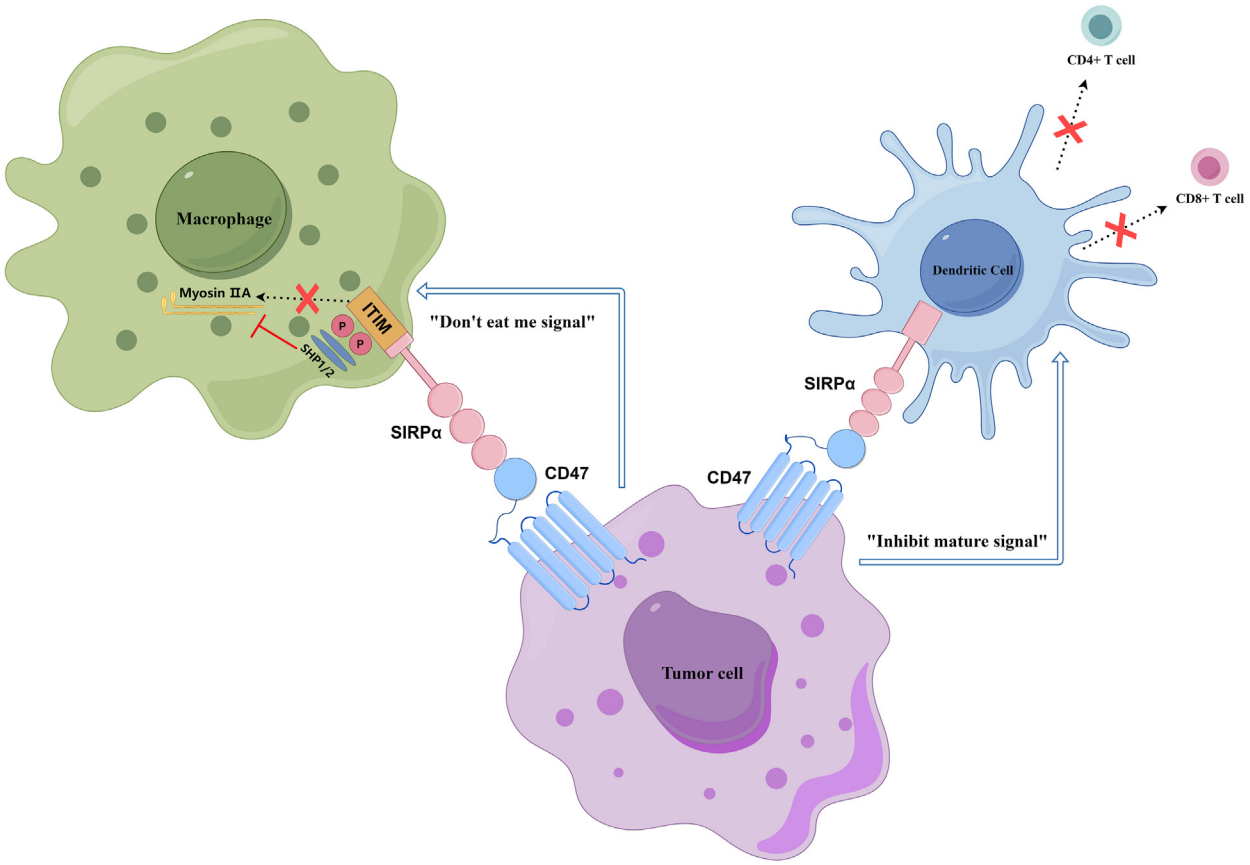

Figure 1. CD47-SIRPα interaction in immune regulation

When CD47 on tumor cells binds to SIRPα on macrophages, it triggers the phosphorylation of immunoreceptor tyrosine-based inhibitory motifs (ITIMs), recruiting Src homology region 2 domain-containing phosphatases (SHP-1 and SHP-2), which inactivate myosin IIA, thereby blocking phagocytosis ("Don't eat me" signal). In dendritic cells, this interaction inhibits maturation and cytokine production, preventing $CD8^+$ and $CD4^+$ T cell activation, allowing tumor cells to evade immune destruction.

immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which serve as docking sites for Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP1) and SHP2.[16] In macrophages, the binding of CD47 to SIRPα leads to phosphorylation of tyrosine residues within the intracellular ITIM domain of SIRPα. Subsequently, phosphatases such as SHP-1 and SHP-2 are recruited to these phosphorylated sites, initiating a cascade of signaling events. This signaling cascade results in the inactivation of myosin IIA and subsequent inhibition of phagocytosis in macrophages.[17] Moreover, in dendritic cells, the CD47-SIRPα signal can inhibit the maturation of DCs, prevent the production of cytokines from mature DCs, and prevent the subsequent activation of $CD8^+$ T cells against tumor antigens presented on dendritic cells.[18] These examples demonstrate the role of CD47 in regulating part of the immune response.

Expression and regulation of CD47 in gastrointestinal tumor cells

The tumor microenvironment (TME) plays a pivotal role in the initiation, progression, and metastasis of gastrointestinal cancers.[19] Interactions within the TME contribute significantly to tumor growth, invasion, immune evasion, and therapy resistance.[20] One of the primary mechanisms by which tumor cells evade the immune system is through the overexpression of immune checkpoint proteins, including CD47.[21] Understanding the expression patterns and regulatory mechanisms of CD47 in gastrointestinal cancers is essential for the development of effective immunotherapeutic strategies. Table 1 summarizes the expression and clinical significance of CD47 across various gastrointestinal tumors.

Esophageal cancer

CD47 has been found to be highly overexpressed across a broad spectrum of solid tumors, as determined by flow cytometry and immunohistochemistry techniques. In esophageal squamous cell carcinoma (ESCC) patients (n = 132), 43.2% exhibited high CD47 expression (n = 57). Furthermore, CD47 expression was found to be higher in ESCC compared to adjacent non-tumor tissues.[22] Additional studies support these findings, revealing that patients with ESCC express significantly higher levels of CD47 in cancerous tissues compared to non-cancerous tissues. Biopsies collected from 14 patients showed that CD47 mRNA expression was markedly higher in cancerous tissues. Immunohistochemistry analysis further confirmed extensive anti-CD47 staining on the surface of tumor cells, stromal cells, and

Table 1. CD47 expression and prognostic significance in gastrointestinal cancers

Cancer Type	Sample Size	High CD47 Expression (%)	Key Findings and Prognostic Significance
Esophageal Squamous Cell Carcinoma	132	43.2%	Higher CD47 expression in cancerous tissues correlating with advanced tumor stages and worse prognosis.
Gastric Cancer	115	49.6%	High CD47 expression correlates with lower survival rates, resistance to chemotherapy, and prevalence in the microsatellite instable subtype.
Colorectal Cancer	269	35.3%	Elevated CD47 expression is linked to reduced overall survival and is associated with lymphatic and perineural invasion.
Hepatocellular Carcinoma	166	21.7%	Increased CD47 expression is associated with large vessel invasion and a high proliferation index, suggesting a target for boosting antitumor immunity.
Gallbladder Cancer	92	17.4%	Elevated CD47 expression correlates with vascular and perineural invasion, highlighting its potential as a therapeutic target to enhance immune response.
Pancreatic Ductal Adenocarcinoma	106	61.3%	High CD47 expression indicates a poor prognosis and adverse clinicopathological features. CD47 may present a more stable expression compared to PD-L1, offering potential as a biomarker for anti-CD47 therapy.

infiltrated cells such as fibroblasts and macrophages in the tumor region, whereas minimal staining was observed in corresponding non-cancerous tissues.[23]

Currently, research on CD47 regulation in ESCC is limited. One prominent study found that miR-133a is downregulated in ESCC tissues and serves as a direct regulator of CD47, lower levels of miR-133a corresponded with increased CD47 expression, which was associated with lymph node metastasis and poor prognosis.[24] Another study identified through bioinformatics analysis that interferon-gamma (IFN-γ) may be involved in the upregulation of CD47 in various cancer types, including esophageal adenocarcinoma, although the specific mechanisms in ESCC remain unclear.[25] These findings underscore the necessity for further research into the regulatory processes governing CD47 expression in ESCC and their implications for cancer progression and therapeutic targeting.

Gastric cancer

Among patients with early gastric cancer (n = 115), 49.6% (n = 57) displayed high CD47 expression, as determined by immunohistochemistry. Notably, CD47-positive patients had a lower 5-year survival rate compared to CD47-negative patients, indicating a potential correlation between CD47 expression and poor prognosis.[26] Additional studies has revealed that CD47 expression is associated with macrophage infiltration and adverse clinical outcomes in gastric cancer. High CD47 expression in gastric cancer patients correlates with poor prognosis and reduced therapeutic responsiveness to fluorouracil-based adjuvant chemotherapy. This suggests that CD47 could serve as an independent prognostic marker for survival outcomes and chemotherapy resistance.

The expression of CD47 in gastric cancer may be regulated by Galectin-3 (Gal-3) and IFN-γ. Gal-3 is correlated with CD47 levels, and its depletion leads to reduced CD47 expression and enhanced immune responses.[27] Furthermore, IFN-γ has been shown to upregulate CD47 expression in cancer cells, increasing its binding affinity to SIRPα and consequently diminishing macrophage phagocytosis.[25] Importantly, CD47 expression is enriched in the microsatellite unstable subtype of gastric cancer and is associated with ARID1A mutations and activation of the FGFR2 signaling pathway. These features provide further insight into the tumor biology and potential therapeutic targets for gastric cancer.[28]

Colorectal cancer

In a study involving primary colorectal cancer patients (n = 269), immunohistochemical analysis showed that 35.3% of the lesions (n = 95) exhibited CD47 expression on the cytomembrane of colorectal cancer cells. Importantly, patients with CD47-positive tumors had a significantly shorter overall survival time than those with CD47-negative tumors, suggesting a potential correlation between CD47 expression and poor prognosis.[29] Earlier research has highlighted the concurrent expression of CD47 and CD44 in colorectal cancer, which promotes malignancy. In a study of 95 cases of stage II-IV colorectal cancer, CD47 and CD44 were overexpressed in 82 and 80 cases, respectively. This overexpression correlated with distant metastasis, and high levels of both CD47 and CD44 were associated with worse disease-free survival in stage III cases.[30] Additional studies have provided further insights into the role of CD47 in colorectal adenocarcinoma. Immunohistochemical analysis of 328 colorectal adenocarcinoma tissues revealed that CD47 expression

was observed in 16.2% (n = 53) of the samples. Positive CD47 expression was significantly associated with adverse clinicopathologic features, including lymphatic invasion, perineural invasion, tumor budding, pathologic N stage, and AJCC stage. These findings underscore the impact of CD47 expression on tumor aggressiveness and patient prognosis.[31] Moreover, transcriptomic and immunophenotypic profiling of colorectal cancer has revealed that CD47 expression is upregulated during malignant transformation. Spatial profiling indicated increased infiltration of myeloid cells and a shift in macrophage populations from pro-inflammatory to immune-suppressive subsets, accompanied by the upregulation of the CD47/SIRPα axis. This shift highlights the role of CD47 in creating an immunosuppressive tumor microenvironment.[32]

The expression of CD47 in colorectal cancer is influenced by hypoxia and oncogenic signaling pathways. Under hypoxic conditions commonly found in tumors, HIF-1α can upregulate CD47 expression.[33] Additionally, the inflammatory cytokine IL-8, secreted by M2 macrophages, enhances CD47 levels and promotes cancer cell migration through its receptors CXCR1.[34] This mechanism contributes to immune evasion in tumors. Furthermore, the long non-coding RNA LINC00460 is significantly overexpressed in colorectal cancer, acting as a molecular sponge for miR-186-3p, leading to the upregulation of MYC, CD47, and PD-L1.[35] MYC also increases LINC00460 expression, creating a positive feedback loop that further elevates CD47 levels. These regulatory mechanisms highlight the potential of CD47 as a therapeutic target, particularly in addressing immune evasion in colorectal cancer.

Hepatocellular carcinoma

In hepatocellular carcinoma patients (n = 166), immunohistochemical analysis identified CD47 expression in 21.7% of patients (n = 36). Notably, this elevated expression was significantly linked to frequent large vessel invasion and a higher Ki-67 proliferation index, suggesting a correlation between CD47 expression and tumor aggressivenes.[36] Additional research involving 76 tissue samples, including 54 cases of cholangiocarcinoma and 22 cases of hepatocellular carcinoma, provides further insights into CD47 expression. The immunohistochemical analysis revealed that high CD47 expression was associated with adverse pathological features and poor clinical outcomes. Moreover, the study demonstrated that inhibiting CD47 increased the phagocytic activity of macrophages, both in cell culture and animal models, indicating that CD47-targeted therapies could be an effective strategy to boost antitumor immunity and inhibit tumor progression in hepatocellular carcinoma.[37]

The expression of CD47 in hepatocellular carcinoma is significantly influenced by inflammatory signaling and the tumor microenvironment. The interleukin-6 (IL-6), secreted by tumor-infiltrating macrophages, upregulates CD47 levels through the activation of the STAT3 signaling pathway.[38] Additionally, inhibition of STAT3 can decrease CD47 expression and promote immunogenic cell death, indicating that targeting this pathway may enhance immune recognition and response against hepatocellular carcinoma.[39] Furthermore, in the context of drug resistance, CD47 expression is further enhanced by the activation of nuclear factor kappa B (NF-κB) in sorafenib-resistant hepatocellular carcinoma cells, contributing to an aggressive tumor phenotype and treatment failure.[40] These regulatory mechanisms underscore the potential of CD47 as a promising therapeutic target in addressing immune evasion and improving treatment outcomes in hepatocellular carcinoma.

Gallbladder cancer

In a study of gallbladder cancer patients (n = 92), immunohistochemical analysis revealed that CD47 expression was observed in 17.4% of patients (n = 16). This elevated expression was significantly associated with adverse clinicopathological features, including vascular invasion and perineural invasion in the advanced stage group, indicating a correlation between CD47 expression and more aggressive tumor behavior.[41] Moreover, experiments involving RNA extraction and quantitative RT-PCR techniques have confirmed the upregulation of CD47 at the gene level in gallbladder cancer tissues. This molecular evidence further emphasizes the significance of CD47 in the pathogenesis and progression of gallbladder cancer. The potential for targeting CD47 in therapeutic strategies to enhance the immune system's ability to combat gallbladder cancer is increasingly being recognize.[42]

Specific regulatory mechanisms of CD47 in gallbladder cancer are not well-defined. Factors such as hypoxia and inflammatory cytokines commonly observed in the tumor microenvironment could potentially influence CD47 levels.[43] Nonetheless, additional research is required to elucidate these mechanisms within the context of gallbladder cancer.

Pancreatic cancer

Among pancreatic ductal adenocarcinoma (PDAC) patients (n = 106), CD47 expression was found to be high in tumor cells in 61.3% patients (n = 65), was associated with adverse clinicopathological features, and worse prognosis.[44] Additionally, in a set of 47 resected specimens of pancreatic neuroendocrine tumors (PanNET), CD47 was found to be highly expressed in the cells derived from human PanNET compared to the surrounding noncancerous pancreatic cells.[45] The notable upregulation of CD47, intriguingly sets the stage for a discussion on another critical immune checkpoint, PD-L1. The expression of PD-L1 through immunohistochemical staining has been widely used as one of the tools in clinical trials to identify responders and non-responders to immunotherapy treatment.[46] However, the expression level of PD-L1 is not the only predictor of immunotherapy response. In the case of pancreatic cancer, the expression level of PD-L1 in PDAC tumor tissues lacks consistent consensus. However, studies have indicated that PD-L1 expression can be detected in approximately 12%–39% of PDAC tumor tissues.[47–49] This partly explains the poor response to anti-PD-1/PD-L1 therapy in pancreatic cancer. Based on literature, it has been hypothesized that the expression of CD47 in tumor cells is potentially higher and more stable than PD-L1 expression in pancreatic cancer. The role of CD47 expression level in tumor tissues as a biomarker of anti-CD47 therapy in pancreatic cancer is hopeful. However, further exploration of additional biomarkers is necessary to screen patients who may benefit from anti-CD47 therapy.

In PDAC, the regulatory mechanisms underlying CD47 expression are currently limited. One significant finding is that mixed lineage kinase domain-like pseudo-kinase (MLKL) driven necroptosis in tumor cells can enhance the 'don't eat me' signal, a

phenomenon associated with increased CD47 expression.[50] In co-culture models involving PDAC cells and tumor-associated macrophages, particularly M1 macrophages, CD47 expression was shown to be upregulated, which facilitated tumor cell escape from macrophage phagocytosis. Notably, this upregulation of CD47 was influenced by cytokines secreted from macrophages during co-culture, with IL-6 emerging as a key player. Elevated levels of IL-6 were associated with increased CD47 expression, indicating a cytokine-mediated regulatory mechanism.[43,50] These findings highlight the complex interplay between PDAC cells and the immune microenvironment in modulating CD47 expression and its implications for immune evasion in pancreatic cancer.

CD47/SIRPα INHIBITORS IN PRECLINICAL RESEARCH OF GASTROINTESTINAL CANCERS

Enhancing immune response through CD47 inhibition in esophageal cancer

In a mouse xenograft model of ESCC, tumor cell transfection with precursor miR-133a led to a significant reduction of CD47 expression and a suppressed tumor progression.[24] Hua et al. reported that treatment with a CD47 antagonist increased the infiltration of $CD8^+$ T cells in a preclinical model of ESCC. Furthermore, they found that this CD47 antagonist therapy improved the effectiveness of anti-PD-1 and CTLA-4 therapies. The study also revealed a significant correlation between the efficacy of anti-CD47 therapy and the functionality of dendritic cells. Notably, there was an inverse relationship between the expression of CD47 in tumor cells and the infiltration of $CD8^+$ T cells. These findings suggest a potential negative impact of CD47 expression on the antitumor immune response in patients with esophageal squamous cell cancer. Based on these results, a combinatory treatment strategy incorporating the administration of anti-CD47 is justified.[51]

Breakthroughs in combined CD47-targeted therapies in gastric cancer

Combining CD47-targeting with antiangiogenic drugs has shown promising results in treating gastric cancer. Interestingly, the upregulation of CD47 during bevacizumab therapy induces immunosuppression. However, blocking CD47 reverses macrophage inhibition, enhances antitumor effects, and inhibits tumor metastasis, effectively overcoming resistance to antiangiogenic therapy. This combined approach could significantly improve patient outcomes by enhancing the efficacy of antiangiogenic treatments.[52] Moreover, CD47 knockdown has been found to inhibit liver metastasis of gastric cancer in mice and enhance macrophage-mediated phagocytosis. Tumor-derived exosomes play a role in phagocytosis by Kupffer cells, and the administration of anti-CD47 antibodies has been shown to inhibit *in vivo* tumor growth. Notably, combining anti-CD47 antibodies with 5-Fu treatment demonstrates a synergistic effect, suggesting this combination as a potential strategy for treating liver metastasis of gastric cancer.[53] Additionally, the dual inhibition of CD47 and Galectin-3 (Gal3) enhances tumor cell phagocytosis and reprograms macrophages, effectively overcoming the immunosuppressive microenvironment. This approach has been effective in suppressing tumor growth in peritoneal metastasis of gastric adenocarcinoma, highlighting the potential of multi-targeted therapies in combating metastatic disease.[27] Furthermore, CD47-blocking antibodies have been observed to inhibit tumor growth in murine gastric cancer cell tumor xenografts. This therapy activates cGAS-STING signaling, leading to the production of type I interferons and consequently activating antitumor immunity in Epstein-Barr virus-associated gastric cancer. These findings underscore the potential of anti-CD47 therapy in promoting antitumor immune responses and inhibiting tumor growth in this specific subtype of gastric cancer.[54] In summary, targeting CD47 in gastric cancer therapy holds significant promise. By combining CD47 inhibition with other therapeutic strategies, such as antiangiogenic drugs, 5-Fu, and Gal3 inhibitors, it is possible to enhance antitumor effects and overcome resistance mechanisms. These advancements provide a strong rationale for further exploration and clinical development of CD47-targeted therapies in gastric cancer.

Enhancing therapeutic strategies through CD47 inhibition in colorectal cancer

CD47 plays a crucial role in promoting cell proliferation and enhancing the metastatic potential of colorectal cancer cells both *in vitro* and *in vivo*. Mechanistically, CD47 interacts with ENO1, inhibiting its FBXW7-mediated ubiquitination. This interaction leads to an upregulation of aerobic glycolysis and increased activity of the MAPK signaling pathway. These processes underscore the importance of CD47 in colorectal cancer progression and highlight its potential as a therapeutic target.[55] In preclinical models, particularly in CT-26 mouse tumors, a triple combination therapy involving FOLFOX, CD47, and PD-L1 blockade has demonstrated potential in enhancing the antitumor immune response and improving outcomes. This regimen shows promising translational prospects for clinical settings.[56] Furthermore, the investigation into the molecular interactions downstream of CD47 has unveiled that SHP2 deneddylation by the SIRPα receptor plays a significant role in mediating immune suppression in colon cancer. This finding provides a deeper understanding of the immune evasion strategies employed by tumor cells and opens new avenues for targeted therapy.[57]

Radiation-induced upregulation of CD47 and PD-L1 via the ataxia-telangiectasia and rad3-related-mediated DNA repair pathway additionally complicates the immune landscape by inhibiting the phagocytosis and antigen presentation capabilities of immune cells.[58] Targeting this pathway could potentially enhance immune system recognition and elimination of tumor cells, improving the efficacy of immunotherapeutic strategies. Moreover, studies involving SIRPα-deficient mice have demonstrated that the absence of this receptor enhances both innate and adaptive immune responses, offering increased resistance to tumor progression independent of CD47. This suggests that inhibiting SIRPα could be a viable strategy to potentiate cancer immunotherapy, offering a dual approach alongside CD47 blockade.[59] In conclusion, the strategic inhibition of CD47 and its signaling partners presents a robust method for combating colorectal cancer. By combining CD47 blockade with targeted therapies against the CD47-SIRPα axis and integrating conventional chemotherapies and other immune checkpoint inhibitors,

there is a significant potential to amplify the antitumor immune response.

Synergistic approaches targeting CD47 in hepatocellular carcinoma

CD47 upregulation in hepatocellular carcinoma has been identified as a key factor contributing to resistance against the commonly used cancer drug sorafenib. Research indicates that by blocking CD47, it is possible to enhance the efficacy of sorafenib, thereby overcoming this resistance and potentially improving treatment outcomes for patients with hepatocellular carcinoma. This synergy suggests that CD47 blockade might be a critical component in the treatment of sorafenib-resistant hepatocellular carcinoma.[40] Further investigations have led to the development of a bispecific antibody that targets both GPC3 and CD47. This novel therapeutic approach has shown superior effectiveness over traditional monotherapy or combined treatments with anti-CD47 and anti-GPC3 monoclonal antibodies in xenograft models of hepatocellular carcinoma. The bispecific antibody not only targets CD47 effectively but also provides a more potent anticancer action by engaging with two distinct antigens simultaneously, enhancing the overall therapeutic impact.[60] Additionally, the inhibition of CD47 has been found to significantly enhance the phagocytic activity of $CD103^+$ DCs and to activate the STING pathway. This activation leads to the secretion of cytokines like CXCL9 and IL-12, which play crucial roles in the recruitment and activation of NK cells. These effects collectively contribute to a heightened immune response against tumor cells, underscoring the potential of targeting both the CD47 pathway and the STING pathway as part of a comprehensive strategy to treat hepatocellular carcinoma.[61] To capitalize on these insights, integrating CD47 antagonists with other immune checkpoint inhibitors has emerged as a promising strategy. This combined approach aims to dismantle the immunosuppressive tumor microenvironment while simultaneously activating multiple facets of the immune response to combat hepatocellular carcinoma more effectively.

Advancing treatment through CD47 inhibition in pancreatic tumor

The downregulation of CD47 expression using miRNAs has been regarded as an effective therapeutic approach for cancer treatment. Xi et al. discovered that miR-128 regulates the expression of CD47 in tumor cells and promotes anti-tumor immunity in pancreatic cancer. *In vitro* assays showed that miR-128 suppresses cell proliferation, clonogenicity, migration, and invasion in Panc02 cells.[62] Additionally, miR-128 induced downregulation of CD47 enhanced macrophage phagocytosis and dendritic cell activity. Furthermore, miR-340, promoted macrophage phagocytosis by targeting CD47 on pancreatic cancer cells. These miRNAs have potential to initiate an effective antitumor T cell response and, consequently, inhibit tumor progression.[63]

CD47 inhibition has also been explored in preclinical animal models. Michaels et al. established a model of pancreatic cancer liver metastasis in NOD-scid-gamma mice by injecting human pancreatic cancer cells into the spleen, It was found that hepatic macrophages could inhibit the progression of liver micrometastasis, while CD47 blockade increased the efficiency of macrophages to remove pancreatic cancer cells, which was related to the surface density of CD47 receptors.[64] Moreover, the study found that the CD47 inhibitor induced macrophages to phagocytose pancreatic cancer stem cells. Similarly, in a xenograft model of PanNET, blocking CD47 resulted in phagocytosis of tumor cells by macrophages, leading to reduced tumor growth and metastasis.[65] In another study using a syngeneic mouse model with the Panc02 cell line, treatment with anti-CD47 monoclonal antibody reduced the proportion of M2 macrophages while significantly increasing the number of M1 macrophages. This shift suggests potential anti-tumor effects and improvements in the tumor microenvironment.[44] Furthermore, the activation of Toll-like receptor agonist CpG induced alterations in the central carbon metabolism of macrophages, allowing macrophages to overcome the immunosuppressive effect of CD47 on PDAC cells. This mechanism effectively achieved anti-tumor activity in a mouse model of pancreatic cancer.[66] It is interesting to note that the continued inhibition of CD47 also caused the apoptosis of pancreatic CSCs. When there is a lack of immune cells, such as macrophages, the CD47 blockade induced cell apoptosis through a non-immune effect in pancreatic cancer.[66,67] Moreover, chimeric antigen receptor (CAR)-T cells targeting the CD47 antigen have been developed, demonstrating significant efficacy in killing ovarian, pancreatic, and other cancer cells, producing high levels of cytokines that positively correlated with CD47 antigen expression.[68] As demonstrated above, these current models positively establish the anti-tumor effect of inhibiting CD47. The diverse mechanisms by which CD47 blockade enhances macrophage activity, induces apoptosis of cancer cells, and improves the tumor microenvironment underscore its promise in developing effective cancer therapies.

CD47/SIRPα INHIBITOR IN CLINICAL STUDIES OF GASTROINTESTINAL CANCERS

Type of CD47/SIRPα inhibitors

CD47-related inhibitors have been rapidly developed, and currently there are four main types of specific inhibitors that have been developed to target the CD47/SIRPα pathway. These include anti-CD47 monoclonal antibodies, bispecific antibodies, anti-SIRPα monoclonal antibodies, and SIRPα recombinant proteins.[69,70]

As of February 2024, a total of 105 clinical trials have been registered on ClinicalTrials.gov to target the CD47/SIRPα pathway (Table S1), including both solid tumors and hematologic malignancies. The goal of these trials is to evaluate the potential clinical significance of inhibiting the CD47/SIRPα pathway. Currently, there are no ongoing clinical trials targeting CD47 using RNA interference therapy due to safety and biological stability concerns associated with miRNA and siRNA therapies.[71] The anti-CD47 monoclonal antibodies being evaluated include: IBI188, Hu5F9-G4, CC-90002, AO-176, AK117, HMPL-A83, AUR103, ZL-1201, TQB2928, STI-6643, SRF231, sB24M IMC-002, Lemzoparlimab, and Gentulizumab. The bispecific antibodies in trials are: TG-1801, IMM2520, IBI322, HX009, BAT7104, PT217, PF-07257876, PT886, NI-1801, SG2501, IMM2902 and CPO107, most of which are anti-CD47/PD-L1 bispecific antibodies. And finally, the recombinant proteins being assessed include: IMM01, TTI-621, TTI-622,

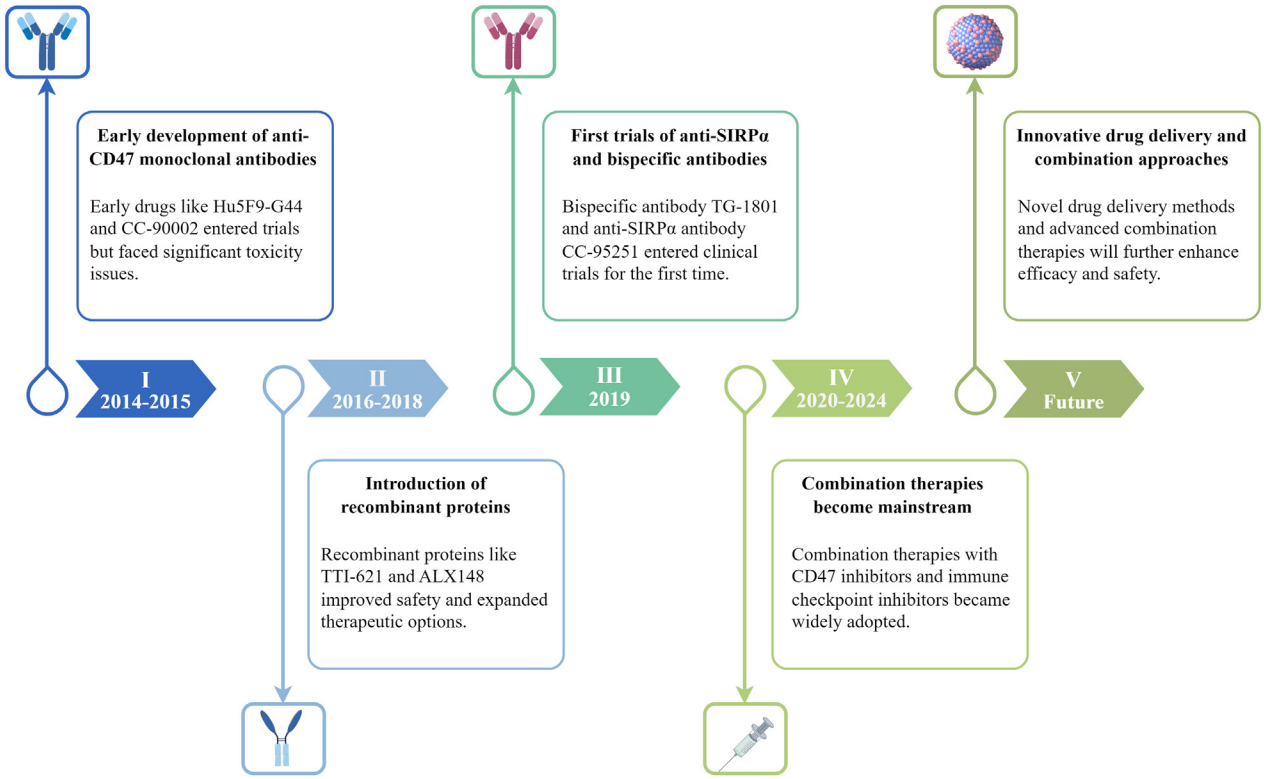

Figure 2. Timeline of CD47/SIRPα clinical trials and key developments

ALX148, IBC0966 and HCB101. While the anti-SIRPα monoclonal antibodies are: DS-1103a, BYON4228, CC-95251, BI765063 and BI770371. Figure 2 offers a visual summary of the timeline for key developments and clinical trials, from the early use of monoclonal antibodies to recent advancements in bispecific antibodies and combination therapies.

Results of clinical trials evaluating CD47/SIRPα inhibitors

Most clinical trials involving CD47/SIRPα inhibitors have focused on hematologic malignancies.[72] Among all the experiments conducted on gastrointestinal tumors, five have yielded research results (NCT02216409,[73] NCT02953782,[74] NCT03013218,[75] NCT03990233,[76] NCT05002127).[77] Detailed information about these trials is presented in Table 2.

Phase I trial of Hu5F9-G4 in advanced malignancies

NCT02216409 was a phase I clinical trial evaluating Hu5F9-G4 in advanced malignancies treatment.[73] The primary objective of the study was to assess the safety and tolerability of Hu5F9-G4, as well as evaluate the pharmacokinetics and pharmacodynamics of Hu5F9-G4. A total of 62 patients were enrolled in the study, out of which 22 had gastrointestinal cancers. Among these patients, 18 had colorectal cancer, and 4 had pancreatic cancer. The results demonstrated that Hu5F9-G4 exhibited excellent tolerability in patients with solid tumors and lymphoma when administered using a priming and maintenance dose regimen. Additionally, two patients diagnosed with ovarian and fallopian tube cancers, respectively, achieved partial remissions.

Combination of Hu5F9-G4 and cetuximab in colorectal cancer

NCT02953782 was a study evaluating the combination of Hu5F9-G4 and cetuximab in previously treated KRAS wild-type and KRAS mutant colorectal cancer patients.[74] The trial enrolled 78 patients and did not reach a maximum tolerated dose. The combination was well-tolerated, with common adverse events including acneiform dermatitis, dry skin, and fatigue. In 30 evaluable KRAS wild-type colorectal cancer patients, 2 achieved confirmed partial responses, for an objective response rate of 6.7%. Among 40 KRAS mutant patients, while no objective responses were seen, 45% achieved stable disease, and median overall survival was 12.4 months in KRAS mutant patients naive to TAS102 or regorafenib, longer than historical controls.

Phase I trial of BI 765063 in advanced solid tumors

NCT03990233 studied BI 765063, an anti-SIRPα monoclonal antibody, in patients with advanced solid tumors.[76] The trial enrolled 50 patients and did not reach a maximum tolerated dose. BI 765063 monotherapy was generally well-tolerated, with common adverse events including infusion reactions, fatigue, and headache. Of 47 evaluable patients, 45% derived clinical benefit, including 1 patient with advanced hepatocellular carcinoma who achieved a durable partial response. Tumor biopsy analysis showed increased intratumoral $CD8^+$ T cells and upregulated PD-L1 expression in the responding patient.

Evaluation of ALX148 in various cancers

NCT03013218 was a phase I clinical trial evaluating ALX148 alone and in combination with pembrolizumab or

Table 2. Overview of clinical trials involving CD47/SIRPα inhibitors for gastrointestinal Cancers

Trial ID	Study Status	Phases	Sample Size	Gastrointestinal Cancer Types & Number	Interventions	Response rate
NCT02216409	Completed	Phase 1	62	Colorectal cancer (18) Pancreatic cancer (4)	Hu5F9-G4 monotherapy	Partial remissions in ovarian cancer
NCT02953782	Completed	Phase I/II	78	Colorectal cancer (78)	Hu5F9-G4 + Cetuximab	Stable disease in 45% of KRAS mutant cases
NCT03990233	Active, not recruiting	Phase I	50	Not reported	BI 765063 monotherapy or BI 765063 + Ezabenlimab	Clinical benefit in 45% of patients; durable partial response in 1 hepatocellular carcinoma patient
NCT03013218	Active, not recruiting	Phase I	110	Gastric/GRJ cancer (25) Colorectal cancer (10) Pancreatic cancer (4)	ALX148 monotherapy or ALX148 + Pembrolizumab or ALX148 + Trastuzumab or ALX148 + Rituximab or ALX148 + Pembrolizumab + 5FU + Platinum or ALX148 + Trastuzumab + Ramucirumab + Paclitaxel	Disease control rate of 26.3% in Gastric/ gastroesophageal junction cancer
NCT05002127	Recruiting	Phase II/III	54 (mid-term analysis)	Gastric/GEJ cancer (54)	Phase II: ALX148 + Trastuzumab + Ramucirumab + Paclitaxel or Trastuzumab + Ramucirumab + Paclitaxel Phase III: ALX148 +Trastuzumab + Ramucirumab + Paclitaxel or Ramucirumab + Paclitaxel	Objective response rate of 52%, surpassing the 28% ORR of the second-line treatment standard (Cyramza with paclitaxel)

ORR: objective response rate.
GEJ: gastroesophageal junction.

trastuzumab.[75] The study enrolled 110 patients to assess the efficacy and safety of these treatment regimens, out of which 38 had gastrointestinal cancers. Among these patients, 25 had gastric or esophagogastric junction cancer, 10 had colorectal cancer, and 4 had pancreatic cancer. In a subgroup analysis, ALX148 plus pembrolizumab was administered to 20 patients with checkpoint inhibitor-naive and checkpoint inhibitor-treated head and neck squamous cell carcinoma (HNSCC), resulting in a disease control rate of 30.0% (n = 6). Additionally, ALX148 plus trastuzumab was administered to 19 patients with HER2-positive gastric or gastroesophageal junction (GEJ) cancer, achieving a disease control rate of 26.3% (n = 5). The data supporting the Food and Drug Administration (FDA) granted ALX148 Fast Track designation for the first-line treatment of HNSCC patients, and second-line treatment of patients with HER2-positive gastric/GEJ carcinoma in early 2020. In October 2023, ALX Oncology reported positive results from the mid-term data of its ASPEN-06 phase II clinical trial (NCT05002127).[77] This trial evaluated the combination of ALX148, Trastuzumab, Ramucirumab, and Paclitaxel for the treatment of HER2 positive gastric/GEJ carcinoma patients. The mid-term results demonstrated an advantage with an objective response rate of 52% compared to the efficacy report of cyramza in combination with paclitaxel (the second-line treatment standard for gastric/GEJ carcinoma) had an ORR of 28% in the RAINBOW study. Furthermore, the safety profile of ALX148 aligns with previous clinical trials, exhibiting good tolerability.[75] We are indeed looking forward to the future evaluation of the CD47 inhibitor ALX148 in clinical trials for other gastrointestinal cancers, such as pancreatic cancer.

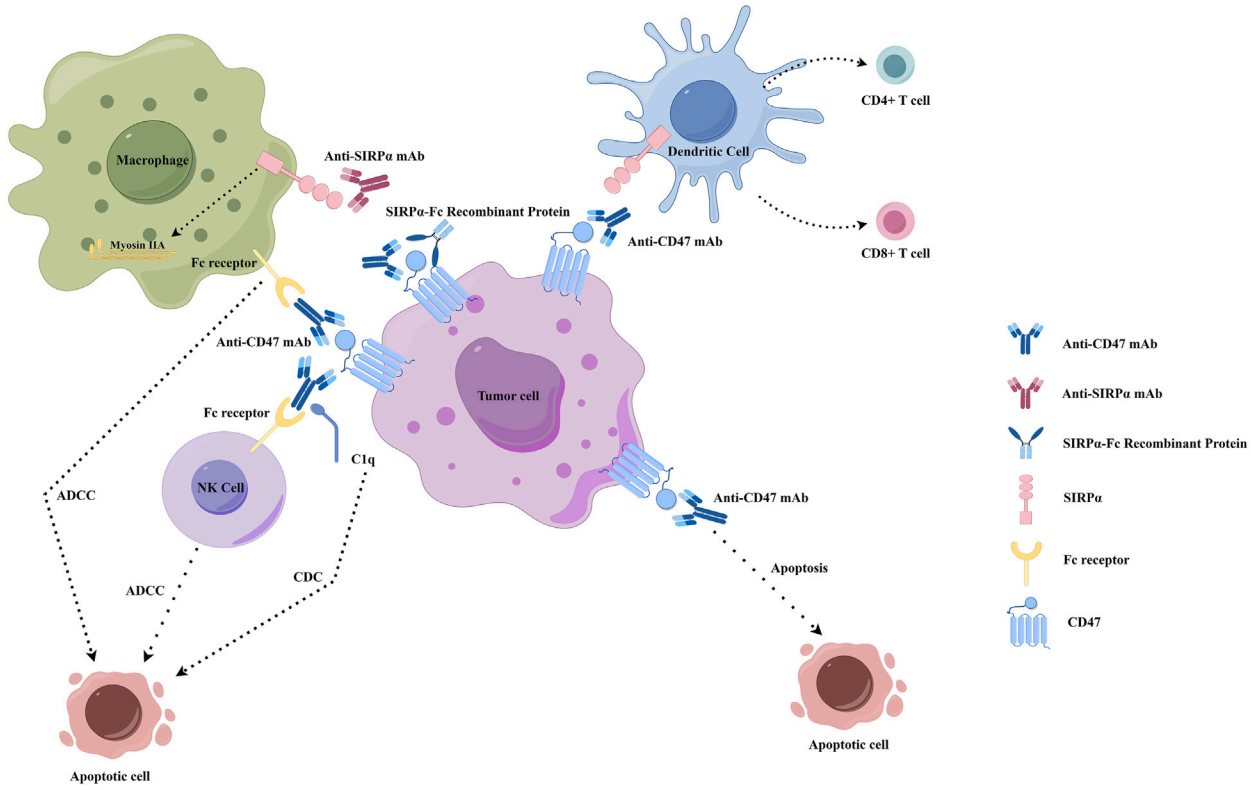

Figure 3. Mechanisms of action for CD47/SIRPα blockade in tumor cells

Anti-CD47 monoclonal antibodies induce additional immune responses, such as antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity, via Fc receptor activation on natural killer cells and complement proteins like C1qsAnti-SIRPα monoclonal antibodies and SIRPα-Fc fusion proteins also enhance macrophage-mediated phagocytosis by preventing CD47 from interacting with SIRPα on immune cells. Moreover, CD47 blockade enhances dendritic cell activation, promoting T cell cross-priming and boosting $CD8^+$ T cell-mediated tumor cell killing. Finally, CD47 inhibition can directly induce tumor cell apoptosis through non-immune pathways.

TOXICITY AND FURTHER IMPROVEMENT OF CD47 INHIBITORS

Mechanisms of action of CD47 inhibitors

As demonstrated earlier, there is an anti-tumor effect to blocking the CD47/SIRPα pathway in gastrointestinal cancer. The downstream effects include: (1) Blocking the "don't eat me" signal from the CD47/SIRPα interaction which then induces macrophage phagocytosis. (2) Traditional effect functions, antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), which is induced by the Fc-terminal of a monoclonal antibody. (3) Activation of dendritic cells to enhance the cross-activation of T cells, including $CD8^+$ cytotoxic T cells. (4) Promotion of tumor cell apoptosis through non-immune response (Figure 3).[8,12,78,79] Van Duijn et al. provided a comprehensive summary of the mechanisms of action for CD47 inhibitors, so we will not go into further detail here.[80]

Toxicity of CD47 inhibitors

The toxicity of CD47 inhibitors primarily arises from their mechanism of action and widespread expression of CD47 on various cell types.[81] This can lead to significant adverse effects, categorized primarily into immune system overactivation and off-target toxicity.[82,83] CD47 antibodies can inadvertently target normal RBCs, triggering macrophages to attack them and causing severe adverse reactions like anemia and hemolysis.[84] Early anti-CD47 drugs, such as CC-90002 and SFR231, had their development terminated due to severe hematological toxicity. Hu5F9-G4 initially showed promise in reducing off-target toxicity by using a priming dose to reduce RBC targeting, providing a potential improvement over other earlier drugs.[73] In clinical practice, this approach involved administering a priming dose of 1 mg/kg to silence RBCs, followed by a maintenance dose, which helped mitigate off-target toxicity. The observed toxicities mainly included anemia, erythrocyte agglutination, thrombocytopenia, headache, fatigue, and nausea. Some of these toxicities reached grade 3 but did not worsen with continued treatment. However, significant side effects persisted in clinical applications, including hemagglutination (41%), leukopenia (38%) and anemia (66%). Moreover, a higher incidence of grade 3 anemia events (17%) was observed. Subsequent experiments with Hu5F9-G4, such as the ENHANCE trial, the world's first phase III trial of CD47 inhibitors, were canceled in July 2023. This decision was made due to imbalances between the study groups caused by suspected unexpected serious adverse reactions reported by researchers. (NCT04313881).

Improvement of CD47 inhibitors

Reducing off-target toxicity through modified dosing regimens

A key strategy to mitigate off-target effects is the use of modified dosing regimens, such as the introduction of a priming dose with drugs like Hu5F9-G4. This initial dose triggers the clearance of senescent red blood cells, leading to temporary mild anemia but also stimulates the maturation of reticulocytes into less sensitive, fresh young RBCs, allowing for higher subsequent treatment doses.[73]

Development of IgG4 type CD47 antibodies

The use of IgG4 type CD47 antibodies, like IBI188 and Hu5F9-G4, which feature IgG4 Fc ends, induces weaker ADCC and CDC effects. This strategy lowers efficacy against tumor cells compared to IgG1 but significantly reduces the risk of hemolysis and other hematologic side effects.[85]

Selective antibody screening

Selective screening for antibodies that do not bind to CD47 on RBCs is another approach to improving safety. Lemzoparlimab utilizes unique drug technologies to select antibodies that target epitopes obscured by glycosylation on RBCs but exposed on tumor cells, thereby minimizing hematologic side effects.[86]

Utilization of SIRPα fusion proteins

The development of SIRPα fusion proteins like TTI-621, IMM01, and ALX148 offers an enhanced safety profile due to weak binding affinity to RBCs. While TTI-621 and IMM01 use IgG1-Fc ends to enhance monotherapy efficacy, ALX148 features an Fc end lacking biological activity, necessitating combination with other drugs to boost anti-tumor efficacy.[75,87]

Development of anti-SIRPα antibodies

Anti-SIRPα antibodies such as BI765063 offer potentially better safety profiles due to the narrower expression spectrum of SIRPα. These antibodies do not bind to RBCs, although this may compromise direct tumor targeting effects.[88]

Advancements in bispecific antibodies

The development of bispecific antibodies, exemplified by PF-07257876, which has a higher affinity for PD-L1 and a lower affinity for CD47, helps reduce binding to non-target cells such as RBCs, thereby enhancing safety.[89]

Innovative drug delivery methods

Introducing new drug delivery methods, such as nanoparticle delivery systems, can provide synergistic effects when combined with other checkpoint inhibitors. Additionally, techniques like masking CD47 antibodies with tumor microenvironment-responsive polymer chains can effectively reduce side effects.[90,91] It is important to note that these advancements are not standalone solutions, the integration of multiple improvements is critical to enhancing clinical outcomes and reducing toxic effects.

ADVANCES IN COMBINATION THERAPIES

Enhanced efficacy through combination therapies

The meta-analysis by Son et al. summarized all clinical trial data on CD47 inhibitors up to 2022, and found that for both CD47 and SIRPα inhibitors, single-agent treatment did not show satisfactory results for solid tumor patients, with ORRs below 3%. However, the ORR for SIRPα blockade in combination therapy for solid tumors was significantly better than monotherapy (28.3% vs. 1.2%).[92] Given the complexity of the tumor immune microenvironment in gastrointestinal cancers, the efficacy of single immune checkpoint inhibitors is limited. Using CD47 inhibitors as adjunct therapy in combination regimens should be a direction for future research.[93] Furthermore, the study by Huang et al. examined the association between CD47 expression and 47 immune checkpoint genes across 33 cancer types. They found that in most cancer types, CD47 expression was positively correlated with immune checkpoint markers, including BLCA, DLBC, KICH, KIRC, LUAD, LUSC, PAAD, PCPG, SKCM, STAD, UCEC and UVM. This further supports the advantages and feasibility of combination therapy approaches.[94]

Novel combination approaches

Since the previous reviews by Son et al. and Ye et al. have already provided excellent summaries of combination therapy data prior to 2022, this article aims to supplement the latest progress and theoretical breakthroughs in CD47-based combination therapies over the past two years.[92,95] Nishiga et al. made a significant finding that the combination of radiation therapy and CD47 blockade enhanced the local anti-tumor effect of radiation in a preclinical model of small cell lung cancer. Furthermore, this combination treatment stimulated the occurrence of a distant anti-tumor effect, indicating the induction of abscopal effects.[96] Zhang et al. developed a novel therapeutic approach using bridging-lipid nanoparticles (B-LNPs) that target both PD-L1 and CD47 in glioblastoma. This dual-targeting system was enhanced with an STING agonist, diABZI, that promoted anti-tumor immunity by improving phagocytosis and T cell recruitment. This approach showed promising preclinical results in brain tumor regression and the induction of immunological memory against glioma.[97] These findings underscore the potential of innovative combination therapies to transform the treatment landscape for challenging cancers.

PERSPECTIVES AND LIMITATIONS

Advancing precision immunotherapy

Although CD47-SIRPα inhibitors have shown promising results in hematological tumors, the complex tumor immune microenvironment of gastrointestinal cancers poses unique challenges. Small molecule drugs possess advantageous properties such as a suitable half-life and effective penetration into tumor tissues, yet they may also inadvertently affect normal tissues due to their systemic distribution, potentially leading to toxic side effects.[98] In contrast, antibody-based therapies offer enhanced tumor targeting, minimizing off-target effects. however, their large molecular size often limits their ability to deeply penetrate tumor masses and they are susceptible to phagocytosis by peripheral immune cells, which can diminish their therapeutic efficacy.[99] Understanding the diversity and complexity of the tumor microenvironment is a challenge and a key to promoting the efficacy of immunotherapies such as anti-CD47. The tumor microenvironment changes, not only during tumor development, but also before and after anti-tumor therapy. Measuring the expression levels of immune checkpoints in tumors can help identify which patients are more likely to respond to the combination treatment

of CD47 inhibitors and checkpoint inhibitors and reduce the potential for toxic effects. The technique of single-cell sequencing, non-invasive imaging, and liquid biopsy has enabled us to understand the component of immune cells and the immune checkpoints in each cancer patient, leading to future precision immunotherapy based on the immune cell landscape of each patient.[100] Currently, single-cell sequencing has been used to reveal the immune cell landscape in solid tumors, such as lung and breast cancer.[101,102] Combining single-cell sequencing with spatial transcriptomics could facilitate the establishment of a comprehensive immune cell atlas for human gastrointestinal cancers. This would deepen our understanding of the tumor immune microenvironment, providing a solid foundation for the development of immunotherapeutic strategies for gastrointestinal cancers.[103,104]

Utilizing preclinical models to enhance cancer research

The development of oncology drugs depends on preclinical animal models. However, replicating the complexities of human tumor immunity remains challenging. NOD mice, commonly used in CD47 research, and subcutaneous tumor models often fall short of mimicking the intricate interactions within the tumor microenvironment. This limits their ability to accurately predict therapeutic outcomes in human tissues.[105] Genetically engineered mouse models provide a closer approximation of human cancer genetics but have restricted use in translational research.[85] Humanized mouse models, incorporating human immune cells, offer better insights into immunotherapies like CD47 blockade but are resource-intensive and technically complex.[106] Recent developments in organoid models, derived from patient tumor tissues, promise more personalized insights into tumor-immune interactions, though they are still evolving for large-scale drug testing.[107]

No model perfectly captures human tumor biology. To advance CD47-targeted therapies for gastrointestinal cancers, it is crucial to combine the strengths of these models with advanced tools to study the tumor microenvironment. This comprehensive approach will help refine immunotherapeutic strategies and improve clinical outcomes.[108]

Emerging therapies and future directions

Recently, targeted therapies such as PARP inhibitors,[109] KRAS-G12^{C} inhibitors,[110] and the combination of MEK inhibitor trametinib and BRAF inhibitor dabrafenib,[111] have shown promising results in the treatment of gastrointestinal cancers. Current combination therapy studies have primarily focused on pairing CD47 inhibitors with traditional chemotherapies or PD-1 inhibitors, but it will be important to explore combining CD47 inhibitors with these new targeted therapies as well. Additionally, cancer vaccines utilizing antigen-specific active immunotherapy are rapidly developing, and exploring the combination of these immunotherapies with CD47 inhibitors holds great promise for achieving durable anti-tumor responses.[112,113] A key challenge in the use of CD47 inhibitors remains how to more precisely target them to tumor cells, in order to reduce off-target effects and destruction of red blood cells. A recent study by Su et al. demonstrated a microfluidics-enabled, tumor acidity-responsive nanovesicle that can selectively deliver and release CD47/PD-L1 antibodies in the acidic tumor microenvironment. This delivery strategy has important implications for reducing immune-related adverse events associated with CD47 inhibitors.[114]

CONCLUSIONS

In summary, we discussed the ongoing research and clinical trials focused on optimizing the utilization of CD47 antibodies, either as monotherapy or in combination, for the treatment of gastrointestinal cancers. This includes investigations into combination therapies and strategies to mitigate adverse effects, with the goal of identifying patients most likely to benefit from this innovative immunotherapeutic approach. The future research should provide insights into the development of next-generation CD47 antibodies, CAR-T therapy, and combination treatments,[115,116] thus paving the way for anti-CD47 treatment in gastrointestinal cancers.

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AUTHOR CONTRIBUTIONS

C.C. conducted the investigation and wrote the original draft. F.L. and H.H. reviewed and edited the manuscript. Y.P. conceptualized the project and provided supervision. All authors approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

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