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Role of *CD47* gene expression in colorectal cancer: a comprehensive molecular profiling study

Hiroyuki Arai ,[1,2] Nishant Gandhi,[3] Francesca Battaglin,[1] Jingyuan Wang,[1] Sandra Algaze,[1] Priya Jayachandran,[1] Shivani Soni,[1] Wu Zhang,[1] Yan Yang,[4] Joshua Millstein,[4] Jae Ho Lo,[1] Davendra Sohal,[5] Richard Goldberg,[6] Michael J Hall,[7] Aaron James Scott,[8] Jimmy J Hwang,[9] Emil Lou ,[10] Benjamin A Weinberg,[11] John Marshall,[11] Sanjay Goel,[12] Joanne Xiu,[3] W Michael Korn,[3] Heinz-Josef Lenz[1]

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For numbered affiliations see end of article.

Correspondence to
Heinz-Josef Lenz; lenz@usc.edu

ABSTRACT

Background In patients with colorectal cancer (CRC), the therapeutic effects of conventional immune checkpoint inhibitors targeting the adaptive immune system are largely limited to those with microsatellite instability-high tumors. Meanwhile, new immunotherapies targeting the innate immune system are attracting increasing attention. CD47 is a representative innate immune checkpoint involved in the evasion of tumor cell phagocytosis by macrophages. This large-scale study comprehensively examined the molecular significance of *CD47* gene expression in CRC.

Methods We analyzed the next-generation sequencing data of DNA and RNA from 14,287 CRC cases included in the data set of a commercial Clinical Laboratory Improvement Amendments-certified laboratory (Caris Life Sciences). The cases were divided into two groups based on the median value of *CD47* gene expression levels. The molecular and immune profiles between the groups were compared, and the relationship between *CD47* expression and survival outcomes was further examined.

Results In *CD47*-high tumors, the proportion of consensus molecular subtypes 1 and 4 was significantly higher than in *CD47*-low tumors. The expression levels of damage-associated molecular pattern-related genes showed a positive correlation with *CD47* expression levels. Major oncogenic pathways, such as mitogen-activated protein kinase, phosphoinositide 3-kinase, angiogenesis, and transforming growth factor beta, were significantly activated in *CD47*-high tumors. Additionally, the expression levels of a panel of adaptive immune checkpoint genes and estimates of immune cells constituting the tumor microenvironment (TME) were significantly higher in *CD47*-high tumors.

Conclusions *CD47* expression in CRC was associated with the activation of several oncogenic pathways and an immune-engaged TME. Our findings may provide valuable information for considering new therapeutic strategies targeting innate immune checkpoints in CRC.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ In the context of the antitumor immune response, the crucial role of the innate immune system has gained attention, and new immunotherapies targeting the phagocytosis checkpoint CD47 are being developed.

WHAT THIS STUDY ADDS

⇒ We conducted a large-scale molecular profiling study using clinical specimens of colorectal cancer and found that *CD47* gene expression levels positively correlate with the activation of the major oncogenic signaling pathway, including the mitogen-activated protein kinase and angiogenesis pathways. Additionally, tumors with high *CD47* gene expression exhibited significantly increased immune cell infiltration within the tumor microenvironment.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ A new combination therapy was suggested for colorectal cancers with elevated *CD47* expression, which involves the simultaneous inhibition of the innate immune system, the adaptive immune system, and related pathways, including the angiogenesis pathway.

INTRODUCTION

Cancer immunotherapies targeting adaptive immune checkpoints, such as programmed cell death protein-1, programmed cell death ligand 1 (PD-L1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), have significantly improved patient outcomes across various metastatic cancer types. However, in metastatic colorectal cancer (CRC), substantial benefits from immune checkpoint inhibitor (ICI) treatment are largely limited to patients with mismatch repair-deficient or microsatellite instability-high (dMMR/MSI-H) tumors.[1–3] Mismatch repair-proficient or microsatellite stable (pMMR/MSS) CRCs are largely unresponsive to ICIs due to the presence of abundant immunosuppressive

cells, such as CD4+T regulatory cells (Tregs), myeloid-derived suppressor cells, and tumor-associated macrophages, as well as elevated levels of cytokines, including transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), interleukin (IL)-4, and IL-10, in the tumor microenvironment (TME). These factors are well-known mechanisms of immunotherapy resistance.[4–7]

Emerging studies have demonstrated the crucial role of the innate immune system in antitumor immune responses.[8] The innate immune system serves as the primary non-specific defense against malignant cell transformations.[9] Additionally, the innate immune response can activate the adaptive immune system through a cross-priming process mediated by phagocytic cells, such as macrophages and dendritic cells.[9] Certain cytotoxic and targeted anticancer agents induce a distinctive type of cancer cell death, referred to as immunogenic cell death (ICD).[10] This process releases "danger" signals, known as tumor-derived damage-associated molecular patterns (DAMPs), from dying cells, which can be recognized by the phagocytic cells.[11] Following phagocytosis, the release of proinflammatory cytokines is triggered, in turn, priming T-cell activities.[8 12]

Malignant cells exhibit elevated levels of both pro-phagocytosis ("eat me") and anti-phagocytosis ("don't eat me") signals compared with normal cells.[13 14] Pro-phagocytosis signals, including DAMPs such as calreticulin (encoded by *CALR*) and HMGB1, have been identified. Conversely, tumor cells rely on the expression of anti-phagocytosis molecules, known as phagocytosis checkpoints, to evade immune-mediated eradication.[8] CD47, the initial phagocytosis checkpoint binding SIRP alpha on macrophages, is notably overexpressed in CRC.[15–17] Recent clinical investigations have been focused on a novel immunotherapy targeting CD47, extending beyond conventional adaptive checkpoint-targeted therapies, particularly in metastatic CRC.[18] However, CD47 has not been evaluated as a predictive or prognostic marker. A profound understanding of CD47's role within molecular pathways is imperative for developing effective immunotherapeutic strategies in CRC. Hence, we conducted a comprehensive molecular profiling study to address this clinical interest.

METHODS

Study population

Formalin-fixed paraffin-embedded (FFPE) tumor samples from patients with CRC (N=14,287) submitted to a commercial Clinical Laboratory Improvement Amendments-certified laboratory (Caris Life Sciences, Phoenix, Arizona, USA) were retrospectively reviewed for molecular profiling (MPC: molecular profiling cohort). The MPC was used for all the molecular and immune analysis performed in the study. Additionally, the prognostic value of *CD47* expression was investigated in a larger (N=25,962) CRC cohort (OC: outcomes cohort) with treatment information and clinical outcomes data obtained from insurance claims. These cohorts were not mutually exclusive with 13,885/14,287 (97%) MPC being represented in the OC.

Genome, transcriptome, and immunohistochemistry analyses

Next-generation sequencing (NGS) using a 592-gene Caris MI TumorSeek panel and whole transcriptome sequencing (WTS) were performed with DNA and RNA extracted from FFPE specimens, respectively (online supplemental methods). In the NGS analysis, gene mutations classified as "pathogenic" or "likely pathogenic" according to the American College of Medical Genetics and Genomics criteria were identified as "mutated", while those deemed "variants of unknown significance", "likely benign", or "benign" were considered "non-mutated" (wild type). Microsatellite instability and mismatch repair status were determined through a combination of immunohistochemistry, fragment analysis, and NGS, with the findings categorized as either dMMR/MSI-H or pMMR/MSS (online supplemental methods). PD-L1 expression was evaluated using immunohistochemistry with the SP142 antibody (Spring Biosciences), where the intensity of staining on tumor cell membranes was graded on a semiquantitative scale: 0 for no staining, 1+ for weak staining, 2+ for moderate staining, and 3+ for strong staining. Tumors were classified as PD-L1 positive if more than 5% of tumor cells showed 2+ or 3+ staining. The consensus molecular subtype (CMS) classification from Caris was generated using RNA sequencing data derived from the WTS platform (online supplemental methods).

Statistical analysis

In the MPC, patients were stratified into *CD47*-high and *CD47*-low groups based on the *CD47* gene expression levels using the median cut-off within the cohort. The clinical and molecular features between *CD47*-high and *CD47*-low groups were compared. The Mann-Whitney U test and the χ^2 test were used for the comparison of continuous variables and categorical variables, respectively. The DAMPs signature, calculated as the composite z-score of the six DAMPs-related genes (*CALR*, *HMGB1*, *ANXA1*, *HSPAA1*, *HSPA1A*, and *CXCL10*), was compared between *CD47*-high and *CD47*-low groups. Additionally, we explored the correlation between *CD47* expression and major oncogenic signaling pathways by comparing the composite z-scores of genes listed in online supplemental table S1 between *CD47*-high and *CD47*-low groups. The Microenvironment Cell Population counter (MCP-counter) was used for the quantification of the abundance of immune and stromal cell populations using WTS data, as described previously.[19] The median gene expression levels were compared between *CD47*-high and *CD47*-low groups.

In the OC, overall survival (OS) was defined as the time from tissue collection to the last clinical contact from an insurance claims repository, assuming that any patient without a claim for more than 100 days had died,

which holds true for more than 95% of patients with a recorded death in the National Death Index. Survival on angiogenesis inhibitors was calculated from the initiation of any of the angiogenesis inhibitors (aflibercept, bevacizumab, ramucirumab, regorafenib or sorafenib) to the last clinical contact. A similar approach was used to calculate survival on ICI (atezolizumab, durvalumab, ipilimumab, nivolumab, or pembrolizumab). OS was estimated using the Kaplan-Meier method and compared between *CDC47*-high and *CDC47*-low patient groups, stratified by the median cut-off in this cohort, using the log-rank test. In addition, a sensitivity analysis was performed using the quartiles of *CD47* expression to compare the top and bottom quartiles in this cohort.

To adjust p values for multiple hypothesis testing, the q values were calculated using the Benjamini-Hochberg method. Patients with any missing data were not included in each analysis. All statistical analyses were two-sided at a significance level set to 0.05 and conducted with SPSS V.23 (IBM SPSS Statistics).

RESULTS

Patient characteristics

Out of 14,287 patients, 7,143 and 7,144 were classified into *CD47*-low and *CD47*-high groups, based on the tumor's *CD47* expression levels. The *CD47*-high group exhibited a higher median age and a lower frequency of right-sided tumors compared with the *CD47*-low group. The distribution of gender was similar between both groups. The frequency of *RAS* mutations was lower in the *CD47*-high group (49.5% vs 54.0%), while the frequency of *BRAF* mutations was comparable between the two groups (8.2% vs 7.8%) (table 1).

NGS-based profiling and clustering of CMS subtypes

The *CD47*-high group exhibited significantly higher frequencies of mutations in *TP53*, *KMT2C*, and *CIC* and had a higher incidence of *NTRK1* fusion, while displaying significantly lower frequencies of *KRAS* mutation, *CDX2* amplification, and *FLT1* amplification, compared with the *CD47*-low group (figure 1A).

The *CD47*-high group showed higher frequencies of CMS1 (17.9% vs 14.5%) and CMS4 (40.1% vs 26.8%), along with lower frequencies of CMS2 (31.1% vs 35.4%) and CMS3 (10.9% vs 23.3%), compared with the *CD47*-low group (q<0.01) (figure 1B).

Associations between DAMPs signature and *CD47* expression

DAMPs signature, calculated as the composite z-score of the six DAMPs-related genes, was significantly activated in the *CD47*-high groups compared with the *CD47*-low groups (online supplemental figure S1A). *CD47* expression levels were positively correlated with gene expression levels of *CALR* (Spearman correlation coefficient=0.63, q<0.01; online supplemental figure S1B) and *HMGB1* (Spearman correlation coefficient=0.66, q<0.01; online supplemental figure S1C), both of which encode major DAMPs components.

Immunotherapy-related markers

The frequency of dMMR/MSI-H was higher in the *CD47*-high group compared with the *CD47*-low group (7.5% vs 5.8%, q<0.01). The frequency of cases with a tumor mutational burden (TMB) of 10/Mb or more (TMB-high) was equivalent in both groups. The PD-L1 positivity rate was also equivalent in both groups. Even when limited to pMMR/MSS cases, the frequency of TMB-high and

Table 1 Patient characteristics compared between *CD47*-low and *CD47*-high groups

		***CD47*-low (N=7,143)**		***CD47*-high (N=7,144)**		**Q value**
Age	Median (Range)	62 (14 to >89)		63 (17 to >89)		<0.01
Sex	Male Female	3,901 3,242	(54.6%) (45.4%)	3,906 3,238	(54.7%) (45.3%)	0.94
Primary tumor sidedness	Left Right Unknown	3,833 2,348 962	(53.7%) (32.9%) (13.5%)	4,011 2,109 1,024	(56.1%) (29.5%) (143%)	<0.01
Location of tumor sampling	Primary Metastatic Unknown	4,167 2,812 164	(58.3%) (39.4%) (2.3%)	3,886 3,121 137	(54.4%) (43.7%) (1.9%)	<0.01
RAS status	Wild type Mutant Unknown	3,259 3,857 27	(45.6%) (54.0%) (0.4%)	3,582 3,537 25	(50.1%) (49.5%) (0.3%)	<0.01
BRAF status	Wild type Mutant Unknown	6,305 559 279	(88.3) (7.8) (3.9)	6,273 589 282	(87.8) (8.2) (3.9)	0.40

Unknown groups were not included in the analysis.

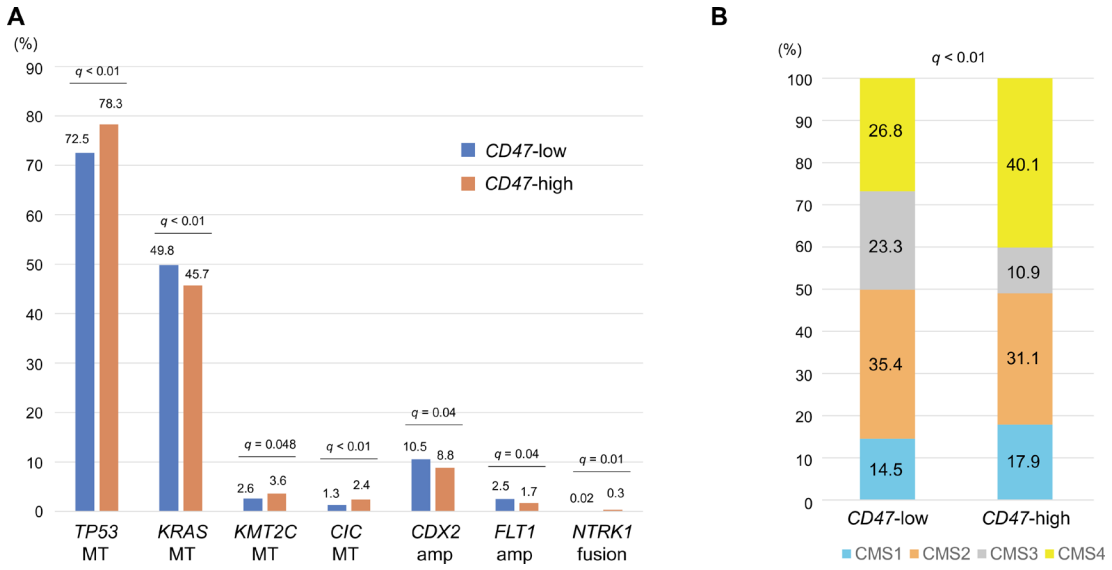

Figure 1 (A) Next-generation sequencing-based profiling and (B) clustering of CMS subtypes compared between *CD47*-high and *CD47*-low tumors. In (A) only alterations with a significant difference in frequency are shown. CMS, consensus molecular subtype.

the PD-L1 positivity rate were equivalent in both groups (table 2).

Signature of oncogenic signaling pathways

The *CD47*-high group had significantly higher activity in all examined oncogenic signaling pathways (mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), angiogenesis, TGF-β, epithelial-mesenchymal transition, and immune-related pathways) compared with the *CD47*-low group (q<0.01) (figure 2). This result was consistent even when limited to pMMR/MSS cases (online supplemental figure S2) or dMMR/MSI-H cases (online supplemental figure S3). All oncogenic signaling pathways evaluated showed a positive correlation with *CD47* expression levels (online supplemental figure S4).

Immune-related genes' expression and cell population in the TME

Compared with the *CD47*-low tumors, the *CD47*-high group had significantly higher expression of multiple adaptive immune checkpoint genes (*CD274*, *CTLA-4*, *HAVCR2*, *IDO1*, *LAG3*, *PDCD1*, *PDCD1LG2*) (q<0.01). Additionally, the *CD47*-high group also showed significantly higher gene expression levels of *IFNG* (q<0.01) (figure 3). These results were consistent even when limited to pMMR/MSS cases (online supplemental figure S5) or dMMR/MSI-H cases (online supplemental figure S6).

According to the MCP-counter analysis, all immune-related cells in the TME (T cells, B cells, natural killer (NK) cells, monocytes, macrophages, dendritic cells, neutrophils, and endothelial cells) were significantly more abundant in the *CD47*-high group compared with the *CD47*-low group (q<0.01) (figure 4). This result was consistent even when limited to pMMR/MSS cases (online supplemental figure S7) or dMMR/MSI-H cases (online supplemental figure S8).

Survival outcomes data

There was no significant difference in OS between patients with *CD47*-high tumors and those with *CD47*-low tumors (*CD47*-high vs *CD47*-low, median OS 29.7 vs 29.7 months, HR 1.03, 95% CI 0.99 to 1.06, p=0.17) (figure 5A). However, among patients treated with therapies including angiogenesis inhibitors, those with *CD47*-high tumors had significantly better OS compared with those with *CD47*-low tumors (median 29.9 vs 28.7 months, HR

Table 2 Comparison of immunotherapy-related markers between *CD47*-low and *CD47*-high groups

	All			pMMR/MSS		
	***CD47*-low (N=7,143)**	***CD47*-high (N=7,144)**	**Q value**	***CD47*-low (N=6,711)**	***CD47*-high (N=6,596)**	**Q value**
dMMR/MSI-H	5.8%	7.5%	<0.01	–	–	–
TMB-H (≥10/Mb)	8.8%	10.3%	0.11	3.4%	3.1%	1
PD-L1>5%	3.3%	4.3%	0.07	2.5%	3.0%	1

dMMR, mismatch repair-deficient; H, high; MSI, microsatellite instability; MSS, microsatellite stable; PD-L1, programmed cell death ligand 1; pMMR, mismatch repair proficient; TMB, tumor mutational burden.

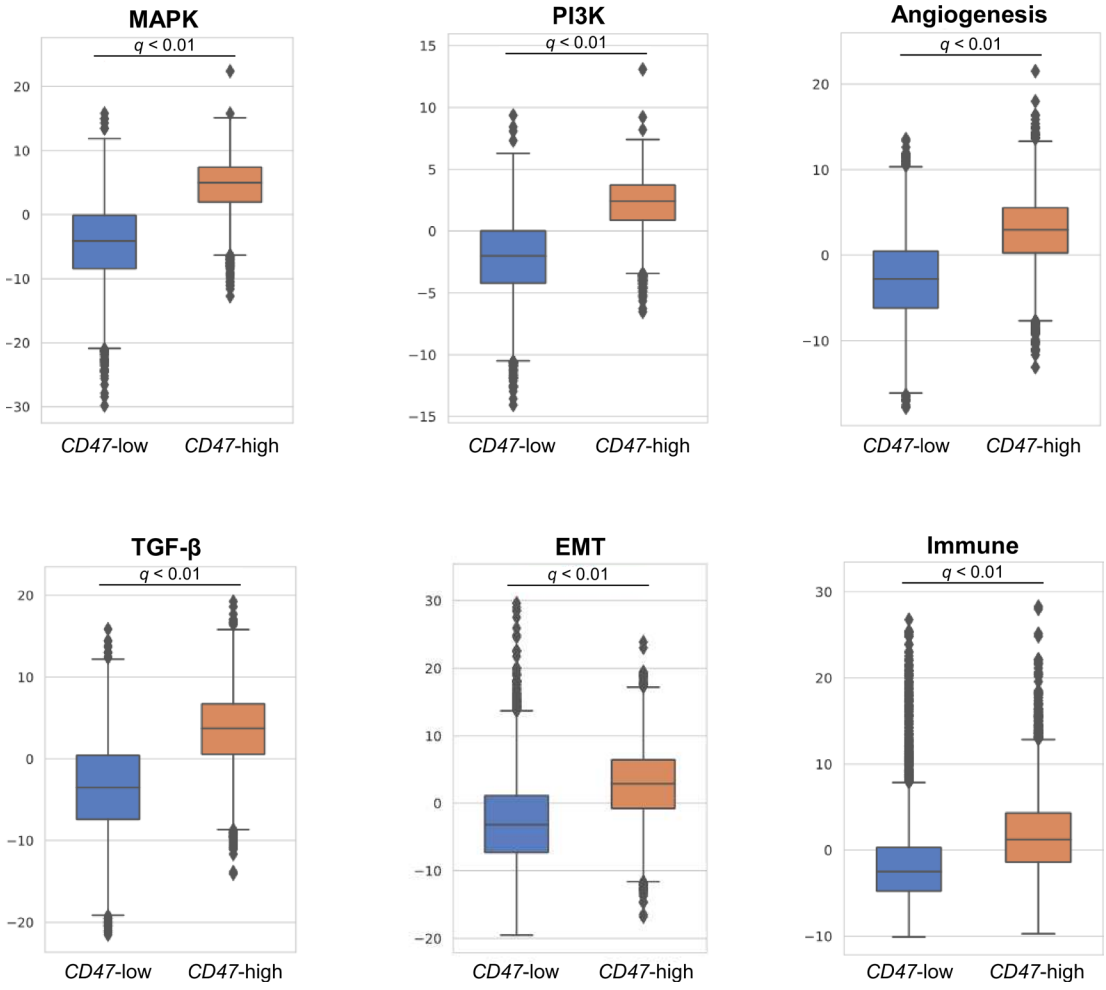

Figure 2 Correlation between *CD47* expression and major oncogenic signaling pathways by comparing the composite z-scores of genes listed in online supplemental tale S1) between *CD47*-high and *CD47*-low tumors. EMT, epithelial mesenchymal transition; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; TGF, transforming growth factor.

0.94, 95% CI 0.89 to 0.99, p=0.02) (figure 5B). Similarly, patients treated with ICIs also showed numerically longer OS in patients if they had *CD47*-high tumors (median 22.2 vs 18.4 months, HR 0.87, 95% CI 0.75 to 1.02, p=0.09) (figure 5C). When analyzed based on quartiles of *CD47* expression levels, a slightly worse prognosis was observed in patients in the top *CD47* quartile compared with those in the bottom quartile (figure 5D). Moreover, the relative prolongation in OS for patients with high *CD47* expression who received treatments including angiogenesis inhibitors or ICI was more pronounced when evaluated based on quartiles rather than the median (figure 5E and F).

DISCUSSION

We here report novel findings from our large-cohort study that elucidate the molecular characteristics linked to gene expression of *CD47*, a pivotal phagocytosis checkpoint in CRC. Notably, our data showed that *CD47*-highly expressed CRC harbors activation of various key oncogenic signaling pathways and significant immune cell infiltration in the TME. This suggests that the phagocytosis checkpoint might be involved in the aggressiveness of CRC, highlighting the potential for novel immunotherapies that inhibit this pathway.

In our study, it was revealed that the expression level of *CD47* in CRC positively correlates with the activity of several oncogenic signaling pathways, including MAPK, PI3K, angiogenesis, and TGF-β. This suggests that the transcriptional regulation of CD47 is influenced by various upstream oncogenic signaling pathways and also that CD47 further enhances downstream signaling pathways. For example, the MYC onco-protein, a master regulator of cellular programs, directly binds to the promoter of *CD47*, thereby upregulating its expression.[20] Since the MYC protein is upregulated by the MAPK, WNT, and NOTCH pathways, the association between *CD47* and these pathways is reasonable.[20] Additionally, HIF-1, known to directly activate the promoter of *CD47*, plays a role in inducing angiogenesis-promoting factors like VEGF under hypoxic conditions and within the TME.[21] Furthermore, CD47's "don't eat me" signal prevents endothelial cells from being phagocytosed by macrophages, thus supporting angiogenesis.[22] Therefore, the

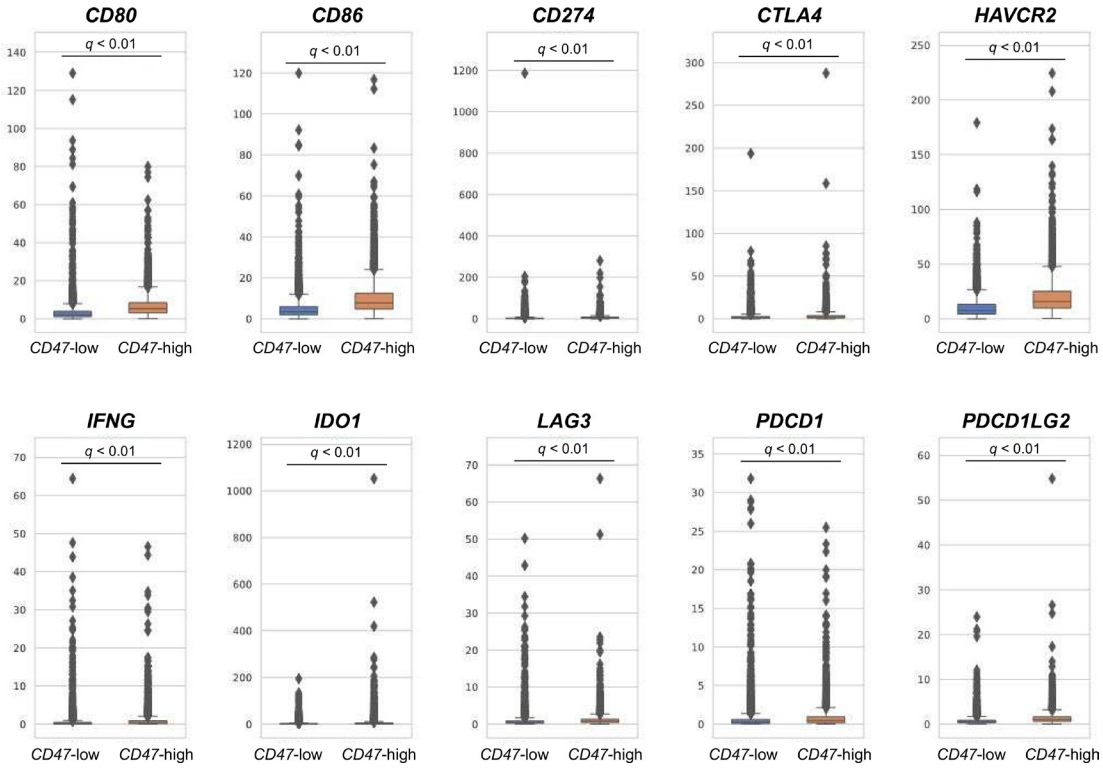

Figure 3 Comparison of immune-related gene expression between *CD47*-high and *CD47*-low tumors.

positive correlation between *CD47* and the angiogenesis pathway can also be explained. Furthermore, basic studies on glioblastoma and endometrial carcinoma have shown that CD47 promotes tumor cell invasion and migration by enhancing PI3K signaling.[23 24] In our study, consistent with the previous reports, a positive correlation between *CD47* and the PI3K pathway was also demonstrated in CRC.

CD47 has been reported to influence the TME by promoting M2 polarization and Tregs, contributing to

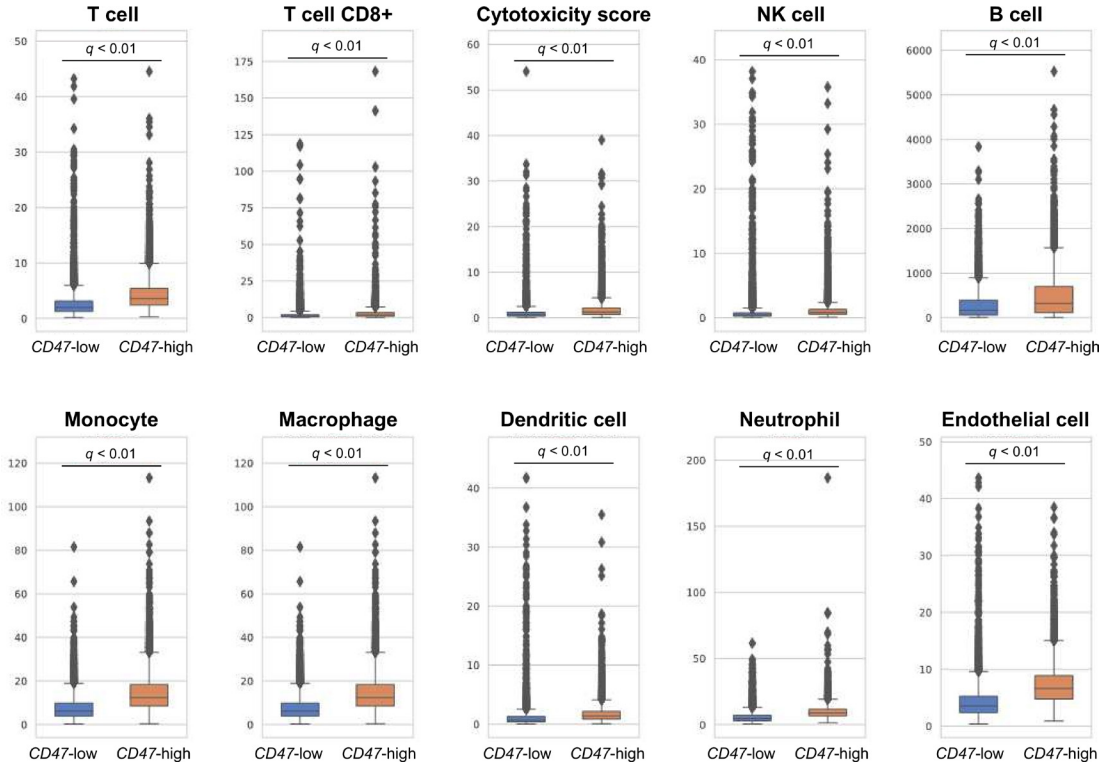

Figure 4 Comparison of immune and stromal cell populations between *CD47*-high and *CD47*-low tumors. NK, natural killer.

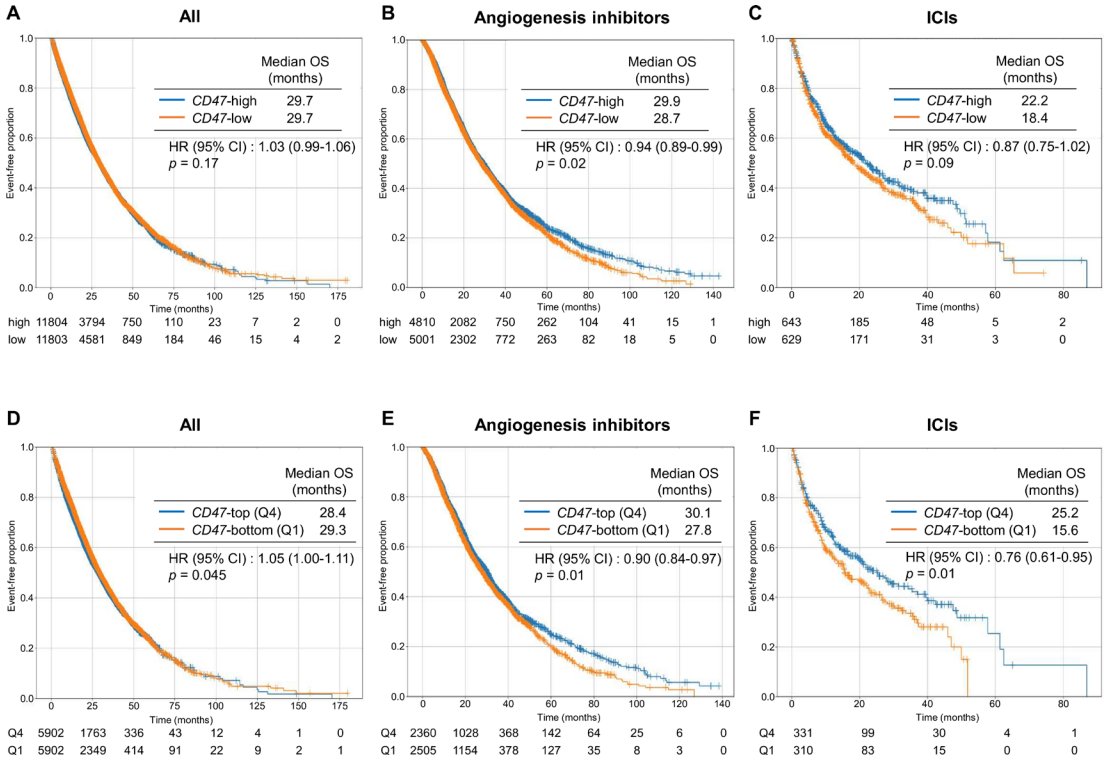

Figure 5 Association between *CD47* expression and overall survival. Comparison between groups divided by the median *CD47* expression level: (A) all patients, (B) patients treated with angiogenesis inhibitors, and (C) patients treated with ICIs. Comparison between the top quartile (Q4) and bottom quartile (Q1) of *CD47* expression levels: (D) all patients, (E) patients treated with angiogenesis inhibitors, and (F) patients treated with ICIs. ICIs, immune checkpoint inhibitors; OS, overall survival.

the creation of an immunosuppressive TME.[25] We showed that high *CD47* expression was associated with a greater amount of several types of immune cells infiltration into the TME. As for the CMS classification, *CD47*-high tumors had a significantly higher proportion of CMS1 and CMS4 than *CD47*-low tumors. CMS1 is characterized by enhanced infiltration of cytotoxic T cells and NK cells, while CMS4 is characterized by increased infiltration not only of T cells but also of fibroblastic cells, endothelial cells, and myeloid cells.[26] Although both CMS1 and CMS4 exhibit increased cell infiltration in the TME, CMS1 tends to respond well to ICI therapy, whereas CMS4 is known to form an immunosuppressive TME due to its inflammatory and angiogenic signature, making it less responsive to ICI therapy.[26] In our study, interestingly, the cells that more extensively infiltrated the TME of *CD47*-high tumors included cytotoxic lymphocytes, monocytic lineage cells, and endothelial cells, regardless of MSI status. These characteristics seem like a mixture of CMS4 and CMS1.[27] Furthermore, in *CD47*-high tumors, the gene expression levels of *INFG* as well as immune checkpoint genes such as *CD274* (encoding PD-L1), *CTLA-4*, *IDO1*, and *HAVCR2* (encoding TIM-3), were significantly higher, regardless of MSI status. These results suggest that in *CD47*-high tumors, interferon-γ is produced by infiltrating immune cells such as CD8+T cells and NK cells within the TME, resulting in the high expression of multiple immune checkpoints to evade the adaptive immune system.[28]

CD47, due to its anti-phagocytosis role, has been associated with aggressive tumor phenotypes that evade elimination by phagocytes. Previously, an analysis based on immunohistochemistry using a small cohort of clinical samples of CRC reported that protein expression of CD47 correlates with poor prognosis.[17] In our analysis, which was based on gene expression, we did not observe a clear prognostic effect of *CD47* expression. Only in the quartile analysis did we find that *CD47* expression was associated with poor prognosis, although this association was weak. On the other hand, it was suggested that there might be a positive correlation between the efficacy of angiogenesis inhibitors or ICIs and *CD47* expression. Further investigation using clinical data is needed to fully understand the prognostic and predictive value of *CD47* expression.

From the results of this comprehensive molecular profiling study, potential therapeutic strategies for aggressive *CD47*-high CRCs can be considered. First, there are some promising data on new and improved ICI therapies targeting the innate immune checkpoint CD47. By blocking the enhanced “don’t eat me” signal due to high CD47 expression, macrophage-mediated phagocytosis can be restored, leading to the release of cytokines and subsequent cross-priming of the adaptive immune system.[29] This forms the basis for the ongoing development of combination therapies using CD47 inhibitors and traditional ICIs targeting the adaptive immune system. Our findings suggest that active tumor immune

cell infiltration in the TME may synergistically benefit the cross-priming of the adaptive immune system when CD47 is inhibited. Currently, a phase I trial using a bispecific antibody targeting CD47 and PD-L1 is ongoing for solid tumors (NCT05780307). Additionally, considering that the angiogenesis pathway is activated in *CD47*-high tumors, the addition of angiogenesis inhibitors could modulate the TME to be more favorable for ICI therapy, potentially enhancing antitumor effects synergistically. Interestingly, our results showed a positive correlation between *CD47* expression and the DAMP signature, particularly the gene expression levels of *CALR* and *HMGB1*, which are "eat me" signals. Previous studies have reported that the antitumor effect of CD47 inhibition is mediated by counterbalancing with DAMP-mediated "eat me" signals.[30] Therefore, the molecular background of *CD47*-high tumors with an elevated DAMP signature may be well-suited for CD47 inhibitors. Additionally, it is known that DAMPs are released from cancer cells by certain cytotoxic anticancer drugs like oxaliplatin, which induce ICD.[31] Thus, combining oxaliplatin to further enhance immunogenicity is also reasonable. In this context, a phase I trial is underway to investigate the combination therapy of a CD47 inhibitor and nivolumab combined with FOLFOX plus a molecular targeted agent (bevacizumab or cetuximab) for first-line treatment of MSS CRC (jRCT2051210038). In summary, for CRCs characterized by elevated CD47 signaling, combining CD47 inhibitors, traditional ICIs, angiogenesis inhibitors, and ICD inducers like oxaliplatin could represent a comprehensive and effective therapeutic strategy.

There are several limitations to this study. First, it is a retrospective study, and the cohort consists of a heterogeneous group of patients with various backgrounds. While the large sample size strengthens the study, it is important to acknowledge that future research with more homogeneous cohorts or specific stratifications may be necessary to confirm these findings. Second, there is a significant lack of clinical information, such as treatment and disease stage. Therefore, particularly in the OS analysis, the results could be greatly influenced by unmeasured prognostic factors, making it difficult to definitively determine the impact of *CD47* expression on prognosis. Lastly, we categorized the gene expression levels of *CD47* into high and low groups using a dichotomous approach based on the median value of the cohort used in this study, which is specific to our data set. While this method simplifies comparisons and effectively highlights broad trends, it may overlook finer details. Future studies could benefit from using more granular or continuous models to better capture the complexity of CD47's role in CRC.

In conclusion, *CD47* gene expression in CRC was associated with the activation of several major oncogenic pathways. Furthermore, tumors with high *CD47* expression showed increased immune cell infiltration and greater expression of adaptive immune checkpoint molecules. These findings may provide valuable information for considering novel therapeutic strategies targeting the innate immune system in CRC.

Author affiliations
[1]Division of Medical Oncology, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California, USA
[2]Department of Clinical Oncology, St Marianna University School of Medicine, Kawasaki, Kanagawa, Japan
[3]Clinical & Translational Research, Medical Affairs, Caris Life Sciences, Caris Life Sciences, Phoenix, Arizona, USA
[4]Department of Population and Public Health Sciences, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California, USA
[5]Division of Hematology/Oncology, University of Cincinnati, Cincinnati, Ohio, USA
[6]West Virginia University Cancer Institute, Morgantown, West Virginia, USA
[7]Department of Clinical Genetics, Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA
[8]Department of Medicine, University of Arizona Cancer Center, Tucson, Arizona, USA
[9]Department of Solid Tumor Oncology, GI Medical Oncology Levine Cancer Institute, Charlotte, North Carolina, USA
[10]Division of Hematology, Oncology and Transplantation, University of Minnesota, Minneapolis, Minnesota, USA
[11]Ruesch Center for the Cure of Gastrointestinal Cancers, Lombardi Comprehensive Cancer Center, Georgetown University, Washington, District of Columbia, USA
[12]Rutgers Cancer Institute of New Jersey, New Brunswick, New Jersey, USA

X Emil Lou @cancerassassin1

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Contributors HA primarily planned, designed, and drafted the manuscript. NG did statistical analyses and visualization. H-JL did supervision, project administration, and funding acquisition, and served as the guarantor. All authors made substantial contributions to data collection, validation, and drafting the manuscript. All authors read and approved the final manuscript.

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Competing interests NG, JX, and WMK are employees of Caris Life Sciences. All remaining authors have declared no conflicts of interest.

Patient consent for publication Not applicable.

Ethics approval This study was conducted in accordance with guidelines of the Declaration of Helsinki, Belmont report, Good Clinical Practice, REMARK, and US Common Rule. In keeping compliance with policy 45 CFR 46.101(b) (4), the part of this study using the Caris data set was performed using retrospective, de-identified clinical data. Therefore, this part was considered Institutional Review Board exempt and no patient consent was necessary.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Availability of data and materials: De-identified data sets analyzed in the current study are available from the corresponding author on reasonable request.

ORCID iDs
Hiroyuki Arai http://orcid.org/0000-0002-1886-7070
Emil Lou http://orcid.org/0000-0002-1607-1386

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