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A Comprehensive Study on the Prognostic Value and Clinicopathological Significance of Different Immune Checkpoints in Patients With Colorectal Cancer: A Systematic Review and Meta-Analysis

Mahdieh Azizi, PhD[1,†], Zahra Mokhtari, MSc[1,†], Shirin Tavana, MSc[1], Peyman Bemani, PhD[1], Zahra Heidari, PhD[2], Roghayeh Ghazavi, PhD[3], Marzieh Rezaei, PhD[1,*]

[1] Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[2] Department of Biostatistics and Epidemiology, Faculty of Health, Isfahan University of Medical Sciences, Isfahan, Iran
[3] Department of Knowledge and Information Science, Faculty of Education and Psychology, Shahid Chamran University of Ahvaz, Ahvaz, Iran

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ABSTRACT

Background: The prognostic significance of immune checkpoint expression in the tumor microenvironment has been widely investigated in colorectal cancers. However, the results of these studies are inconsistent and limited to some immune checkpoints.
Objective: The study aimed to investigate the correlation between different immune checkpoint expression and clinicopathological features and prognostic parameters.
Methods: We conducted a systematic review and meta-analysis of the published literature in PubMed, Web of Science-Core Collection, Scopus, Embase, and Cochrane databases to summarize the association between various immune checkpoints expression on both tumor cells and immune cells with clinicopathological features and prognostic parameters in patients with colorectal cancer.
Results: One hundred four studies incorporating 22,939 patients were included in our meta-analysis. Our results showed that among the B7 family, the high expression of B7H3, B7H4, PD-1, and PD-L1 on tumor cells and tumor tissue was significantly associated with higher T stage, advanced tumor, node, metastasis (TNM) stage, presence of vascular invasion, and lymphatic invasion. In addition, patients with high expression of B7H3, B7H4, PD-1, PD-L1, and PD-L2 were associated with shorter overall survival. High expression of PD-1 and PD-L1 in immune cells correlated with the absence of lymph node metastasis, lower TNM stage, early T stage, poor overall survival, and disease-free survival, respectively. Moreover, we found significant positive correlations between CD70 and Galectin-3 expression with advanced T stage. HLA-II overexpression was correlated with the absence of lymph node metastasis (odds ratio = 0.21, 95% CI = 0.11–0.38, $P < 0.001$) and early TNM stage (odds ratio = 0.35, 95% CI = 0.26–0.47, $P < 0.001$).
Conclusions: Overexpression of B7H3, B7H4, PD-1, PD-L1, PD-L2, CD70, and Galectin-3 on tumors is significantly associated with unfavorable clinicopathological characteristics and poor prognostic factors. Hence, these immune checkpoints can serve as predictive biomarkers for prognosis and the clinicopathological features of colorectal cancer because this is essential to identify patients suitable for anticancer therapy with immune checkpoint inhibitors.

Introduction

Colorectal cancer (CRC) is the third most common malignancy and fourth most common cause of cancer-related death worldwide.[1] CRC comprises a heterogeneous group of diseases with complex genetic and epigenetic changes.[2] The heterogeneity of CRC has made challenging the diagnosis, prognosis, and selection of the

* Address correspondence to: Marzieh Rezaei, Ph.D, Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Hezar Jarib Street, Postal Code 8174673461, Isfahan, Iran.
E-mail address: ma.rezaei@med.mui.ac.ir (M. Rezaei).
† These authors are co-equal first author.

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appropriate treatment for this disease.[3] However, screening can reduce the incidence and mortality rate from this cancer; therefore, it is urgent to explore available biomarkers for early cancer detection and prognosis evaluation.

The tumor microenvironment (TME) is largely responsible for the response to therapy and is of great value in predicting prognosis and evaluating useful biomarkers for successful immunotherapeutic approaches.[4] One of the important components of TME is immune cells and compounds produced by these cells. The state of the immune system in the TME and the function of immune cells are important factors in tumor progression.[5] Analysis of the interactive relationships between tumor cells and the immune system components in the TME has received much attention.[6] One of the critical molecules in the relationship between tumor cells and the immune cells are immune checkpoints (ICPs) that regulate the infiltrated immune cell functions. ICPs are effective factors in regulating the function of immune cells, especially T cells and can transmit inhibitory or stimulatory signals through the interaction of these molecules with their cognate receptors on target and effector cells, respectively.[7] Checkpoint receptors, such as programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte-activation gene 3 protein (LAG3), T-cell immunoglobulin and mucin 3 domain (TIM3), T-cell immune receptor with Ig and ITIM domains (TIGIT), and others, are immunosuppressive molecules, as they negatively regulate activation of the immune effector cells.[8] Additionally other ICPs such as CD40, CD27, OX40, CD137 (4-1BB), and glucocorticoid-induced TNFR-related protein (GITR), which are members of the tumor necrosis factor receptor (TNFR) superfamily, as well as checkpoint receptors belonging to the B7-CD28 superfamily such as CD28 and the inducible T-cell stimulator (ICOS) stimulate the immune response.[9]

The prognostic significance of ICP expression in the TME has been widely investigated in various cancers. Although some studies have reported a significant correlation between ICP expression and patient survival, others have found no prognostic value for ICPs expression.[10,11] Regarding the role of these molecules in CRC, there are a few studies, and their results are inconsistent and conflicting; hence, there is a need for more investigation. As these ICPs are of high therapeutic value, it would be of great interest to investigate whether their assessment in the TME has prognostic significance. To address these issues, we carried out a comprehensive meta-analysis to investigate the association between the expression of all activatory or inhibitory ICPs on both tumor cells and immune cells and clinicopathological features and prognostic parameters (overall survival [OS], disease-free survival [DFS], and recurrent-free survival [RFS]) in patients with CRC.

Materials and Methods

This meta-analysis was performed according to the preferred reporting items for systematic reviews and meta-analysis statements. Our study was based on data from previously published studies; therefore, ethical approval was not necessary.

Search strategy

In order to search for articles related to the role of ICPs in CRC, a list of all of them (including receptors and related ligands) was retrieved from reputable sources. According to the division of these ICPs, these cases can be considered in 3 superfamilies: B7-CD28 superfamily, immunoglobulin superfamily, and TNF-superfamily. Each of the receptors and related ligands would be searched separately. The ICP search line was configured based on their synonyms retrieved from Medical Subject Headings and Embase Subject Headings as well as related review articles. Subjects related to CRC were also retrieved in a similar manner. The databases searched included PubMed, Web of Science-Core Collection (all indexes included SCI-Expanded, SSCI, AHCI, ESCI and books, and conference papers), Scopus, Embase, and Cochrane. Both controlled and free searches were used in the PubMed, Embase, and Cochrane databases. The endpoint for search items was February 22, 2021. To increase the sensitivity of document retrieval, the search was made in the fields of titles, abstracts, and keywords of the documents. Additionally, at this stage, no restrictions were considered in terms of the document type, publication date, and language of the documents. Due to the length of the ICPs search strategies, they are presented separately in the Supplemental Table 1.

Study selection and eligibility criteria

The retrieved results from the mentioned databases were entered into Endnote reference management software, and duplicates were removed. A separate library was created for each of the ICPs to allow for the comparison of results. The selection of suitable studies was conducted in 2 phases. In the first phase, titles and abstracts of papers were screened, and irrelevant papers were excluded. In the second phase, the full text of identified papers was explored deeply to select only relevant papers. Both screening phases were done by 2 independent reviewers (Z.M. and S.T). Discrepancies were resolved by consultation and consensus. The PICOTS (Population, Index prognostic factors, Comparator prognostic factors, Outcomes, Timing, and Setting) system was utilized to identify the studies that were included in the analysis.

Target population and treatment: patients with histologically confirmed CRC.
Index Prognostic factors: ICP expression in CRC tissue was detected by immunohistochemistry (IHC).
Comparator prognostic factors: not applicable for this review.
Outcome of interest: the relationship between expression of ICPs and gender, clinicopathological features (lymphatic metastasis, differentiation, TNM stage, tumor location, etc), and outcomes parameters (OS, DFS, and RFS).
Timing: ICP expression was assessed before immunotherapy and chemotherapy.
Setting: Cancer hospitals and treatment centers.

Finally, observational cohort and cross-sectional studies that were published as a full paper in English were included in this review. We mainly excluded duplicate studies, letters, abstracts, reviews, meta-analysis, case reports, cell and animal studies, articles lacking sufficient information, and articles using other detection methods of ICP expression such as RNA-resequencing or microarray data from public databases. The number of searched articles and articles in each step for 3 superfamilies was defined in the preferred reporting items for systematic reviews and meta-analysis diagram (Supplemental Figures 1–3).

Data extraction and quality assessment

All relevant article data were extracted by 3 independent reviewers (Z.M., S.T., and M.A.). The information extracted from each study included: the first author, country, year of publication, number of patients, gender, expression pattern of ICPs (sample used, detection method, cutoff values, and positive or high expression rate of ICPs), clinicopathological features, and prognostic parameters. Any disagreements in the literature selection and data extraction were resolved by consensus from all authors. In addition, quality assessments were conducted independently for each study

by 3 reviewers (Z.M., S.T., and M.A.) using the National Heart, Lung, and Blood Institute quality assessment tool for observational studies. Each positive response carried 1 point, and no point was given for negative or unclear responses. Discrepancies in scoring were resolved by discussion and consensus. A study that received a score of 5 or higher was considered a high-quality study.

Statistical analysis

Meta-analysis was performed using STATA software package (version 11.0) (Stata Corp LP, College Station, Texas). The cutoff value of ICPs extracted from the articles has divided patients into positive and negative groups. Pooled odds ratio (ORs) and their 95% CIs were used to determine the association between ICP expression and clinicopathological parameters, and hazard ratio (HRs) and their 95% CIs were used to evaluate the association between ICP expression and OS, DFS, and RFS. HR or OR higher than 1 indicated a worse prognosis or a significant correlation between ICPs and clinicopathological parameters, respectively. Heterogeneity among research was tested using the χ^2-based Q-test and I^2 test. P value < 0.05 with $I^2 > 50\%$ was defined as a significant heterogeneity; then, a random-effects model was applied to calculate the pooled effect. Otherwise, a fixed-effects model was applied. To identify the possible sources of heterogeneity within these studies, a subgroup analysis was performed. Subgroup analyses were conducted by comparing available data from the studies grouped by the origin of the marker on the tumor cells, tumor tissue, and immune cells to reduce the impact of heterogeneity to a certain extent. Egger's test was used to assess the publication bias of all enrolled studies ($P > 0.05$ indicating no publication bias). Moreover, we utilized the trim and fill analysis when publication bias was present to adjust the publication bias, thereby confirming the reliability of our results. Additionally, sensitivity analysis was utilized to check the contribution of each study to the pooled estimate by excluding individual studies one at a time and recalculating the pooled HR and OR estimates for the remaining studies. Sensitivity analysis was not conducted for studies grouped by the origin of the marker. P values < 0.05 were considered statistically significant. All P values and 95% CI were two-sided.

Result

Description of the studies

In this study, we finally determined that 104 studies met our inclusion criteria and thus were included in this meta-analysis. All studies were published from 1999 to 2021. As shown in Table 1,[12–114] 45 studies were conducted in China, 15 studies were performed in Japan, 10 studies were carried out in Korea, 5 studies were performed in United States, 4 studies were performed in Taiwan, 2 studies were conducted in Egypt, Turkey, Australia, Austria, and Switzerland, and 1 study was carried out in UK, Denmark, Finland, Germany, France, Iraq, Sweden, Italy, Iran, Brazil, Norway, Belgium, the Netherlands, Indonesia, and Malaysia, respectively. All the studies were published in English. The sample sizes of these studies ranged from 36 to 1420 patients, and a total of 22,939 patients were enrolled in these studies. Among all ICPs in 3 superfamilies just B7H3, B7H4, CD70, CEACAM1, PD1, PD-L1, PD-L2, LAG3, TIM3, HLA-II, Galectin-3, and CTLA-4 had enough data to be included in this study. In all the included studies, ICP expression in CRC tissues was evaluated using IHC. The origin of the investigated markers in the enrolled studies is as follows: in 63 studies, the origin of markers was tumor cells, in 27 studies was immune cells, in 25 studies was tumor tissue, and in 1 study, the origin of the expressed marker was mentioned the cancer-associated fibroblasts. Each article had an independent cutoff value used to define the criterion for ICPs positive. Regarding ICPs type, 61 studies examined the expression of PD-L1, 12 studies examined the expression of PD-1, 11 studies examined the expression of Galectin3, 10 studies examined the expression of B7H3, 6 studies examined the expression of B7H4 and PD-L2, 4 studies examined the expression of TIM3, 5 studies examined the expression of HLA-II, 3 studies examined the expression of LAG3 and CTLA4, and finally, 2 studies examined the expression of CD70 and CEACAM1. In addition, 99 studies reported the relationship between expression of ICPs and clinicopathological features, and 45 studies assessed the association between ICPs and OS, RFS, and DFS. National Heart, Lung, and Blood Institute scores ranged from 5 to 11, revealing the high quality of the entire study. The quality of the included studies was good, with a score ≥ 5.

Correlation between ICP expression and tumor clinicopathological parameters

To comprehensively analyze the role of ICP expression as prognostic biomarkers in CRC, we investigated the relationship between ICP expression and gender and clinicopathological parameters including tumor location, TNM stage, T stage, N stage, M stage, vascular invasion, lymphatic invasion, lymphovascular invasion, and microsatellite stability (MSS)/microsatellite instability (MSI) status.

B7—CD28 superfamily

B7H3

The pooled results of 10 studies comprising 2819 patients indicated that high B7H3 expression was associated with colon cancer (OR = 1.24, 95% CI = 1.03–1.50, $P = 0.021$), higher T stage (OR = 1.69, 95% CI = 1.32–2.17, $P < 0.001$), advanced TNM stage (OR = 1.31, 95% CI = 1.09–1.58, $P = 0.003$), and the presence of vascular invasion (OR = 1.63, 95% CI = 1.05–2.55, $P = 0.031$). Meanwhile, B7H3 overexpression had no significant association with gender (male vs female, OR = 0.99, 95% CI = 0.84–1.18, $P = 0.94$) and N stage (presence vs absence, OR = 1.21, 95% CI = 0.97–1.50, $P = 0.77$). First, we evaluated the general expression of markers on all the cells without consideration of the expression on the specific cells; then, we performed a subgroup analysis according to the origin of the marker to reduce the heterogeneity of studies. Interestingly, subgroup analysis showed that B7H3 overexpression on tumor cells was associated with colon cancer (OR = 1.28, 95% CI = 1.03–1.59, $P = 0.02$), higher TNM stage (OR = 1.32, 95% CI = 1.08–1.63, $P = 0.008$), and higher T stage (OR = 1.78, 95% CI = 1.31–2.42, $P < 0.001$). Furthermore, B7H3 expression on tumor tissue increased in patients with higher T stage (OR = 1.53, 95% CI = 1.01–2.33, $P = 0.047$) and positive lymph node metastasis (OR = 1.51, 95% CI = 1.02–2.24, $P = 0.037$). No significant correlation between high B7H3 expression with gender and vascular invasion was found in the subgroup analyses (Table 2).

B7H4

The pooled data suggested that B7H4 overexpression could predict the advanced TNM stage (OR = 3.29, 95% CI = 1.36–7.97, $P = 0.008$) in CRC. However, we detected no significant relationships between B7H4 overexpression and gender (male vs female, OR = 0.95, 95% CI = 0.76–1.19, $P = 0.66$), tumor location (colon vs rectum, OR = 1.03, 95% CI = 0.80–1.34, $P = 0.79$), N stage (presence vs absence, OR = 2.00, 95% CI = 0.36–11.26, $P = 0.43$), and T stage (III/IV vs I/II, OR = 2.62, 95% CI = 0.38–18.00, $P = 0.32$). We were not able to evaluate the expression of B7H4 according to the origin of the marker in subgroup analysis due to limited data (Table 2).

Table 1
Main characteristics of the included studies.[12–114]

First author	Year of study	Country	No. of patient	Origin of marker	Marker	Marker + (%)	Cut-off	Outcome	Score of study	Reference
Wu	2018	China	225	Tumor cell	B7H3	197(87.6)	NR	OS, Gender, Tumor location, TNM stage, T stage, N stage, M stage, VI	9	12
Zhang	2018	China	223	Tumor tissue	B7H3	157(70.4)	Score >3 (intensity + area)	OS, Gender, Tumor location, TNM stage, T stage, N stage, M stage, VI	9	13
Zhang	2020	China	213	Tumor cell	B7H3	136 (63.8)	Score ≥3 (intensity + area)	OS, Gender, Tumor location, TNM stage, T stage, N stage, M stage, VI	9	14
Liu	2018	China	231	Tumor tissue	B7H3	138 (59.7)	IHC Scores ≥3 (intensity + area)	OS, Gender, Tumor location, TNM stage, T stage, N stage, VI	8	15
Sun	2010	China	102	Tumor cell	B7H3	54 (52.94)	Grade ≥ 2 (intensity)	Gender, Tumor location, T stage, N stage, M stage	7	16
Ingebrigtsen	2014	Norway	731	Tumor cell	B7H3	637 (87)	≥10% (area)	Gender, Tumor location, TNM stage, T stage, N stage	10	17
Bin	2014	China	104	Tumor cell	B7H3	59 (56.73)	NR	Gender, T stage, N stage, M stage	9	18
Jiang	2016	China	87	Tumor cell	B7H3	71 (81.6)	≥25% (area)	Gender, TNM stage, T stage, N stage	5	19
Lu	2020	China	805	Tumor cell	B7H3	410 (50.9)	NR	OS, Gender, Tumor location, TNM stage, Microsatellite status	10	20
Qiu	2018	China	98	Tumor cell	B7H3	45 (45.9)	IHC scores >2.75 (intensity + area)	Gender, Tumor location, T stage, N stage, M stage	8	21
Ding	2020	China	110	Tumor cell	B7H4	56 (50.9%)	Score≥2 (area)	Gender, OS	8	22
Cao	2019	China	118	Tumor tissue	B7H4	66 (55.93)	Score ≥ 4 (intensity + area)	Gender, Tumor location, TNM stage, T stage, N stage	7	23
Lu	2020	China	805	Tumor cell	B7H4	234 (29.1)	NR	Gender, Tumor location, TNM stage, Microsatellite status	10	20
Qiu	2018	China	98	Tumor cell	B7H4	32 (32.7)	IHC scores >5.75 (intensity + area)	Gender, Tumor location, T stage, N stage, M stage	8	21
Liang	2013	China	185	Tumor cell	B7H4	117 (63.2)	Score≥4 (intensity + area)	Gender, TNM stage, T stage, N stage, OS	9	24
Zhao	2014	China	56	Tumor cell	B7H4	27 (48.2)	Score≥4 (intensity + area)	Gender, Tumor location, T stage, N stage	7	25
Jacobs	2018	Belgium	51	cancer-associated fibroblasts	CD70	25 (49)	NR	Gender, Tumor location, T stage, N stage, M stage, Microsatellite status	8	26
Inoue	2019	Japan	269	cancer-associated fibroblasts	CD70	40 (14.86)	NR	Gender, Tumor location, T stage, N stage, M stage, Microsatellite status	8	27
Kang	2007	Taiwan	99	Tumor tissue	CEACAM1	75 (75.7)	NR	N stage	5	28
Song	2011	Korea	123	Tumor cell	CEACAM1	93 (75.6)	NR	N stage	9	29
Omura	2020	Japan	131	Tumor cell	CTLA4	27 (20.6)	NR	Gender, TNM stage, N stage	11	30
Lee	2018	South Korea	89	Immune cell	CTLA4	74 (83.1)	Moderate-to-strong intensity in more than 5%	TNM stage	9	31
Teng	2015	China	62	Tumor cell	CTLA4	31 (50)	H-score > 20	Gender, N stag	10	32
Endo	2005	Japan	121	Tumor tissue	Galectin-3	79 (65.2)	>20% (area)	Gender, Tumor location, TNM stage, T stage, N stage, M stage, VI, LI	9	33
Tsuboi	2007	Japan	108	Tumor tissue	Galectin-3	72 (66.6)	NR	Gender, TNM stage, T stage, N stage, VI, LI	5	34

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Table 1 (*continued*)

First author	Year of study	Country	No. of patient	Origin of marker	Marker	Marker + (%)	Cut-off	Outcome	Score of study	Reference
Povegliano	2011	Brazil	75	Tumor tissue	Galectin-3	32 (42.6)	≥50% (area)	TNM stage, M stage	10	35
Rashed	2015	Egypt	50	Tumor tissue	Galectin-3	44 (88%)	>20% (area)	Gender, Tumor location, TNM stage, N stage	6	36
Saravi	2015	Iran	130	Tumor tissue	Galectin-3	61 (46.9)	≥50% (area)	Gender, Tumor location, TNM stage, N stage, M stage	9	37
Wang	2015	China	45	Tumor cell	Galectin-3	33 (73.3)	>25% (area)	N stage	5	38
Gopalan	2016	Australia	73	Tumor tissue	Galectin-3	69 (95)	≥30% (area)	Gender, Tumor location, TNM stage, T stage, N stage, M stage	6	39
Huang	2016	China	117	Tumor cell	Galectin-3	51 (43.6)	Score ≥ 2 (intensity + area)	Gender, T stage	9	40
Tao	2017	China	61	Tumor cell	Galectin-3	38 (62.5)	Score ≥ 4 (intensity + area)	Gender, Tumor location	8	41
Lu	2017	China	57	Tumor tissue	Galectin-3	43 (75.43)	Score≥ 2 (intensity)	Gender, Tumor location, TNM stage, N stage	7	42
Nakamura	1999	Japan	117	Tumor tissue	Galectin-3	36 (30.8)	Score > 1 (intensity)	T stage, N stage, LI, VI	7	43
de Bruin	2008	Netherlands	1016	Tumor cell	HLA-DR	216 (21)	≥20% (area)	Gender, TNM stage	10	44
Walsh	2009	Australia	270	Tumor cell	HLA-DR	163 (60.4)	NR	Gender, VI	9	45
Morita	1995	Japan	148	Tumor cell	HLA-DR	50 (33.78)	>50% (area)	T stage, N stage, LI, VI	6	46
Warabi	2000	Japan	76	Tumor tissue	HLA-II	32 (42)	>50% (area)	N stage, LI	5	47
Sconocchia	2014	Italy	220	Tumor cell	HLA-II	55 (25)	>15 cells	TNM stage	7	48
Lee	2018	South Korea	89	Immune cell	LAG3	44 (49.4)	Moderate-to-strong intensity in more than 5%	TNM stage	9	31
Al-Badran	2020	UK	387	Immune cell	LAG3	191 (49)	NR	Gender, Tumor location, TNM stage, T stage, N stage	8	49
Al-Badran	2020	UK	413	Tumor cell	LAG3	160 (39)	NR	Gender, Tumor location, TNM stage, T stage, N stage	8	49
Lee	2018	South Korea	89	Immune cell	PD-1	39 (43.8)	Moderate-to-strong intensity in more than 5%	DFS, TNM stage, LVI	9	31
Gruber	2020	Austria	75	Immune cell	PD-1	8 (10.7)	NR	OS, DFS, Gender	7	50
Zengin	2021	Turkey	212	Tumor tissue	PD-1	98 (46.2)	NR	OS, RFS, Gender, Tumor location, T stage, LVI, Microsatellite status	9	51
Lee	2016	USA	395	Immune cell	PD-1	76 (19)	>1.43 tumor-infiltrating lymphocytes of 1+ or 2+ intensity per square millimeter	RFS, Gender, Tumor location, TNM stage, T stage, N stage, Microsatellite status	8	52
Li	2016	China	276	Immune cell	PD-1	106 (38.4)	Score > 4 (intensity + area)	OS, DFS, Gender, Tumor location, TNM stage, T stage, N stage, M stage, Microsatellite status	9	53
Berntsson	2018	Sweden	526	Immune cell	PD-1	298 (56.7)	≥10% (area)	OS, Gender, Tumor location, T stage, N stage, M stage, Microsatellite status	8	54
Al-Badran	2020	UK	719	Immune cell	PD-1	411 (57)	NR	Gender, Tumor location, TNM stage, T stage, N stage	8	49
Al-Badran	2020	UK	722	Tumor cell	PD-1	223 (31)	NR	Gender, Tumor location, TNM stage, T stage, N stage	8	49

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Table 1 (*continued*)

First author	Year of study	Country	No. of patient	Origin of marker	Marker	Marker + (%)	Cut-off	Outcome	Score of study	Reference
Kuai	2020	China	73	Tumor tissue	PD-1	31 (42.5)	Score> 3 (intensity + area)	Gender, Tumor location, TNM stage, T stage, N stage	9	55
Zhou	2020	China	60	Tumor tissue	PD-1	19 (31.7)	NR	Gender, TNM stage	9	56
Enkhbat	2018	Japan	116	Immune cell	PD-1	39 (33.6)	> 20% (area)	Gender, Tumor location, TNM stage, T stage	8	57
Wei	2018	China	422	Immune cell	PD-1	69 (16.4)	NR	OS, DFS	10	58
Ahtiainen	2019	Finland	190	Immune cell	PD-1	125 (64.2)	55 cells/mm^2	OS, DFS, Gender, Tumor location, TNM stage, LVI, Microsatellite status	8	59
Zhu	2015	China	120	Tumor cell	PD-L1	30 (25)	Score ≥ 4 (intensity + area)	OS, Gender, Tumor location, T stage, N stage, M stage, VI	8	60
Omura	2020	Japan	131	Tumor cell	PD-L1	100 (23.7)	NR	Gender, TNM stage, T stage, N stage, LI, VI, Microsatellite status	11	30
Lee	2018	South Korea	89	Immune cell	PD-L1	56 (62.9)	Moderate-to-strong intensity in more than 5%	TNM stage, LVI	9	31
Wu	2019	China	204	Tumor tissue	PD-L1	84 (41.2)	Score ≥ 6 (intensity + area)	OS, Gender, Tumor location, TNM stage, T stage, N stage, M stage	11	61
Wyss	2018	Switzerland	279	Tumor cell	PD-L1	170 (60.9%)	NR	Gender, Tumor location, TNM stage, T stage, N stage, M stage, LVI, VI, Microsatellite status	9	62
Yomoda	2018	Japan	132	Immune cell	PD-L1	24 (18.2)	NR	Gender, Tumor location, TNM stage, T stage, N stage, LI, VI	10	63
Elfishawy	2020	Egypt	60	Immune cell	PD-L1	23 (38.3)	≥5% (area)	Gender, Tumor location, TNM stage, T stage, N stage, M stage, LVI	7	64
Elfishawy	2020	Egypt	60	Tumor cell	PD-L1	15 (28)	≥5% (area)	Gender, Tumor location, TNM stage, T stage, N stage, M stage, LVI	7	64
Gruber	2020	Austria	75	Immune cell	PD-L1	18 (24)	NR	OS, DFS, Gender	7	50
Jiang	2020	China	65	Tumor cell	PD-L1	44 (68)	≥ 6% (area)	OS, DFS, Gender, Tumor location, TNM stage, N stage, LI, VI	9	65
Onwe	2020	Malaysia	91	Tumor cell	PD-L1	6 (6.5)	NR	Gender, Tumor location, TNM stage	8	66
Zhao	2020	China	181	Tumor cell	PD-L1	31 (17.1)	Score> 2 (intensity + area)	OS, Gender, Tumor location, TNM stage, LVI	10	67
Waleed Aziz Al-hayali	2020	Iraq	99	Immune cell	PD-L1	32 (32.3)	≥5% (area)	Gender, Tumor location, TNM stage, T stage, N stage, M stage	7	68
Waleed Aziz Al-hayali	2020	Iraq	99	Tumor cell	PD-L1	14 (14.1)	≥5% (area)	Gender, Tumor location, TNM stage, T stage, N stage, M stage	7	68
Boustani	2020	France	74	Tumor cell	PD-L1	37 (50)	NR	OS, Gender, T stage, N stage	10	69
Huang	2020	China	633	Tumor cell	PD-L1	234 (37)	≥5% (area)	OS	8	70
Huemer	2020	Austria	65	Tumor cell	PD-L1	48 (73.8)	>1% (area)	OS, DFS, Gender, TNM stage, T stage, N stage, LVI, VI	10	71
Jung	2020	Korea	58	Tumor cell	PD-L1	18 (31.03)	≥1% (area)	Gender, Tumor location, T stage, N stage, M stage, LVI, Microsatellite status	8	72
Lee	2016	USA	395	Tumor cell	PD-L1	19 (5)	>1% of tumor cells staining with 2+ intensity	RFS, Gender, Tumor location, TNM stage, T stage, N stage, Microsatellite status	8	52
Li	2016	China	276	Tumor cell	PD-L1	138 (50)	Score > 4 (intensity + area)	OS, DFS, Gender, Tumor location, TNM stage, T stage, N stage, M stage, VI, Microsatellite status	9	73
Rosenbaum	2016	USA	181	Tumor cell	PD-L1	16 (9)	≥ 5% (area)	Gender, TNM stage, T stage, N stage, VI, Microsatellite status	8	74

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Table 1 (*continued*)

First author	Year of study	Country	No. of patient	Origin of marker	Marker	Marker + (%)	Cut-off	Outcome	Score of study	Reference
Saigusa	2016	Japan	90	Tumor cell	PD-L1	36 (40)	Score >2 (intensity)	OS, RFS, Gender, TNM stage, T stage, N stage, LI, VI	11	75
Wang	2016	China	262	Immune cell	PD-L1	54 (21)	≥5% (area)	RFS, Gender, Tumor location, TNM stage, T stage, N stage	10	76
Wang	2016	China	262	Tumor cell	PD-L1	NR	≥5% (area)	RFS	10	76
Koganemaru	2017	Japan	235	Immune cell	PD-L1	36 (15.3%)	≥5% (area)	DFS, Gender, Tumor location, T stage, N stage	7	77
Koganemaru	2017	Japan	235	Tumor cell	PD-L1	19 (8.1%)	≥5% (area)	DFS, Gender, Tumor location, T stage, N stage	7	77
Bae	2018	Korea	175	Tumor tissue	PD-L1	93 (53.1)	>50% (area)	OS, DFS, Gender, Tumor location, TNM stage, T stage, N stage, LVI	8	78
Berntsson	2018	Sweden	536	Immune cell	PD-L1	297 (55.4)	≥10% (area)	OS, Gender, Tumor location, T stage, N stage, M stage, VI, Microsatellite status	8	54
Berntsson	2018	Sweden	536	Tumor cell	PD-L1	107 (20)	≥1% (area)	Gender, Tumor location, T stage, N stage, M stage, VI, Microsatellite status	8	54
Huang	2018	Taiwan	864	Tumor cell	PD-L1	384 (44%)	NR	Gender, Tumor location, TNM stage, T stage, N stage, LVI, Microsatellite status	9	79
Korehisa	2017	Japan	36	Immune cell	PD-L1	26 (72.2)	≥1% (area)	Gender, Tumor location, TNM stage, T stage, N stage, LI, VI	7	80
Korehisa	2017	Japan	36	Tumor cell	PD-L1	13 (36.1)	≥1% (area)	Gender, Tumor location, TNM stage, T stage, N stage, LI, VI	7	80
Liu	2018	China	60	Immune cell	PD-L1	26 (43.3)	NR	OS, Gender, Tumor location	7	81
Ogura	2017	Japan	281	Immune cell	PD-L1	89 (31.7)	Moderate-to-strong intensity in more than 5%	Gender, T stage, N stage, LVI	9	82
Feng	2019	China	168	Immune cell	PD-L1	96 (57.1)	Score≥ 2 (intensity)	OS, RFS, Gender, Tumor location, T stage, VI, Microsatellite status	8	83
Ho	2019	Taiwan	238	Tumor cell	PD-L1	13 (5.5)	H-score ≥ 10 (intensity + area)	OS	9	84
Li	2019	China	90	Tumor tissue	PD-L1	45 (50)	NR	OS, Gender, TNM stage, T stage, N stage, M stage	8	85
Shan	2019	China	80	Tumor tissue	PD-L1	46 (57.5)	> 10% (area)	Gender, T stage, N stage, M stage	10	86
Sudoyo	2019	Indonesia	98	Tumor tissue	PD-L1	18 (18.37)	≥ 5% (area)	Gender, Tumor location, Microsatellite status	6	87
Pyo	2019	Korea	265	Immune cell	PD-L1	47 (17.7)	≥10% (area)	Gender, Tumor location, TNM stage, T stage, N stage, M stage, LI, VI	7	88
Pyo	2019	Korea	265	Tumor cell	PD-L1	25 (9.4)	≥10% (area)	Gender, Tumor location, TNM stage, T stage, N stage, M stage, LI, VI	7	88
Lu	2020	China	805	Tumor cell	PD-L1	235 (29.2)	NR	Gender, Tumor location, TNM stage, Microsatellite status	10	20
Qiu	2018	China	98	Tumor cell	PD-L1	45 (45.9)	IHC scores >6.25 (intensity + area)	Gender, Tumor location, T stage, N stage, M stage	8	21
Noh	2020	China	489	Tumor cell	PD-L1	179 (36.6)	Score ≥3 (intensity + area)	OS, Gender, Tumor location, TNM stage, T stage, N stage, LVI, Microsatellite status	9	89

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Table 1 (*continued*)

First author	Year of study	Country	No. of patient	Origin of marker	Marker	Marker + (%)	Cut-off	Outcome	Score of study	Reference
Hecht	2016	Germany	103	Tumor tissue	PD-L1	40 (38.8)	NR	OS	9	90
Kim	2016	Korea	208	Immune cell	PD-L1	62 (29.8)	Moderate-to-strong intensity in more than 5%	TNM stage	8	91
Kim	2016	Korea	208	Tumor cell	PD-L1	26 (12.5)	Moderate-to-strong intensity in more than 5%	Gender, TNM stage, LVI	8	91
Droeser	2013	Switzerland	1420	Tumor cell	PD-L1	433 (36)	Score > 2 (intensity)	OS, Gender, Tumor location, T stage, N stage, VI	8	92
Inaguma	2017	Japan	454	Tumor cell	PD-L1	54 (12)	≥5% (area)	Gender, Tumor location, Microsatellite status	4	93
Lee	2017	Korea	186	Immune cell	PD-L1	107 (57.5)	NR	OS, Gender, Tumor location, TNM stage, T stage, N stage, M stage, LI, VI	10	94
Lee	2017	Korea	153	Immune cell	PD-L1	47 (30.7)	NR	OS, Gender, Tumor location, TNM stage, T stage, N stage, M stage, LI, VI	10	94
Lee	2017	Korea	186	Tumor cell	PD-L1	43 (23.1)	>5% (area)	Gender, Tumor location, TNM stage, T stage, N stage, M stage, LI, VI	10	94
Lim	2017	Korea	123	Tumor cell	PD-L1	35 (28.5)	NR	Gender, Tumor location, T stage, N stage, LI, VI, Microsatellite status	10	95
Masugi	2017	USA	823	Tumor cell	PD-L1	281 (34.1)	NR	Gender, Tumor location, TNM stage, T stage, N stage, M stage, Microsatellite status	8	96
Shi	2013	China	143	Tumor cell	PD-L1	64 (44.8)	Score ≥ 2 (intensity)	OS, Gender, Tumor location, TNM stage, T stage, N stage, M stage	10	97
Shao	2017	China	68	Tumor cell	PD-L1	7 (10.3)	≥1% (area)	OS, DFS, Gender, T stage, N stage, M stage, VI	10	98
El Jabbour	2017	USA	104	Immune cell	PD-L1	13 (12)	≥10% (area)	TNM stage, T stage, N stage, Microsatellite status	6	99
El Jabbour	2017	USA	104	Tumor cell	PD-L1	18 (17%)	≥5% (area)	TNM stage, T stage, N stage, Microsatellite status	6	99
Enkhbat	2018	Japan	116	Tumor cell	PD-L1	52 (44.8)	Score > 3 (intensity + area)	DFS, Gender, Tumor location, TNM stage, T stage, VI, LI	8	57
Lee	2018	Korea	336	Immune cell	PD-L1	152 (45.2)	> 5% (area)	OS, DFS, Gender, Tumor location, TNM stage, T stage, N stage, M stage, LI, VI, Microsatellite status	9	100
Lee	2018	Korea	336	Tumor cell	PD-L1	15 (4.5)	>1% (area)	OS, DFS, Gender, Tumor location, TNM stage, T stage, N stage, M stage, LI, VI, Microsatellite status	9	100
Liang	2013	China	185	Tumor cell	PD-L1	102 (55.1)	Score ≥ 4 (intensity + area)	OS, DFS, Gender, TNM stage, T stage, N stage	9	24
Wang	2017	China	254	Immune cell	PD-L1	46 (18.1)	NR	RFS	8	101
Wei	2018	China	422	Tumor tissue	PD-L1	188 (44.5)	≥1% of tumor-infiltrating immune cells and/or ≥5% of tumor cells (area)	OS, DFS, Gender, Tumor location, TNM stage, T stage, N stage, M stage, VI, Microsatellite status	10	58

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Table 1 (*continued*)

First author	Year of study	Country	No. of patient	Origin of marker	Marker	Marker + (%)	Cut-off	Outcome	Score of study	Reference
Zhong	2018	China	87	Tumor cell	PD-L1	69 (79.3)	NR	Gender, TNM stage, T stage, N stage	9	102
Ahtiainen	2019	Finland	194	Immune cell	PD-L1	79 (40.7)	Moderate-to-strong intensity in more than 5%	OS, DFS, Gender, Tumor location, TNM stage, LVI, Microsatellite status	8	59
Ahtiainen	2019	Finland	194	Tumor cell	PD-L1	22 (11.3)	Moderate-to-strong intensity in more than 5%	Gender, Tumor location, TNM stage, LVI, Microsatellite status	8	59
Calik	2019	Turkey	157	Immune cell	PD-L1	85 (54.1)	>5% (area)	DFS, Gender, Tumor location, T stage	8	103
Calik	2019	Turkey	157	Tumor cell	PD-L1	72 (45.9)	>5% (area)	DFS, Gender, Tumor location, T stage	8	103
Chen	2019	Taiwan	112	Tumor cell	PD-L1	56 (50)	>5% (area)	DFS, Gender, TNM stage, N stage, M stage	13	104
Chiang	2019	China	104	Tumor cell	PD-L1	53 (51)	>5% (area)	DFS, Gender, TNM stage, N stage, M stage, Microsatellite status	12	105
Eriksen	2019	Denmark	572	Tumor cell	PD-L1	35 (6)	>5% (area)	OS, RFS, Gender, Tumor location, T stage, N stage, VI, Microsatellite status	8	106
Zhao	2014	China	56	Tumor cell	PD-L1	27 (48.21)	Score ≥ 4 (intensity + area)	Gender, Tumor location, T stage, N stage	7	25
Li	2015	China	57	Tumor cell	PD-L1	26 (45.61)	Score ≥ 1 (intensity + area)	Gender, T stage, N stage	9	107
Chen	2018	China	240	Tumor tissue	PD-L1	12 (5)	NR	Gender, TNM stage, LI	8	108
Huang	2020	China	1264	Tumor cell	PD-L2	390 (30.9)	Score ≥ 4 (intensity + area)	OS, Gender, Tumor location, TNM stage, Tstage, N stage, M stage, LVI, Microsatellite status		72
Zengin	2021	Turkey	212	Tumor tissue	PD-L2	90 (42.5)	NR	OS, Gender, Tumor location, T stage, LVI, Microsatellite status	9	51
Guo	2018	China	348	Tumor cell	PD-L2	143 (41.1)	Score ≥ 2 (intensity + area)	Gender, Tumor location, TNM stage, N stage, M stage	8	109
Pyo	2019	Korea	264	Immune cell	PD-L2	46 (17.4)	≥10% (area)	Gender, Tumor location, TNM stage, T stage, N stage, M stage	9	110
Masugi	2017	USA	823	Tumor cell	PD-L2	613 (74.5)	>20% (area)	Gender, Tumor location, TNM stage	9	111
Wang	2017	China	124	Immune cell	PD-L2	28 (23)	NR	OS, Gender, Tumor location, TNM stage, T stage, N stage, M stage	8	112
Wang	2017	China	124	Tumor cell	PD-L2	48 (38.7)	Score≥2 (intensity + area)	OS, Gender, Tumor location, TNM stage, T stage, N stage, M stage	8	112
Al-Badran	2020	UK	457	Immune cell	TIM3	271 (59)	NR	Gender, Tumor location, TNM stage, T stage, N stage, VI, Microsatellite status	8	49
Zhou	2015	China	201	Tumor tissue	TIM3	118 (58.70)	Score ≥ 200 (stain intensity × the percentage of the stain cells)	Gender, T stage, N stage, M stage, TNM stage	11	113
Yu	2016	China	112	Tumor tissue	TIM3	47 (41.96)	NR	Gender, TNM stage, M stage	5	114
Kuai	2020	China	73	Tumor tissue	TIM3	41 (56.16)	Score> 3 (intensity + area)	Gender, Tumor location, TNM stage, T stage, N stage	9	55

DFS = disease-free survival; IHC = immunohistochemistry; No. = number; NR = Not reported; OS = overall survival; RFS = recurrent-free survival; TNM = Tumor, Node, Metastasis.

Table 2
Relationship between B7—CD28 Superfamily expression and clinicopathological features of colorectal cancer.

Factor	Studies (N)	Analytical method	OR (95% CI)	*P*	Heterogeneity		Publication bias (egger test)
					I^2 (%)	*P*	*P*
B7H3							
Gender (male vs female) (overall)	10	FEM	0.99 (0.84–1.18)	.94	32.06%	.15	.55
Gender (male vs female) (tumor cell)	8	FEM	0.98 (0.81–1.18)	0.86	45.90%	0.07	-
Gender (male vs female) (tumor tissue)	2	FEM	1.04 (0.70–1.54)	0.83	0.00%	0.62	-
Location (colon vs rectum) (overall)	7	FEM	1.24 (1.03–1.50)	0.021*	0.00%	0.91	0.45
Location (colon vs rectum) (tumor cell)	5	FEM	1.28 (1.03–1.59)	0.02*	0.00%	0.88	-
Location (colon vs rectum) (tumor tissue)	2	FEM	1.11 (0.74–1.66)	0.59	0.00%	0.46	-
Location (right vs left) (overall)	3	FEM	1.19 (0.86–1.66)	0.25	2.69%	0.36	0.16
N stage (N+ vs N−) (overall)	9	FEM	1.21 (0.97–1.50)	0.77	6.26%	0.38	0.31
N stage (N+ vs N−) (tumor cell)	7	FEM	1.10 (0.85–1.43)	0.46	0.33%	0.42	-
N stage (N+ vs N−) (tumor tissue)	2	FEM	1.51 (1.02–2.24)	0.037*	0.00%	0.39	-
T stage (III/IV vs I/II) (overall)	9	FEM	1.69 (1.32–2.17)	0.000‡	12.69%	0.33	0.025*
T stage (III/IV vs I/II) (tumor cell)	7	FEM	1.78 (1.31–2.42)	0.000‡	26.44%	0.23	-
T stage (III/IV vs I/II) (tumor tissue)	2	FEM	1.53 (1.01–2.33)	0.047*	0.00%	0.42	-
TNM stage (III/IV vs I/II) (overall)	7	FEM	1.31 (1.09–1.58)	0.003†	41.60%	0.11	0.15
TNM stage (III/IV vs I/II) (tumor cell)	5	FEM	1.32 (1.08–1.63)	0.008†	60.87%	0.04	-
TNM stage (III/IV vs I/II) (tumor tissue)	2	FEM	1.30 (0.88–1.92)	0.18	0.00%	0.83	-
Vascular invasion (VI+ vs VI−) (overall)	4	FEM	1.63 (1.05–2.55)	0.031*	0.00%	0.76	0.39
Vascular invasion (VI+ vs VI−) (tumor cell)	2	FEM	1.68 (0.83–3.38)	0.14	0.00%	0.58	-
Vascular invasion (VI+ vs VI−) (tumor tissue)	2	FEM	1.60 (0.90–2.58)	0.10	0.00%	0.36	-
B7H4							
Gender (male vs female) (overall)	6	FEM	0.95 (0.76–1.19)	0.66	35.10%	0.17	0.29
Location (colon vs rectum) (overall)	4	FEM	1.03 (0.80–1.34)	0.79	0.00%	0.58	0.79
N stage (N+ vs N−) (overall)	3	REML	2.00 (0.36–11.26)	0.43	90.01%	0.00	0.96
T stage (III/IV vs I/II) (overall)	3	REML	2.62 (0.38–18.00)	0.32	88.76%	0.00	0.45
TNM stage (III/IV vs I/II) (overall)	3	REML	3.29 (1.36–7.97)	0.008†	85.89%	0.0002	0.15
CTLA4							
Gender (male vs female) (overall)	2	REML	2.73 (0.08–91.10)	0.57	96.21%	0.00	-
N stage (N+ vs N−) (overall)	2	CEM	0.83 (0.41–1.68)	0.61	-	-	-
TNM stage (III/IV vs I/II) (overall)	2	FEM	0.82 (0.40–1.68)	0.59	0.00%	0.97	-
PD-1							
Gender (male vs female) (overall)	11	FEM	0.95 (0.82–1.10)	0.54	0.00%	0.69	0.35
Gender (male vs female) (tumor tissue)	3	FEM	0.99 (0.64–1.55)	0.98	45.97%	0.16	-
Gender (male vs female) (immune cell)	7	FEM	0.92 (0.77–1.10)	0.34	0.00%	0.82	-
Location (colon vs rectum) (overall)	6	FEM	0.76 (0.63–0.91)	0.0033†	22.98%	0.26	0.72
Location (colon vs rectum) (immune cell)	4	FEM	0.86 (0.69–1.06)	0.173	0.00%	0.86	-
Location (right vs left) (overall)	8	REML	1.35 (0.96–1.91)	0.08	68.65%	0.004	0.95
Location (right vs left) (tumor tissue)	2	REML	0.92 (0.53–1.59)	0.77	5.94%	0.30	-
Location (right vs left) (immune cell)	5	REML	1.52 (0.95–2.46)	0.083	75.5%	0.002	-
M stage (M+ vs M−) (overall)	2	FEM	0.43 (0.28–0.64)	0.000‡	0.00%	0.73	-
N stage (N+ vs N−) (overall)	6	REML	0.98 (0.45–2.09)	0.96	94.63%	0.00	0.055
N stage (N+ vs N−) (immune cell)	4	REML	0.62 (0.47–0.81)	0.001‡	44.68%	0.12	-
T stage (III/IV vs I/II) (overall)	8	REML	0.88 (0.58–1.33)	0.55	72.59%	0.0003	0.34
T stage (III/IV vs I/II) (tumor tissue)	2	REML	1.43 (0.63–3.27)	0.39	41.98%	0.19	-
T stage (III/IV vs I/II) (immune cell)	5	REML	0.59 (0.46–0.76)	0.000‡	0.00%	0.59	-
TNM stage (III/IV vs I/II) (overall)	8	REML	1.21 (0.58–2.51)	0.59	92.55%	0.000	0.029*
TNM stage (III/IV vs I/II) (tumor tissue)	2	REML	5.81 (2.64–12.79)	0.000‡	0.00%	0.40	-
TNM stage (III/IV vs I/II) (immune cell)	5	REML	0.69 (0.37–1.26)	0.223	82.39%	0.002	-
Lymphovascular invasion (LVI+ vs LVI−) (overall)	3	FEM	0.53 (0.34–0.82)	0.0045†	40.73%	0.18	0.099
MSS/MSI (MSS vs MSI) (overall)	5	REML	1.88 (0.62–5.72)	0.26	94.57%	0.000	0.17
MSS/MSI (MSS vs MSI) (immune cell)	4	REML	2.79 (0.99–7.87)	0.053*	92.11%	0.00	-
PD-L1							
Gender (male vs female) (overall)	71	FEM	0.89 (0.83–0.96)	0.0032†	9.12%	0.26	0.44
Gender (male vs female) (tumor cell)	46	FEM	0.86 (0.79–0.95)	0.003†	21.14%	0.10	-
Gender (male vs female) (tumor tissue)	6	FEM	1.02 (0.79–1.31)	0.85	4.53%	0.38	-
Gender (male vs female) (immune cell)	19	FEM	0.92 (0.79–1.06)	0.26	0.00%	0.78	-
Location (colon vs rectum) (overall)	25	FEM	1.14 (1.01–1.29)	0.029*	46.60%	0.15	0.031*
Location (colon vs rectum) (tumor cell)	15	FEM	1.24 (1.06–1.45)	0.007†	19.67%	0.234	-
Location (colon vs rectum) (tumor tissue)	2	FEM	0.91 (0.66–1.25)	0.58	28.71%	0.236	-
Location (colon vs rectum) (immune cell)	8	FEM	1.07 (0.85–1.34)	0.55	69.31%	0.002	-
Location (right vs left) (overall)	36	REML	1.86 (1.45–2.39)	0.000‡	77.27%	0.000	0.000‡
Location (right vs left) (tumor cell)	22	REML	2.15 (1.47–3.14)	0.000‡	85.16%	0.000	-
Location (right vs left) (tumor tissue)	2	REML	1.29 (0.56–2.93)	0.53	65.63%	0.088	-
Location (right vs left) (immune cell)	12	REML	1.55 (1.15–2.10)	0.004†	40.62%	0.085	-
M stage (M+ vs M−) (overall)	27	REML	0.92 (0.56–1.50)	0.74	80.83%	0.000	0.52
M stage (M+ vs M−) (tumor cell)	17	REML	1.03 (0.57–1.89)	0.901	78.26%	0.00	-
M stage (M+ vs M−) (tumor tissue)	3	REML	1.66 (0.48–5.73)	0.418	76.77%	0.030	-
M stage (M+ vs M−) (immune cell)	7	REML	0.47 (0.15–1.47)	0.196	80.38%	0.010	-
N stage (N+ vs N−) (overall)	54	REML	1.01 (0.82–1.24)	0.89	77.60%	0.00	0.014*
N stage (N+ vs N−) (tumor cell)	35	REML	1.19 (0.95 –1.49)	0.114	67.54%	0.00	-
N stage (N+ vs N−) (tumor tissue)	5	REML	1.44 (0.48–4.24)	0.507	93.02	0.00	-

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Table 2 (*continued*)

Factor	Studies (N)	Analytical method	OR (95% CI)	*P*	Heterogeneity		Publication bias (egger test) *P*
					I^2 (%)	*P*	
N stage (N+ vs N−) (immune cell)	14	REML	0.59 (0.44–0.79)	0.000‡	54.25%	0.012	-
T stage (III/IV vs I/II) (overall)	45	REML	0.94 (0.74–1.18)	0.59	70.89%	0.00	0.0044†
T stage (III/IV vs I/II) (tumor cell)	30	REML	1.20 (0.91–1.58)	0.185	66.11%	0.000	-
T stage (III/IV vs I/II) (tumor tissue)	3	REML	0.86 (0.41–1.78)	0.696	66.14%	0.053	-
T stage (III/IV vs I/II) (immune cell)	12	REML	0.55 (0.39–0.79)	0.001‡	58.54%	0.004	-
TNM stage (III/IV vs I/II) (overall)	44	REML	1.05 (0.83–1.34)	0.65	80.61%	0.00	0.13
TNM stage (III/IV vs I/II) (tumor cell)	25	REML	1.45 (1.10–1.90)	0.008†	72.60%	0.000	-
TNM stage (III/IV vs I/II) (tumor tissue)	6	REML	1.13 (0.61–2.08)	0.693	78.87%	0.000	-
TNM stage (III/IV vs I/II) (immune cell)	13	REML	0.55 (0.38–0.79)	0.002†	66.28%	0.001	-
Lymphatic invasion (LI+ vs LI−) (overall)	17	REML	1.27 (0.86–1.86)	0.22	68.68%	0.00	0.063
Lymphatic invasion (LI+ vs LI−) (tumor cell)	10	REML	1.85 (1.29–2.66)	0.001‡	29.17%	0.138	-
Lymphatic invasion (LI+ vs LI−) (immune cell)	6	REML	0.60 (0.45–0.80)	0.001‡	0.00%	0.35	-
Vascular invasion (VI+ vs VI−) (overall)	28	REML	1.03 (0.72–1.47)	0.85	75.50%	0.00	0.57
Vascular invasion (VI+ vs VI−) (tumor cell)	19	REML	1.50 (1.01–2.21)	0.040*	68.24%	0.00	-
Vascular invasion (VI+ vs VI−) (immune cell)	8	REML	0.40 (0.28–0.57)	0.000‡	10.54%	0.31	-
Lymphovascular invasion (LVI+ vs LVI−) (Overall)	16	REML	1.28 (0.94–1.78)	0.11	63.26%	0.0016	0.080
Lymphovascular invasion (LVI+ vs LVI−) (tumor cell)	10	REML	1.43 (0.93–2.19)	0.10	63.99%	0.01	-
Lymphovascular invasion (LVI+ vs LVI−) (immune cell)	6	REML	1.12 (0.68–1.84)	0.64	64.27%	0.01	-
MSS/MSI (MSS vs MSI) (overall)	25	REML	3.82 (2.43–6.00)	0.000‡	85.86%	0.00	0.77
MSS/MSI (MSS vs MSI) (tumor cell)	19	REML	3.73 (2.10–6.63)	0.000‡	86.83%	0.00	-
MSS/MSI (MSS vs MSI) (immune cell) PD-L2	5	REML	4.41 (2.05–9.49)	0.000‡	76.18%	0.01	-
Gender (male vs female) (overall)	8	FEM	0.95 (0.82–1.10)	0.52	7.51%	0.37	0.76
Gender (male vs female) (tumor cell)	5	FEM	0.99 (0.85–1.15)	0.85	27.08%	0.24	-
Gender (male vs female) (immune cell)	2	FEM	0.81 (0.49–1.35)	0.41	0.00%	0.37	-
Location (colon vs rectum) (overall)	4	FEM	0.84 (0.63–1.1)	0.25	0.00%	0.71	0.68
Location (right vs left) (overall)	6	REML	1.23 (0.65–2.33)	0.52	92.27%	0.00	0.01*
Location (right vs left) (tumor cell)	4	REML	0.92 (0.70–1.20)	0.55	50.55%	0.11	-
M stage (M+ vs M−) (overall)	5	REML	0.61 (0.28–1.3)	0.20	52.24%	0.079	0.78
M stage (M+ vs M−) (tumor cell)	3	REML	0.78 (0.32–1.88)	0.57	68.65%	0.03	-
M stage (M+ vs M−) (immune cell)	2	REML	0.20 (0.04–1.06)	0.058*	0.00%	0.63	-
N stage (N+ vs N−) (overall)	5	REML	0.98 (0.55–1.74)	0.95	71.31%	0.0021	0.49
N stage (N+ vs N−) (tumor cell)	3	REML	1.40 (0.79–2.46)	0.248	53.79%	0.11	-
N stage (N+ vs N−) (immune cell)	2	REML	0.58 (0.28–1.18)	0.134	44.38%	0.18	-
T stage (III/IV vs I/II) (overall)	5	REML	0.58 (0.24–1.41)	0.23	77.98%	0.0016	0.62
T stage (III/IV vs I/II) (tumor cell)	2	REML	1.15 (0.45–2.97)	0.77	18.92%	0.27	-
T stage (III/IV vs I/II) (immune cell)	2	REML	0.19 (0.098–0.37)	0.000‡	0.00%	0.78	-
TNM stage (III/IV vs I/II) (overall)	6	FEM	1.033 (0.87–1.21)	0.69	0.00%	0.52	0.87
TNM stage (III/IV vs I/II) (tumor cell)	4	FEM	1.028 (0.86–1.22)	0.74	21.63%	0.28	-
TNM stage (III/IV vs I/II) (immune cell)	2	FEM	1.077 (0.62–1.84)	0.78	0.00%	0.56	-
Lymphovascular invasion (LVI+ vs LVI−) (overall)	3	REML	0.71 (0.50–1.00)	0.051*	59.56%	0.094	0.043*
MSS/MSI (MSS vs MSI) (overall)	3	REML	0.168 (0.051–0.55)	0.0033†	72.38%	0.027	0.11

CEM = common-effect model; FEM = fixed-effect model; MSI = microsatellite instable; MSS = microsatellite stable; OR = odds ratio; REML = random-effects model; TNM = tumor, node, metastasis.

* $P < 0.05$.

† $P < 0.01$.

‡ $P < 0.001$.

CTLA4

Three studies were eligible for estimation of the relationship between CTLA4 expression and clinicopathological features (gender, N stage, and TNM stage) of CRC (Table 2).

The pooled results indicated that high CTLA4 expression was not significantly associated with gender (male vs female, OR = 2.73, 95% CI = 0.08–91.10, $P = 0.57$), N stage (presence vs absence, OR = 0.83, 95% CI = 0.41–1.68, $P = 0.61$), and TNM stage (III/IV vs I/II, OR = 0.82, 95% CI = 0.40–1.68, $P = 0.59$).

PD-1

The association between PD-1 expression and clinicopathological characteristics was evaluated in 12 studies comprising 4064 patients. Heterogeneity was identified in the analysis of PD-1 expression with tumor location (right vs left) ($P = 0.004$, $I^2 = 68.65\%$), lymph node metastasis ($P < 0.001$, $I^2 = 94.63\%$), T stage ($P < 0.001$, $I^2 = 72.59\%$), TNM stage ($P < 0.001$, $I^2 = 92.55\%$), and MSS cancer ($P < 0.001$, $I^2 = 94.57\%$). Therefore, a random-effects model was used in the above analysis, and other subgroup analyses were performed in a fixed-effects model (Table 2).

The data exhibited that highly expressed PD-1 was firmly related to rectum cancer (OR = 0.76, 95% CI = 0.63–0.91, $P = 0.0033$), absence of distance metastasis (OR = 0.43, 95% CI = 0.28–0.64, $P < 0.001$), and absence of lymphovascular invasion (OR = 0.53, 95% CI = 0.34–0.82, $P = 0.0045$), but not correlative with patient's gender, tumor location (Right vs Left), N stage, T stage, TNM stage, and microsatellite instability. In the following, for a better understanding correlation between PD-1 expression and these characteristics, we divided the studies into 3 groups according to the origin of the marker on tumor cells, tumor tissue, and immune cells and performed subgroup analysis. Subgroup analysis showed that PD-1 overexpression on tumor tissue was associated with a higher TNM stage (OR = 5.81, 95% CI = 2.64–12.79, $P < 0.001$). Furthermore, high expression of PD-1 in immune cells correlated with the absence of

lymph node metastasis (OR = 0.62, 95% CI = 0.47–0.81, $P = 0.001$), lower T stage (OR = 0.59, 95% CI = 0.46–0.76, $P < 0.001$), and MSS tumor (OR = 2.79, 95% CI = 0.99–7.87, $P = 0.053$) in CRC.

PD-L1

The pooled results of 61 studies including 18,813 CRC patients indicated that there were significant positive correlations between PD-L1 expression and gender, tumor location, and MSS tumor. However, in the following, we used subgroup analysis based on the origin of the marker to be able to show this relationship better. Subgroup analysis also showed that PD-L1 overexpression on tumor cell was associated with female gender (OR = 0.86, 95% CI = 0.79–0.95, $P = 0.003$), colon cancer (OR = 1.24, 95% CI = 1.06–1.45, $P = 0.007$), right-sided colon cancer (OR = 2.15, 95% CI = 1.47–3.14, $P < 0.001$), advanced TNM stage (OR = 1.45, 95% CI = 1.10–1.90, $P = 0.008$), presence of lymphatic invasion (OR = 1.85, 95% CI = 1.29–2.66, $P = 0.001$), presence of vascular invasion (OR = 1.50, 95% CI = 1.01–2.21, $P = 0.040$), and MSS tumor (OR = 3.73, 95% CI = 2.10–6.63, $P < 0.001$). Interestingly, high expression of PD-L1 in immune cells also correlated with right-sided colon cancer (OR = 1.55, 95% CI = 1.15–2.10, $P = 0.004$), absence of lymph node metastasis (OR = 0.59, 95% CI = 0.44–0.79, $P < 0.001$), early T stage (OR = 0.55, 95% CI = 0.39–0.79, $P = 0.001$), lower TNM stage (OR = 0.55, 95% CI = 0.38–0.79, $P = 0.002$), absence of lymphatic invasion (OR = 0.60, 95% CI = 0.45–0.80, $P = 0.001$), absence of vascular invasion (OR = 0.40, 95% CI = 0.28–0.57, $P < 0.001$), and MSS tumor (OR = 4.41, 95% CI = 2.05–9.49, $P < 0.001$) (Table 2).

PD-L2

High PD-L2 expression in 6 studies was associated with the MSI tumor (OR= 0.168, 95% CI = 0.051–0.55, $P = 0.0033$) and showed a weak trend toward the absence of lymphovascular invasion (OR = 0.71, 95% CI = 0.50–1.00, $P = 0.051$). However, no significant association was found with gender, tumor location, M stage, N stage, T stage, and TNM stage. Subgroup analysis revealed a significant correlation between high PD-L2 expression and some of the clinicopathological features. In this analysis, PD-L2 overexpression on immune cells was associated with early T stage (OR = 0.19, 95% CI = 0.098–0.37, $P < 0.001$). In addition, the results indicated that high PD-L2 expression implied a weak trend toward the absence of distance metastasis (OR = 0.20, 95% CI = 0.04–1.06, $P = 0.058$) was seen in immune cells (Table 2).

Immunoglobulin superfamily

CEACAM1

Two studies reported the correlation between CEACAM1 and lymph node metastasis. The pooled data (OR = 0.97, 95% CI = 0.27–3.43, $P = 0.96$, [fixed-effect model]) suggested that CEACAM1 overexpression was not significantly associated with the presence of lymph node metastasis (Table 3).

Galectin-3

Our results indicated that there were significant positive correlations between Galectin-3 expression and M stage (presence vs absence, OR = 2.09, 95% CI = 1.19–3.68, $P = 0.01$), T stage (III/IV vs I/II, OR = 3.95, 95% CI = 1.76–8.86, $P < 0.001$), lymphatic invasion (presence vs absence, OR = 2.12, 95% CI = 1.21–3.71, $P = 0.008$), and vascular invasion (presence vs absence, OR = 3.77, 95% CI = 2.26–6.30, $P < 0.001$). However, the pooled data suggested no significant association between Galectin-3 expression and gender, tumor location, N stage, and TNM stage (Table 3).

HLA-II

Five studies reported the relationship between HLA-II expression and clinicopathological features in CRC. As shown in Table 3, HLA-II expression was correlated with the absence of lymph node metastasis (OR = 0.21, 95% CI = 0.11–0.38, $P < 0.001$, fixed-effect model) and lower TNM stage (OR = 0.35, 95% CI = 0.26–0.47, $P < 0.001$, fixed-effect model), but there was no potential correlation between HLA-II overexpression and gender, lymphatic invasion, and vascular invasion. We were not able to evaluate the expression of HLA-II according to the origin of the marker in subgroup analysis due to the lack of data.

LAG3

A total of 3 studies described the association between LAG3 expression and clinicopathological factors (gender, tumor location, N stage, T stage, and TNM stage) in CRC. No significant association was found between LAG3 expression and these factors (Table 3).

TIM3

A total of 4 eligible studies were evaluated for the correlation between Galectin-3 expression and clinicopathological parameters (gender, tumor location, M stage, N stage, T stage, and TNM stage) in CRC. Because of significant heterogeneity between TIM3 expression M stage ($P = 0.021$; $I^2 = 81.08\%$), N stage ($P < 0.001$; $I^2 = 85.18\%$), and TNM stage ($P < 0.001$; $I^2 = 88.92\%$), a random-effects model was utilized. The other analyses were performed using a fixed-effect model (Table 3). Overall, no significant association was revealed between TIM3 expression and clinicopathological features in our data.

TNF-superfamily

CD70

The synthesized data showed that there was a statistically significant connection between CD70 expression and advanced T stage (OR = 14.95, 95% CI = 4.61–48.47, $P < 0.001$) and MSI tumor (OR = 0.21, 95% CI = 0.046–0.98, $P = 0.04$). However, the pooled data suggested no significant association of CD70 with gender, tumor location, M stage, and N stage. We were not able to evaluate the expression of CD70 according to the origin of the marker in subgroup analysis due to limited data.

Correlation between ICP expression and the prognostic parameters (OS, DFS, and RFS)

We also evaluated the association between ICP expression and prognostic parameters (OS, DFS, and RFS) in CRC. Among the ICPs, only markers for which the number of included studies was adequate were examined for prognostic parameters. In addition to the overall analysis of studies, because heterogeneity existed, subgroup analysis was performed based on the origin of the marker for some of ICPs.

The correlation of B7H3 expression and OS rate of CRC patients was significant in 5 studies (Figure 1A), and subgroup analysis revealed that B7H3 overexpression on tumor cells and tumor tissue was associated with shorter OS, respectively (HR = 3.68, 95% CI = 2.75–4.94, $P < 0.001$, fixed-effect model) and (HR = 4.17, 95% CI = 2.14–8.14, $P < 0.001$, fixed-effect model). Overexpression of B7H4 was linked to poor OS in CRC patients, according to 2 studies using a fixed-effects model (HR = 4.83, 95% CI = 2.86–8.17, $P < 0.001$). As depicted in Figure 1B, in 6 studies, PD-1 overexpression was significantly associated with increased total mortality risk among patients. In the subgroup analysis based on markers origin, poor OS was related to high PD-1 expression on the immune cells (HR = 1.81, 95% CI = 1.58–2.07, $P < 0.001$). Data for the association between PD-L1 expression and OS were reported in 31 studies, and their results demonstrate that poor OS was significantly associated with high PD-L1 expression. In subgroups

Table 3
Relationship between immunoglobulin superfamily expression and clinicopathological features of colorectal cancer.

Factor	Studies (N)	Analytical method	OR (95% CI)	*P* value	Heterogeneity		Publication bias (egger test)
					I^2 (%)	*P*	*P*
CEACAM1							
N stage (N+ vs N−) (overall)	2	REML	0.97 (0.27–3.43)	0.96	73.63%	0.05	–
Galectin-3							
Gender (male vs female) (overall)	8	FEM	0.90 (0.65–1.26)	0.54	0.00%	0.66	0.38
Gender (male vs female) (tumor cell)	2	FEM	0.87 (0.47–1.59)	0.64	38.52%	0.20	–
Gender (male vs female) (tumor tissue)	6	FEM	0.92 (0.61–1.37)	0.67	0.00%	0.65	–
Location (colon vs rectum) (overall)	6	FEM	1.35 (0.85–2.14)	0.19	0.00%	0.83	0.58
Location (colon vs rectum) (tumor tissue)	5	FEM	1.34 (0.80–2.23)	0.25	0.00%	0.72	–
Location (right vs left) (overall)	3	FEM	0.91 (0.50–1.64)	0.76	45.62%	0.16	0.27
M stage (M+ vs M−) (overall)	4	FEM	2.09 (1.19–3.68)	0.01†	19.00%	0.29	0.87
N stage (N+ vs N−) (overall)	8	REML	2.30 (0.79–6.71)	0.12	82.97%	0.00	0.17
N stage (N+ vs N−) (tumor tissue)	7	REML	2.09 (0.63–6.90)	0.22	85.48%	0.000	–
T stage (III/IV vs I/II) (overall)	3	FEM	3.95 (1.76–8.86)	0.000‡	0.00%	0.57	0.81
TNM stage (III/IV vs I/II) (overall)	7	REML	1.46 (0.50–4.26)	0.48	83.11%	0.0001	0.03*
TNM stage (III/IV vs I/II) (tumor tissue)	7	REML	1.96 (0.63–6.04)	0.23	79.72%	0.001	–
Lymphatic invasion (LI+ vs LI−) (overall)	3	FEM	2.12 (1.21–3.71)	0.008†	0.00%	0.57	0.29
Vascular invasion (VI+ vs VI−) (overall)	3	FEM	3.77 (2.26–6.30)	0.000‡	0.00%	0.84	0.80
HLA-II							
Gender (male vs female) (overall)	2	FEM	0.92 (0.70–1.21)	0.57	47.81%	0.16	–
N stage (N+ vs N−) (overall)	2	FEM	0.21 (0.11–0.38)	0.000‡	0.00%	0.87	–
TNM stage (III/IV vs I/II) (overall)	2	FEM	0.35 (0.26–0.47)	0.00‡	6.21%	0.30	–
Lymphatic invasion (LI+ vs LI−) (overall)	2	REML	0.08 (0.004–1.73)	0.10	75.60%	0.04	–
Vascular invasion (VI+ vs VI−) (overall)	2	REML	0.34 (0.1–1.47)	0.16	88.73%	0.002	–
LAG3							
Gender (male vs female) (overall)	2	FEM	1.09 (0.82–1.44)	0.53	0.00%	0.67	–
Location (colon vs rectum) (overall)	2	FEM	1.12 (0.81–1.57)	0.47	0.00%	0.68	–
Location (right vs left) (overall)	2	FEM	1.17 (0.85–1.62)	0.31	0.00%	0.41	–
N stage (N+ vs N−) (overall)	2	FEM	1.10 (0.83–1.47)	0.48	0.00%	0.40	–
T stage (III/IV vs I/II) (overall)	2	REML	1.03 (0.42–2.49)	0.94	82.09%	0.01	–
TNM stage (III/IV vs I/II) (overall)	3	FEM	1.094 (0.83–1.43)	0.51	0.00%	0.68	0.74
TIM3							
Gender (male vs female) (overall)	4	FEM	1.23 (0.93–1.63)	0.13	0.00%	0.47	0.23
Gender (male vs female) (tumor tissue)	3	FEM	0.97 (0.64–1.47)	0.89	0.00%	0.91	–
Location (colon vs rectum) (overall)	2	FEM	0.84 (0.56–1.27)	0.43	0.00%	0.75	–
Location (right vs left) (overall)	2	FEM	1.02 (0.68–1.53)	0.89	0.00%	0.82	0.82
M stage (M+ vs M−) (overall)	2	REML	2.56 (0.32–20.09)	0.37	81.08%	0.021	–
N stage (N+ vs N−) (overall)	3	REML	1.25 (0.50–3.12)	0.62	85.18%	0.00	0.95
N stage (N+ vs N−) (tumor tissue)	2	REML	1.65 (0.44–6.18)	0.45	82.39%	0.017	–
T stage (III/IV vs I/II) (overall)	3	FEM	0.72 (0.47–1.10)	0.13	0.00%	0.38	0.74
T stage (III/IV vs I/II) (tumor tissue)	2	FEM	0.78 (0.36–1.70)	0.54	45.23%	0.17	–
TNM stage (III/IV vs I/II) (overall)	4	REML	1.89 (0.7–5.14)	0.20	88.92%	0.00	0.55
TNM stage (III/IV vs I/II) (tumor tissue)	3	REML	2.6 (0.83–8.28)	0.099	82.70%	0.007	–

FEM = fixed-effect model; REML = random-effects model; TNM = tumor, node, metastasis.
* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$.

analysis, a weak significant association was revealed between PD-L1 expression on the tumor cells and OS in patients with CRC (HR = 2.64; 95% CI = 2.13–3.27; $P = 0.08$, random-effect effect). Results gained with the fixed-effect model revealed that PD-L1 overexpression also was associated with shorter OS than the absence of PD-L1 expression on tumor tissue and immune cells in CRC patients, respectively (HR = 2.07, 95% CI = 1.64–2.62, $P < 0.001$) and (HR = 1.57, 95% CI = 1.39–1.76, $P < 0.001$) (Figure 1C). Four studies showed that PD-L2 expression significantly reduced the OS of CRC patients (HR = 3.26; 95% CI = 1.57–5.83; $P = 0.001$, random-effect effect) (Figure 1D).

The association between PD-1 expression on immune cells and DFS in patients with CRC showed that high PD-1 expression was significantly associated with shorter DFS (HR = 1.64, 95% CI = 1.37–1.96, $P < 0.001$, fixed-effect model) (Figure 2A). Moreover, the correlation between PD-L1 and DFS was presented in 21 studies, and the results indicated that PD-L1 overexpression was associated with unfavorable DFS (HR = 1.68, 95% CI = 1.45–1.94, $P < 0.001$, $I^2 = 56.2\%$, $P = 0.001$, random-effect model) (Figure 2B). Data for the association between PD-L1 expression on the tumor cell and DFS were reported in 13 studies. PD-L1 overexpression was associated with shorter DFS (HR = 1.56, 95% CI = 1.39–1.74, $P < 0.001$, random-effect model). Six studies provided the correlation between PD-L1 expression in the immune cells and DFS parameters. Results showed that poor DFS was significantly associated with high PD-L1 expression in immune cells in CRC patients (HR = 1.62, 95% CI = 1.20–2.19, $P = 0.002$). In this regard, it has been shown in 2 other studies that the overexpression of PD-L1 in tumor tissue was associated with unfavorable DFS (HR = 1.67, 95% CI = 1.41–1.99, $P < 0.001$).

The correlation of PD-L1 expression and RFS was significant in 7 studies (HR = 3.97, 95% CI = 2.75–5.73, $P < 0.001$, fixed-effect model) (Figure 3), and subgroups analysis revealed that overexpression of PD-L1 on tumor cells and immune cells was significantly related to unfavorable RFS, respectively (HR = 5.14, 95% CI = 2.34–11.29, $P < 0.001$, fixed-effect model and HR = 3.69, 95% CI = 2.44–5.59, $P < 0.001$, fixed-effect model).

Publication bias

Egger's test and funnel plot were used to evaluate the publication bias of OS, DFS, and RFS analysis. The shape of the funnel plot did not appear dissymmetric, and Egger's test also showed no publication bias among the studies analyzing the prognostic pa-

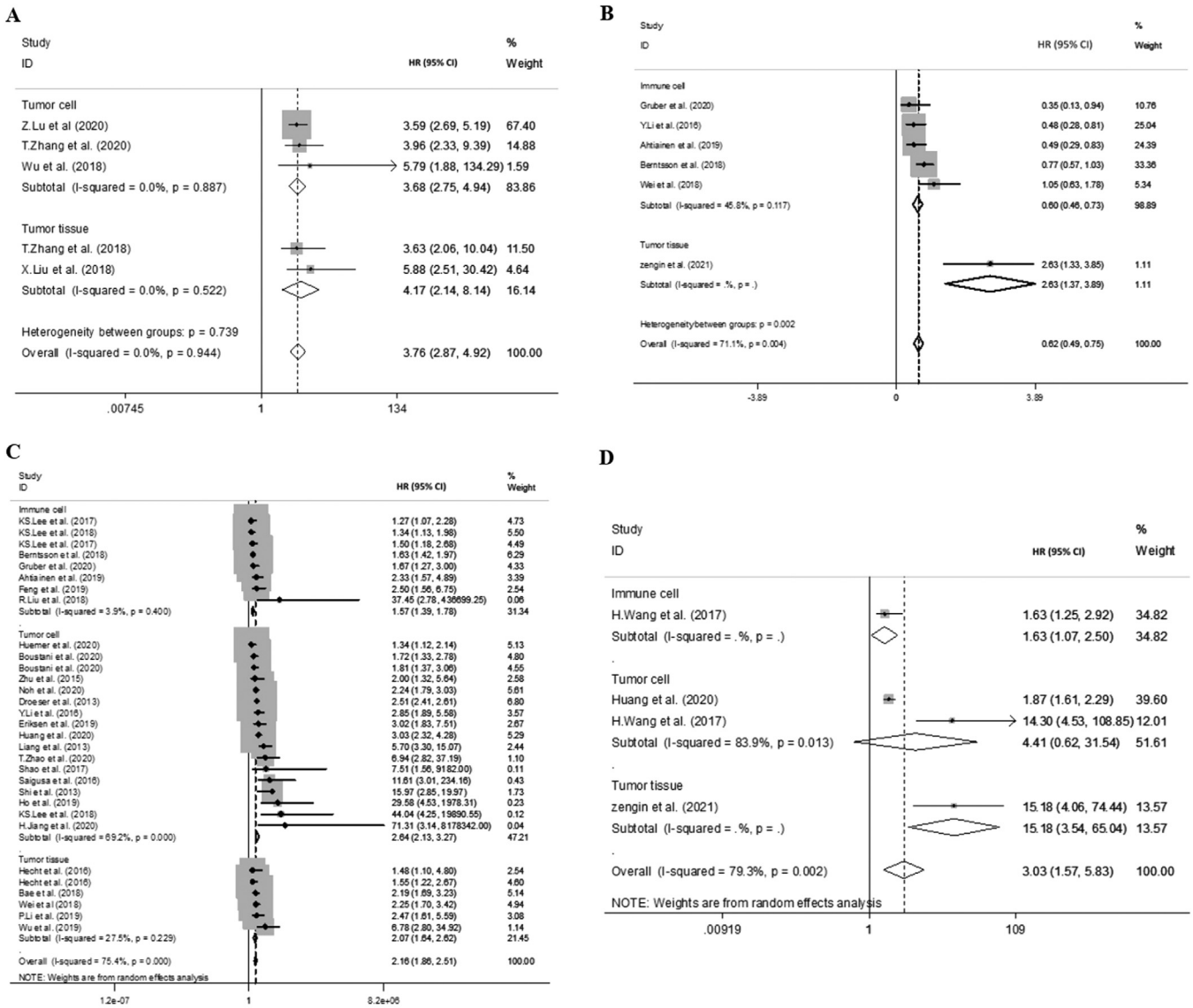

Fig. 1. Forest plot of HR with 95% CI for correlation between ICPs positive expression versus ICPs negative expression and OS. (A) B7H3, (B) PD-1, (C) PD-L1, and (D) PD-L2. B7H3 = B7 homolog 3 protein; HR = hazard ratio; ICP = immune checkpoint; OS = overall survival; PD-1 = programmed cell death 1; PD-L1= PD-1 ligand 1; PD-L2= PD-1 ligand 2.

rameters. The *P* value for these tests was > 0.05. Furthermore, we combined the funnel plot and Egger's test to evaluate whether a publication bias existed for overall clinicopathological features. No significant asymmetry was observed in the funnel plot for most ICPs with clinicopathological features. Moreover, the conclusion was confirmed by Egger's test. However, the funnel plots and Egger's test suggested a publication bias for some ICPs with clinicopathological parameters. Then, the trim and fill method was used to determine the effect of publication bias on the pooled results, which further proved that the results were stable. No study was trimmed or filled in the output results, leaving the pooled results unchanged, which supported the stability of the results.

Publication bias was not analyzed for the correlation of ICP expression and clinicopathological features in subgroups because the number of included studies was low in most groups due to the low sensitivity of the qualitative and quantitative tests.

Sensitivity analyses

A sensitivity analysis was conducted to assess the influence of each study on the synthetic results of the meta-analysis by omitting 1 study at a time. The results show that deleting any single study did not significantly affect the ORs for clinicopathological features and the HRs for OS and DFS/RFS; hence, this meta-analysis of the results is credible (Supplemental 3). The file of Supplemental 3 is available and will be send for journal.

Discussion

The B7 family is an important cancer player, and its study will be very promising in the field of malignancy research. According to available data in this study, among the B7 family, we found that high B7H3 expression is associated with colon cancer, advanced TNM stage and T stage, more vessel invasion, and shorter survival that is consistent with another meta-analysis in different types of cancer,[115,116] but it is in contrast with Fan et al[117] meta-analysis in patients with CRC. Recently, Mielcarska et al[118] have reported a positive correlation between B7H3 tumor concentration and the T parameter that can be associated with the proliferation and invasion potential of tumor cells in CRC. They also performed a survival analysis by using The Cancer Genome Atlas Colon Adenocarcinoma (TCGA) cohort and shown high B7H3 expression was associated

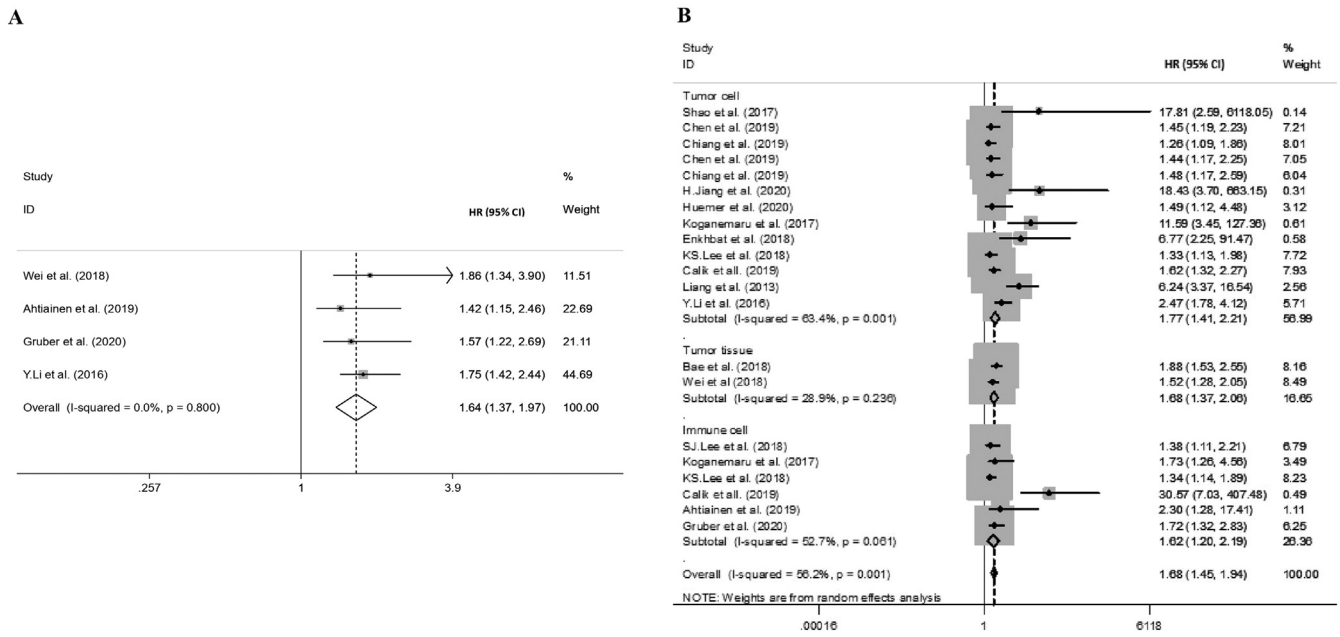

Fig. 2. Forest plot of HR with 95% CI for correlation between ICPs positive expression versus ICPs negative expression and DFS. (A) PD-1 and (B) PD-L1. DFS = disease-free survival; HR = hazard ratio; ICP = immune checkpoint; PD-1 = programmed cell death 1; PD-L1= PD-1 ligand 1.

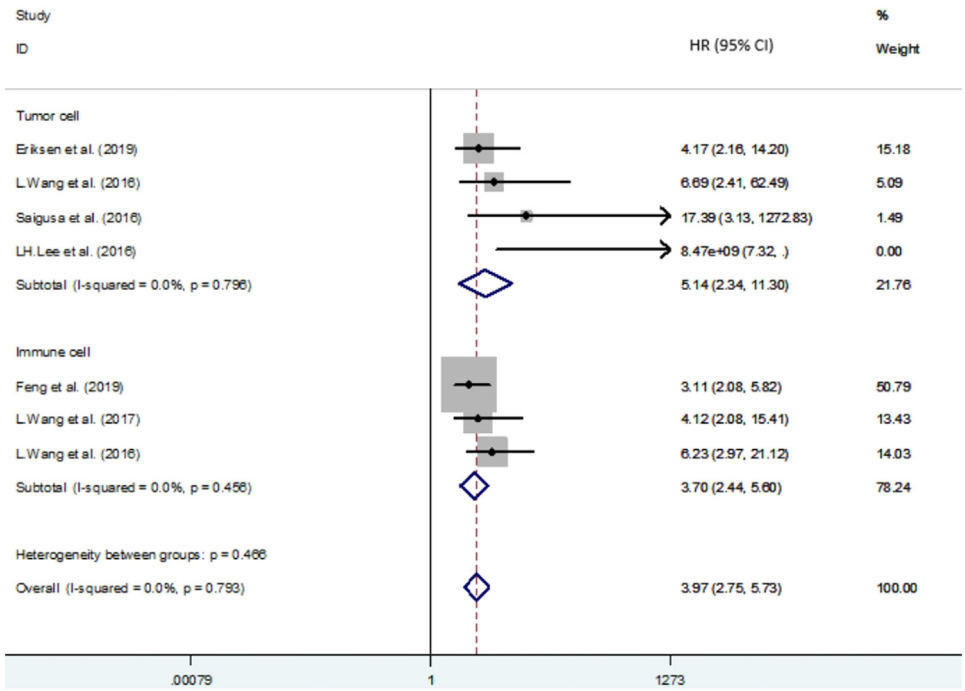

Fig. 3. Forest plot of HR with 95% CI for correlation between PD-L1 positive expression versus PD-L1 negative expression and RFS. HR = hazard ratio; PD-L1 = programmed cell death 1 ligand 1; RFS= relapse-free survival.

with decreased survival. B7H4 overexpression was significantly associated with advanced TNM stage and poor OS, which is consistent with another meta-analysis in non–small cell lung cancer and other cancers.[119,120] Furthermore, a cohort study has reported that overexpression of B7H4 was positively correlated with lymph node metastasis, advanced TNM stage, poor tumor differentiation, and shorter OS in patients with CRC.[121] In our study, CTLA-4 overexpression was not significantly associated with clinicopathological features of CRC. In this regard, a systematic review and meta-analysis done by Hu et al[122] investigated the prognostic value of CTLA-4 in a variety of malignancies and found no relevance when analyzing the overall effect of CTLA-4 expression on OS in several cancers. However, recently one study has reported that high CTLA-4 expression in CRC tissue was highly correlated to the old age group, large tumor size, advanced TNM stage, the presence of distant metastasis, and shorter OS and DFS.[123] Recently, a growing number of studies have investigated the clinicopathological and prognostic implication of PD-1, PD-L1, and PD-L2 protein expressions in tumors among patients with solid tumors, and the results are still controversial.[124] Our results in this study showed that high expression of PD-1 is correlated with rectum cancer, absence of metastasis and lymphovascular invasion, increased total mortality

risk among patients, and shorter DFS. In this regard, we did a cohort study and investigated the prognostic value of the PD-1, TIM3 expression, and PD-1/TIM3 co-expression in patients with CRC. Our findings showed that high PD-1 expression in the invasive margin of the tumor was associated with lower T stage, absence of distance metastasis, lower TNM stage, lack of recurrence, and conversely with larger tumor size (≥5 cm). Our results showed that high PD-1 expression in the invasive margin of the tumor was associated with better OS; however, it was not statistically significant.[125] It seems that the lower TNM stage, absence of metastasis, and lymphovascular invasion are due to the expression of PD-1 on immune cells. A study has shown that a higher density of PD-L1 on macrophages and their spatial proximity with PD-1 expressing T cells are associated with prolonged survival of CRC patients.[126] Zhang et al[127] have reported a significant correlation between high PD-1 expression and poor DFS, whereas they found no significant correlation between PD-1 expression and clinicopathological characteristics or OS in hepatocellular carcinoma patients. In the present meta-analysis, the overexpression of PD-L1 on tumor cells was significantly correlated with right colon CRC, female gender, advanced TNM stage, MSS tumor, presence of lymphatic invasion, and vascular invasion. Other meta-analyses in CRC patients have reported that the overexpression of PD-L1 was dependent on the presence of lymphatic metastasis and vascular invasion, colon, higher TNM stage, and female.[128–133] In addition, the pooled results of OS, RFS, and DFS showed that PD-L1 expression was significantly correlated with unfavorable clinical outcomes in CRC, which concurred with previous studies.[129,131,134,135] Similar to our results, some studies have demonstrated that the overexpression of PD-L1 on immune cells in colon cancer microenvironment is significantly associated with early T stage, lower American Joint Committee on Cancer stage, absence of lymph node metastasis, and vascular invasion. These studies also reported a significant improvement in OS and DFS at a high level of PD-L1 expression in the immune cell, which is contradictory to our survival results.[93,136] In contrast to PD-L1, there is less research on the association of PD-L2 expression and clinicopathological outcomes in CRC. However, based on the available data, our results showed no significant association between PD-L2 expression and gender, tumor location, M stage, N stage, T stage, and TNM stage in CRC. However, high PD-L2 expression was associated with the MSI state and poorer OS. In accordance with our study, Yang et al[137] have revealed that PD-L2 overexpression was related to unfavorable prognosis figures such as shorter OS, DFS, and progression-free survival in solid cancer patients, especially in hepatocellular carcinoma. In another meta-analysis, PD-L2 overexpression in GI cancers after surgery was associated with poor OS, lymphatic metastasis, and tumor metastasis, especially in hepatocellular carcinoma and CRC.[138] However, Kuol et al[135] have reported that PD-L2 overexpression was not associated with clinical stage, tumor progression, and survival outcome in patients with CRC.

CD70 is a member of the tumor necrosis factor family that is aberrantly expressed in different malignancies, and due to the lack of constitutive expression in normal tissues, may be an attractive therapeutic target for tumors.[139] Our data showed a significant relationship between CD70 expression and advanced T stage and MSI tumor in patients with CRC but no significant association of CD70 with gender, tumor location, M stage, and N stage. Additionally, research revealed that OS was significantly lower in individuals with CD70-positive tumors; hence, high expression of CD70 is a poor prognostic factor for tumors.[27,139]

Molecules belonging to the immunoglobulin superfamily (Ig-SF) are frequently involved in cell-cell adhesion, and a number of these molecules also have been linked to cancer progression or suppression. As the ICP, we investigated the roles of some of the members of this family including CEACAM1, Galectin-3, HLA-II, LAG3, and TIM3 in CRC patient outcomes. In our results, no significant association was found between clinicopathological characteristics and the expression of CEACAM1 and LAG3 in patients with CRC. Of course, it should be considered that the amount of data available on the expression of these markers in CRC was limited. However, the association between high LAG3 expression and improved OS in several solid tumors was reported.[140,141] Yılmaz et al[136] have shown that high expression of LAG3 on immune cells is associated with improved DFS in CRC. In contradiction with these studies, another study has reported that the presence of LAG3-positive tumor-infiltrating lymphocytes in TME is highly correlated with larger tumor size, higher tumor grade, lymph node metastasis, and shorter DFS and OS.[124] The results of our study in CRC patients' tissue showed a positive relationship between high expression of LAG3 on tumor-infiltrating lymphocytes in the invasive margin of colon tumor and tumor progression (higher T stage and larger tumor size) and the absence of tertiary lymphoid structure formation, whereas a low score of LAG3 was significantly associated with no metastasis and no recurrence.[142]

HLA-II molecules from Ig-SF are expressed by tumor cells and have a significant impact on their immunogenicity because the downregulation of expression of them significantly correlated with shorter survival in patients with tumors and reduced immunogenicity of the affected tumor cells.[143] In this study, HLA-II overexpression was correlated with the absence of lymph node metastasis and early TNM stage. These findings are consistent with research showing that HLA-II expression is linked to longer survival in the majority of cancer types.[144,145]

Galectin-3 is a tumor cell transformation, migration, invasion, and metastasis mediator. According to research, Galectin-3 levels are significantly elevated in cancer tissues and are linked to CRC metastasis and prognosis.[146] In confirmation of this, our results indicate that high Galectin-3 expression was significantly associated with the presence of metastasis, advanced T stage, and the presence of lymphatic and vascular invasion. Several studies have also found that increased expression of Galectin-3 is associated with reduced survival and poor clinicopathological characteristics in solid tumors such as CRC.[145–149] Thus, Galectin-3, in conjunction with other biomarkers, can be used simultaneously in prognostic outcome analyses.

TIM3 is essential for CTL suppressing and Th1 responses, as well as the expression of anti-tumor cytokines.[150] Preclinical evidence showed that dual blockade of the TIM3 and PD-1 pathways effectively restricted tumor growth. However, the prognostic value of TIM3 in predicting the outcome of various cancers is still debated.[151] No significant association was revealed between TIM3 expression and clinicopathological features in our data, which contrasts with Abdelrahman et al,[124] Zang et al,[150] Qin et al,[151] and Zhang et al[152] meta-analysis. They have reported that TIM3 protein overexpression in TME was relevant to lymph node metastasis, higher grade tumors, advanced tumor stage, and poor prognosis in patients with different solid tumors. As mentioned above, in a cohort study, we investigated the prognostic value of the PD-1, TIM3 expression, and PD-1/TIM3 co-expression in patients with CRC. Our findings showed that TIM3 expression was upregulated in tumor tissues and was associated with higher M stage (M1) in left-sided CRC and shorter OS, whereas TIM3 expression on immune cells at the invasive margin was correlated to improved OS and absence of metastasis in patients. Additionally, PD-1 and TIM3 co-expression had no synergistic effects on predicting OS, which may be due to the small sample size.[125] Similarly, Al-Badran et al[49] showed that TIM3 expression on stromal immune cells was associated with a better CRC prognosis. However, some of studies indicate that overexpression of TIM in TME has inhibitory effects on immune responses against tumors.[153,154]

This study is associated with some limitations. First, the sample size and number of the included studies were relatively small for some of the ICPs. Second, because of the lack of uniform cutoff values for the ICP expression report, different cutoff values were used in research, which could result in bias. In addition, the patient origin, publication year, sample size, follow-up time, specimen type, tumor stage, location, or mismatch repair (MMR) status, use of different antibodies and dilution in the IHC method, and origin of the marker are additional potential causes for heterogeneity. These factors varied between studies, leading the significant heterogeneity in some results. However, we attempted to reduce the impact of heterogeneity resulting from the origin of the marker through subgroup analysis. However, due to limited data availability, subgroup analyses were not performed on some ICPs. Third, few studies included sufficient data to examine the relationship between ICPs and OS, DFS, and RFS, and some of them did not provide the HRs directly. Hence, we extracted HRs and 95% CIs based on survival curves, which may influence the accuracy of data. Hence, larger, more well-designed cohort studies are needed to better assess the relationship between the ICPs overexpression and with clinicopathological characteristics and prognostic parameters in CRC patients and resolve the abovementioned contradictions. In spite of the fact that our study had some limitations, but to the best of our knowledge, this work is the largest meta-analysis that evaluates the association between all of the inhibitory and activatory ICP expression with clinicopathological characteristics and prognostic parameters in CRC patients based on their expression on tumor cells, tumor tissues, and immune cells. The current study provides a comprehensive assessment of the utility of ICP expression status (high vs low) as robust and useful biomarkers for prognostic and clinicopathological factors that can facilitate the better management of individual patients and identify patients suitable for anticancer therapy with immune checkpoint inhibitors.

Declaration of competing interest

The authors have no competing interests to declare.

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Author Contributions

M. Rezaei, Z. Mokhtari, and S. Tavana designed the outline of the article. R. Ghazavi searched and Z. Mokhtari and S. Tavana selected studies. All relevant studies data were extracted by Z. Mokhtari, S. Tavana, and M. Azizi, P. Bemani, and Z. Heidari performed the data analyses. M. Azizi and Z. Mokhtari wrote the manuscript, and M. Rezaei and P. Bemani revised the paper. All authors read, reviewed and approved the final manuscript.

Ethical Approval and Consent to Participate

Not applicable.

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Supplementary materials

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