Prognostic value of the immune target CEACAM6 in cancer: a meta-analysis

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Abstract

Background: Identification of membrane proteins differentially expressed on tumor cells is a key step in drug development. The carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) is a cell adhesion protein belonging to the immunoglobulin superfamily. Here, we explore the prognostic role CEACAM6 expression on patient outcome in cancer.

Methods: A systematic search for studies evaluating the association between tumor expression of CEACAM6 and overall survival (OS) and disease-free survival (DFS) was performed. Hazard ratios (HR) were pooled in a meta-analysis using generic inverse variance and random effect modeling. Subgroup analyses were conducted based on tumor type and method of HR extraction.

Results: Sixteen studies met the inclusion criteria. CEACAM6 expression was associated with worse OS [HR = 1.96, 95% confidence interval (CI) = 1.51–2.53], and DFS (HR = 2.49, 95% CI = 2.01–3.07) with subgroup analysis showing no significant differences between disease site subgroups.

Conclusions: High expression of CEACAM6 is associated with worse OS and DFS in different malignancies. CEACAM6 is a target for the future development of novel therapeutics.

Keywords: CEACAM6, disease outcome, immunotherapy, meta-analysis

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Background

Cancer immunotherapy has gained momentum with the development of immune checkpoint inhibitors.[1] Inhibition of PD1 or PD-L1 has shown clinical benefit in different tumor types.[2] Inhibition of these pathways which suppress the immune response and maintain immunologic tolerance plays a key role in cancer treatment. In addition, other coinhibitory checkpoints like Lymphocyte Activating 3 (LAG3), T-cell immunoreceptor with Ig and ITIM domains (TIGIT), B- and T-lymphocyte attenuator (BTLA), or T-cell immunoglobulin domain and mucin domain 3 (TIM3) have been considered as appropriate targets. Agents against these targets are currently in clinical development with early signs of clinical activity.[3] Most of these strategies either block inhibitory signals or stimulate activating receptors such as OX40, with the goal to augment the immune response through the activation of the innate or adaptive immunity.[4] Indeed, most of these targets are membrane receptors either expressed in immune cells or in tumor cells, so antibodies can easily bind to these proteins. Other mechanisms to take advantage of the immune system in order to target tumor cells have reached the clinical setting. These include vaccines or cell therapy, among others.[1] For any of the previously described treatments, identification of proteins differentially expressed on tumor cells including those on the membrane is a necessary step in drug development.

To avoid the immune response, tumor cells can change the degree of expression of specific antigens on the cell surface with the main objective to repress the activation of immune cells that would otherwise recognize and attack the transformed

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cells. One of those membrane proteins is the carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family, which are highly glycosylated proteins from the immunoglobulin superfamily.[5] They comprise 12 proteins capable of homo- or hetero-dimerization with other CEACAM members[6] or to other membrane proteins such as integrins.[7] They play a role in non-transformed tissues, from phagocytosis or signal transduction, to regulation of cell–cell recognition and adhesion.[8] Moreover, they have been described as bacterial pathogen receptors,[9] and their implication in proliferation, invasiveness, or apoptotic resistance has been described in relation to the oncogenic processes.[10] In addition, these proteins have been proposed as immunotherapy targets in different tumors.[11]

CEACAM6 is a member of the carcinoembryonic antigen molecules, widely distributed in epithelial and myeloid cells.[12] It acts as an intercellular cell adhesion molecule to maintain tissue architecture through interactions with other CEACAM proteins.[13] It has been described as a modulator of cancer progression due to its effects on differentiation and cell growth, resistance to anoikis, treatment resistance, invasiveness and metastasis.[14] Indeed, CEACAM6 is upregulated preferentially at the apical/luminal membranes of many tumors.[12] This effect was first observed in leukemia[15] and subsequently in colorectal,[16] pancreatic,[17] gastric,[18] lung,[19] and many others.[20–23]

Recently, CEACAM6 has been suggested as a target for different cancer immunotherapies given the fact that its membrane expression is highly specific of tumor cells.[11] In models of non-small cell lung cancer, different antibodies against CEACAM6 block cell viability, invasion, and migration through inhibition of Src/FAK phosphorylation.[24] In models of pancreatic adenocarcinoma, the use of monoclonal antibodies (mAb) against this protein sensitizes cells to cytotoxicity using secondary antibodies.[25] A different immunotherapy strategy is the development of humanized single-chain antibody variable fragments (scFv) based on CEACAM6,[26] or specific CEACAM6-single domain antibodies (sdAB) that have demonstrated to inhibit cell growth.[27] CEACAM6 has also been used to create antibody–drug conjugates (ADC), using a tubulin inhibitor as a payload, showing preclinical efficacy in pancreatic cancer.[28]

Beyond the involvement of CEACAM6 in invasion and metastasis, its role as an immune regulator has led to the development of antibodies that inhibit CEACAM6 with the aim to boost the immune response. CEACAM6 has been considered as a novel immune checkpoint, as its inhibition activates the T-cell response. An increase in CEACAM6 expression in multiple myeloma inhibited cytotoxic T-cell reactivity, therefore reducing the immune effect against tumor cells, and treatment with anti-CEACAM6 monoclonal antibodies augmented the T CD8+ activity against malignant cells.[29]

In this context, CEACAM6 has been proposed as a predictor of survival and recurrence in different tumors given its involvement in cancer transformation and the new role in immune regulation.[10] In this article, we report a systematic review and meta-analysis of the prognostic association of CEACAM6 overexpression with outcome in various malignancies. We hypothesize that CEACAM6 overexpression is associated with a worse prognosis.

Methods

This systematic review and meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[30] and was conducted following the Cochrane Handbook for Systematic Reviews of Interventions recommendations.[31] This study was registered in PROSPERO (registration number: CRD42021266217)

Search strategy

The systematic search of the studies was carried out using Cochrane Central Register of Controlled Trials, MEDLINE (Host: PubMed), Scopus, and Web of Science from inception to 11 May 2021. The following keywords were used: 'CEACAM6' OR 'CD66c' OR 'CEAL' OR 'NCA' OR 'KOR-SA3544 antigen' OR 'NCA-50/90' OR 'carcinoembryonic antigen-related cell adhesion molecule 6' OR 'Carcinoembryonic Antigen Related Cell Adhesion Molecule 6' OR 'Normal Cross-Reacting Antigen' OR 'Cluster Of Differentiation 66c' OR 'CD66c Antigen' OR 'Normal Cross-Reacting Antigen' OR 'Carcinoembryonic Antigen-Related Cell Adhesion Molecule 6 (Non-Specific Cross Reacting Antigen)' OR 'Non-Specific Crossreacting

Antigen' AND 'CANCER'. To improve the sensitivity of the search strategy, we reviewed citation lists of included articles, as well as previous systematic reviews or meta-analyses. The full electronic search strategy is detailed in the Supplemental Figure 'electronic search strategy'.

Study selection

Eligibility studies included (1) studies of humans (adults and children); (2) patients with hematological or solid tumors; (3) reporting of a hazard ratio (HR) for overall survival (OS) and/or disease-free survival (DFS; defined as the length of time from primary treatment of an early-stage cancer to death or any signs or symptoms of recurrent cancer) or survival curves allowing estimation of the HR for OS or DFS; (4) English language publication. Case reports, conference abstracts, and letters to editors were excluded. The titles identified by the initial search were evaluated, and potentially relevant publications were retrieved in full. Two authors (MB and EMGM) independently reviewed the full articles for eligibility. Disagreements were resolved by consensus.

Data extraction and risk of bias assessment

The following data were extracted: name of first author, year of publication, tumor type, detection method, agent used, cutoff to define positive expression, sample size, percentage of positive CEACAM6, and outcome.

The outcomes of interest were OS and DFS in patients both with and without CEACAM6 expression as defined by individual studies. The hazard ratio (HR) for OS was extracted whenever available. In cases where the HR was not reported, it was estimated from survival curves using the methods described by Parmar *et al.*[32] We applied a hierarchal approach to the collection of HRs, preferring those reported from multivariable analyses to univariable HR, and preferring both over HRs estimated from survival plots.

The Quality in Prognosis Studies (QUIPS) tool was used to evaluate the risk of bias in six domains: study participation (sampling bias), study attrition (attrition bias), prognostic factor measurement, outcome measurement (ascertainment bias), confounding measurement and accounting and analysis and reporting. Studies were considered to have a low, moderate, or high risk of bias, if they satisfied five to six, three to four, or one to two of the six domains, respectively.[33]

Data extraction was conducted by two independent reviewers (MB and EMGM), and quality assessment was conducted by two independent reviewers (ICR and CAB). Disagreements were solved by consensus or with discussion with a third reviewer (EA)

Data synthesis and statistical analysis

Data were reported descriptively where appropriate. Extracted data were pooled using RevMan 5.4 analysis software (Cochrane Collaboration, Copenhagen, Denmark). In light of substantial clinical heterogeneity (e.g. different tumor types), estimates for HRs were pooled and weighted by generic inverse variance and computed by random effect modeling irrespective of statistical heterogeneity. Statistical heterogeneity was reported using the Cochran's Q. Inconsistence was estimated using I^2 which was considered not important (<30%), moderate (30–50%), substantial (50–75%), or considerable (>75%). In addition, the corresponding p values for Cochran's Q and I^2 statistics were considered. Subgroup analyses were performed for different disease sites and to compare studies with low risk of bias with those with moderate risk of bias. Differences between the subgroups were assessed using methods described by Deeks *et al.*[34] Sensitivity analysis was performed to exclude studies which assessed CEACAM6 by methods other than immunohistochemistry. Publication bias was assessed using visual inspection of the funnel plot for the most commonly reported outcome (OS). All of the statistical tests were two-sided, and statistical significance was defined as $p < 0.05$. No corrections were applied for multiple statistical testing.

Results

Selection of studies

Our search identified 26 articles from which 16 were included in the review. The other 10 studies were excluded due to different reasons: exclusively use of genomic data from the TCGA database,[35–39] data limited to a specific cancer subgroup,[40] data from combined analysis of different genes,[41,42] or studies not reporting survival information.[43,44] Eligible studies included in the analysis were retrospective studies published between 2003 and 2020 and comprised 2441 pat

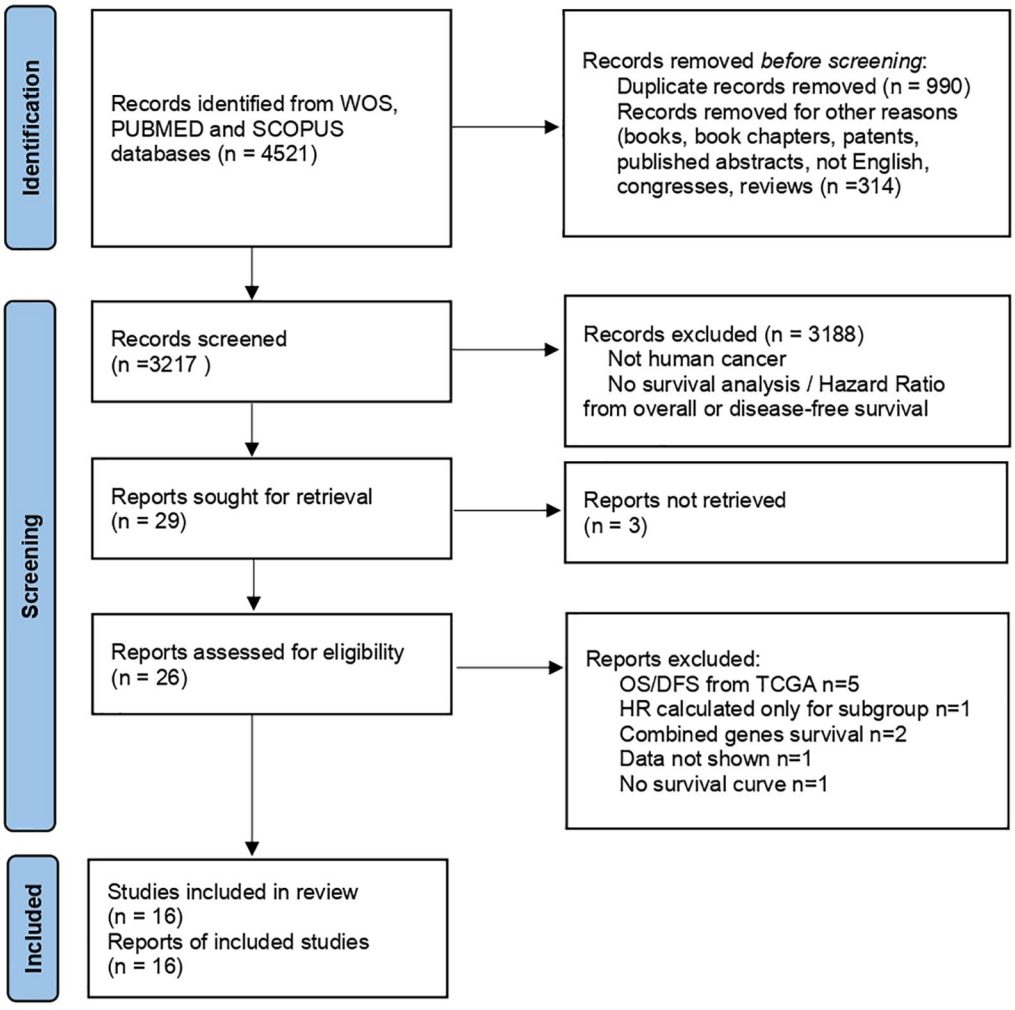


Figure 1. PRISMA of the study selection process for CEACAM6.

ients.[17,21,45–58] Figure 1 shows the study selection schema. CEACAM6 was reported as expressed in 1,535 patients (65%). The characteristics of the included studies are shown in Table 1. For OS, three studies evaluated pancreatic cancer, four gastric cancer, two colorectal cancer, and one each for lung adenocarcinoma, breast cancer, gallbladder cancer, and osteosarcoma. For DFS, two studies evaluated colorectal cancer, and one each for lung adenocarcinoma, breast cancer, intrahepatic cholangiocarcinoma, acute lymphoblastic leukemia (ALL), and gastric cancer.

Risk of bias

The risk of bias, assessed using the QUIPS tool, was low for 10 articles and moderate for 6 (Table S1). The funnel plot was generally symmetrical suggesting low risk of publication bias.

Overall survival

Data for the association between CEACAM6 and OS were reported or calculated in eight studies. All studies utilized immunohistochemistry to assess CEACAM6. CEACAM6 expression was associated with worse OS [HR = 1.96, 95% confidence interval (CI) = 1.51–2.53, $p < 0.001$, see Figure 2(a)]. Heterogeneity was significant (Cochran Q $p < 0.001$, $I^2 = 65\%$). Subsequent subgroup analyses demonstrated that there were no significant differences between cancer type subgroups (subgroup difference $p = 0.8$, Figure 2(b)) or between the methods used for the calculation of HR between subgroups (subgroup difference $p = 0.91$, Supplemental Figure 1). In addition, study quality did not impact on results. There was no difference in the estimate for the association between CEACAM6 and OS for studies with a low risk of bias (HR = 2.13, 95% CI = 1.53–2.97) and those

Table 1. Characteristics of the studies.

	Tumor	Detection method	Agent used	Positive cutoff	n	% CEACAM6 +
Chen *et al.*[48]	Pancreatic	IHC	ab: monoclonal (Abcam)	positive	99	91
Duxbury *et al.*[17]	Pancreatic	IHC	ab: monoclonal By114 (Imgenex)	positive staining >3 (0–3)	89	91
Gebauer *et al.*[49]	Pancreatic	IHC	ab: clone 9A6 (Sigma)	2+ in >70% cells, +3>30% cells (0,1+, 2+, 3+ intensity)	137	72
Deng *et al.*[50]	Gastric	IHC	ab: polyclonal (Sigma)	positive staining >2 (0–3)	75	69
Roy *et al.*[51]	Gastric	IHC	ab: clone9A6 (abcam)	median	106	50
Ru *et al.*[52]	Gastric	IHC	ab: (abcam)	positive reviewed by independent pathologists	436	51
Zang *et al.*[46]	Gastric	IHC	ab: (abcam)	positive staining >3 (0–3)	160	59
Jantscheff *et al.*[53]	Colorectal	IHC	ab: mab 13H10	positive staining >2 (0–3)	243	69
Kim *et al.*[45]	Colorectal	IHC	ab: (R&D)	median	143	54
Han *et al.*[54]	Lung adenocarcinoma	IHC	ab: 9A6 (Santa cruz)	positive staining >2 (0–3)	51	86
Kobayashi *et al.*[55]	Lung adenocarcinoma	IHC	ab: polyclonal (Aviva Systems Biology)	>40% positive carcinoma cells	115	46
Ieta *et al.*[21]	Intrahepatic cholangiocarcinoma	RT-PCR	Specific primers	CEACAM6/GAPDH<2 relative mRNA expression	23	57
Kalina *et al.*[56]	Acute lymphoblastic leukemia	Flow cytometry	ab: KOR-SA3544 labeled to FITC (Immunotech)	>20%	254	43
Maraqa *et al.*[47]	Breast	IHC	ab: clone9A6 (abcam)	positive staining >1 (0–3)	351	81
Tian *et al.*[57]	Gall bladder	IHC	ab: ab78029 (Abcam)	positive cell ratio >50%	68	71
Wang *et al.*[58]	Osteosarcoma	IHC	ab: ab154614 (Abcam)	Staining index (SI) score, >6 (0–12)	91	52

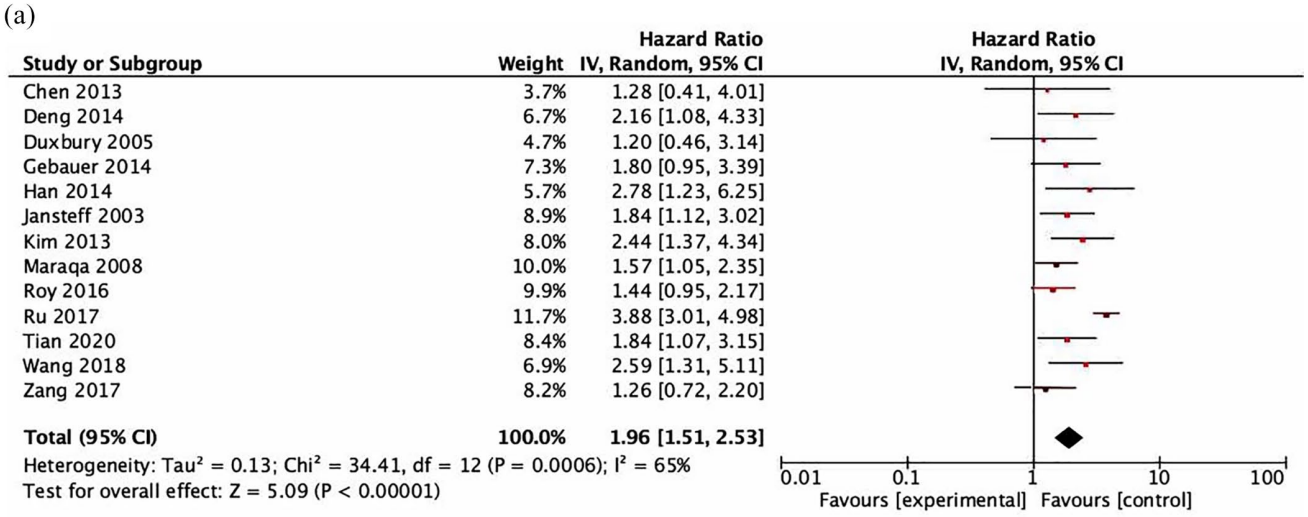
CEACAM6, carcinoembryonic antigen-related cell adhesion molecule 6; FITC, fluorescein-5-isothiocyanate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IHC, immunohistochemistry; RT-PCR, reverse transcription polymerase chain reaction; SI, staining index.

with a moderate risk of bias (HR = 1.65, 95% CI = 1.28–2.12; p for difference = 0.23).

Disease-free survival

Data for the association between CEACAM6 and DFS were reported or calculated in eight studies. CEACAM6 expression was associated with detrimental DFS (HR = 2.49, 95% CI = 2.01–3.07, $p < 0.001$, see Figure 3(a)). The test for heterogeneity was not significant (Cochran Q p = 0.16, I^2 = 34%). Sensitivity analysis excluding the two studies which did not use immunohistochemistry to assess DFS[21,56] showed similar results (HR = 2.79, 95% CI = 2.35–3.30, $p < 0.001$). The subgroup analysis showed that there were no significant differences between cancer type subgroups (subgroup difference p = 0.38, Figure 3(b)) or between methods used for the HR calculation of the different subgroups (subgroup difference p = 0.61, Supplemental Figure 2). There was no difference in the estimate for the association between CEACAM6 and DFS for studies with a low risk of bias (HR = 2.75, 95% CI = 2.23–3.39) and those with a moderate risk of bias (HR = 2.22, 95% CI = 1.42–3.47; p for difference = 0.40).

(a)



(b)

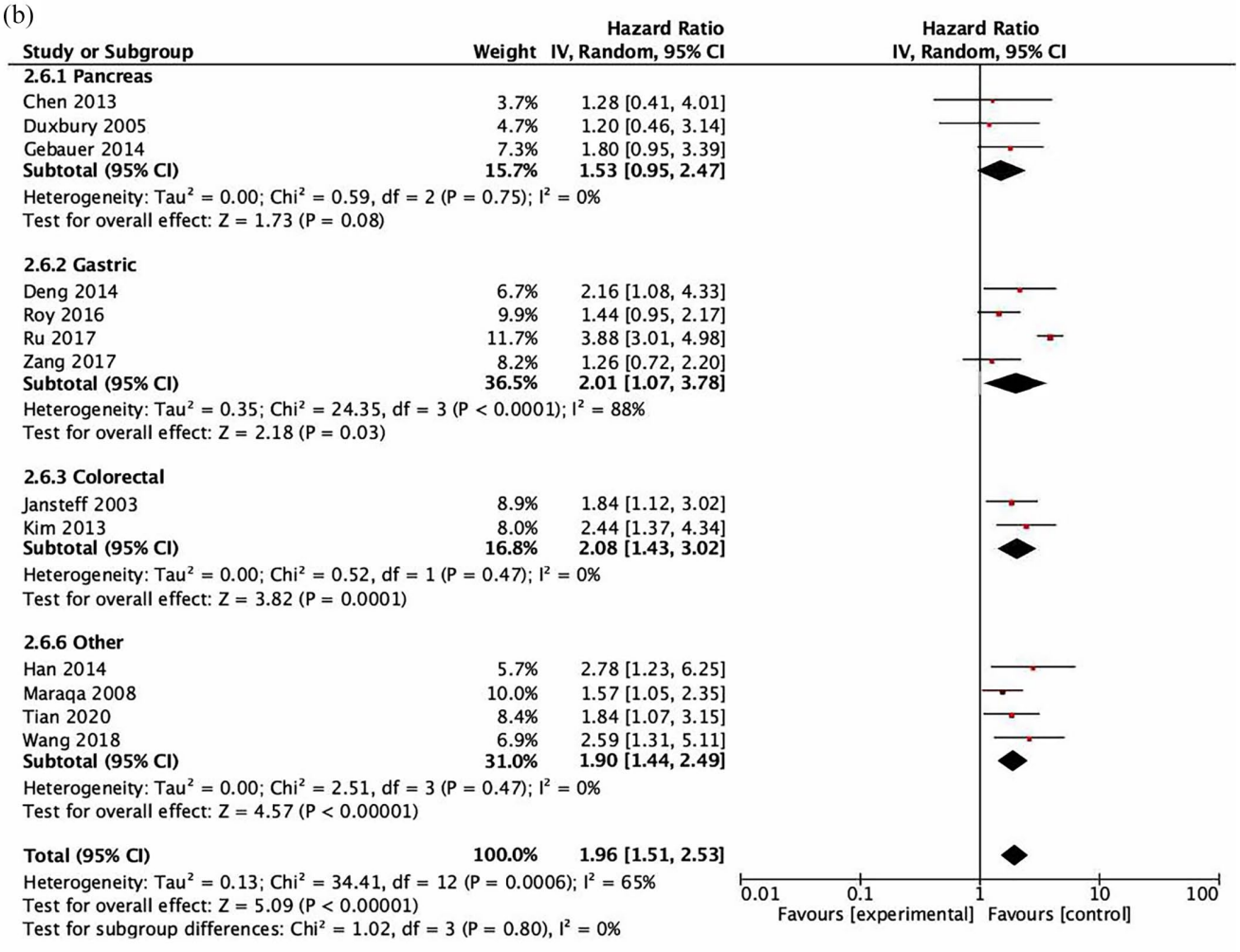


Figure 2. Forest plots showing hazard ratios for overall survival: CEACAM6 overall (a) and by subgroups based on disease site (b). Hazard ratios for each study are represented by squares: the size of the square represents the weight of the study in the meta-analysis; the horizontal line passing through the square represents the 95% confidence interval. All statistical tests were two-sided. The diamonds represent the estimated pooled effect. Test for overall effect based on z-test. All *p* values are two-sided. (a) CEACAM6 OS Overall. (b) CEACAM6 OS by disease site.

CI, confidence interval; OR, odds ratio.

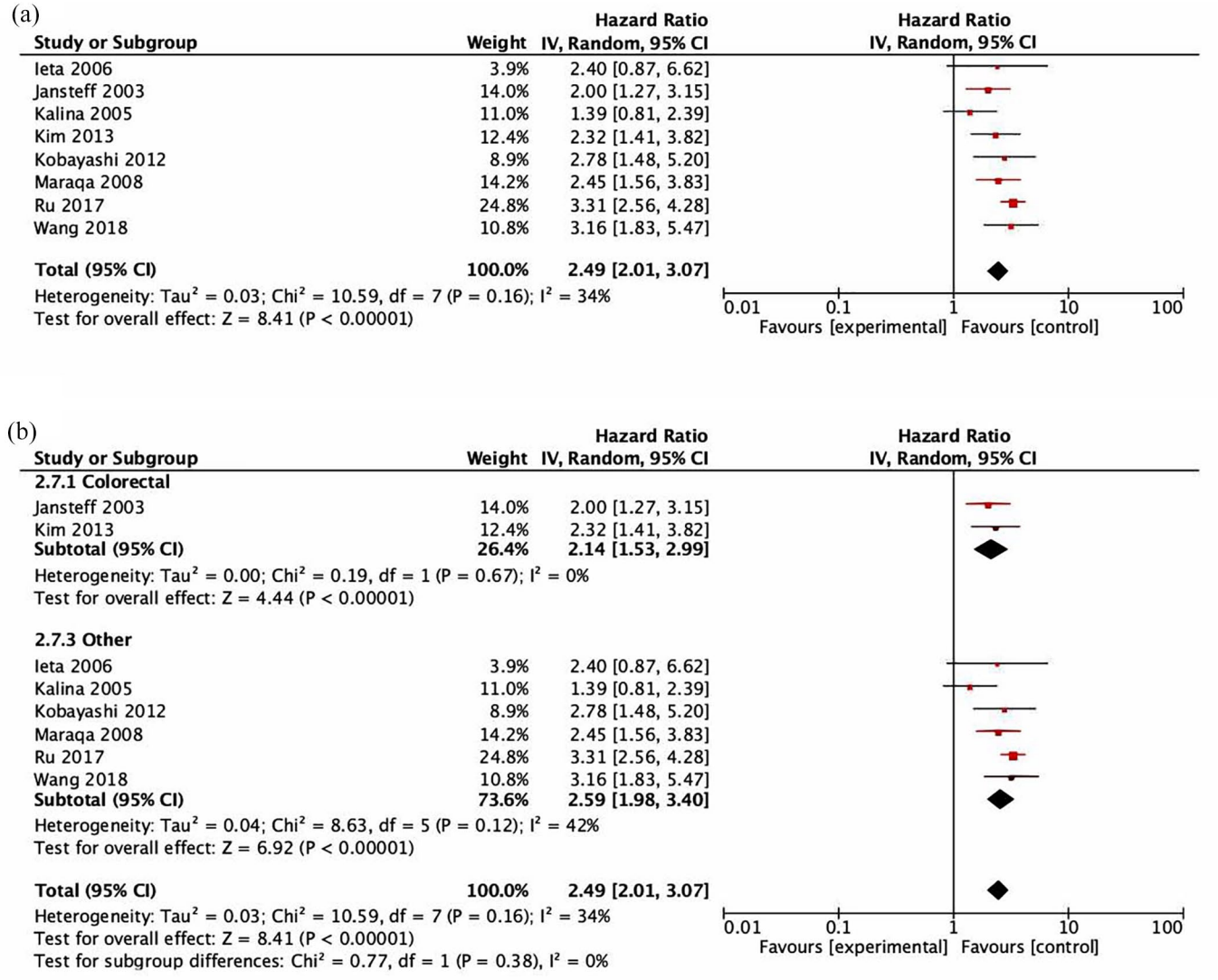


Figure 3. Forest plots showing hazard ratios for disease-free survival (DFS): CEACAM6 overall (a) and by subgroups based on disease site (b). Hazard ratios for each study are represented by squares: the size of the square represents the weight of the study in the meta-analysis; the horizontal line passing through the square represents the 95% confidence interval. All statistical tests were two-sided. The diamonds represent the estimated pooled effect. Test for overall effect based on z-test. All *p* values are two-sided. (a) CEACAM6 DFS overall. (b) CEACAM6 DFS by disease site.
CI, confidence interval; OR, odds ratio.

Discussion

In the present study, we describe the association of CEACAM6 expression with outcome in cancer by performing a meta-analysis of published data. Our results show a large association between the expression of this protein and detrimental survival across a wide range of malignancies.

CEACAM6, a carcinoembryonic antigen molecule, is a highly glycosylated protein from the immunoglobulin superfamily, principally expressed in the cellular membrane.[5] Its presence is observed in nontransformed tissues, and it has a key role in different functions from phagocytosis or signal transduction, to regulation of cell–cell recognition and adhesion.[8]

Data have linked the expression of this protein with the oncogenic transformation at different levels, from differentiation and migration, to cell proliferation and survival.[14] Overall, the CEACAM family of proteins have been involved in several functions related to cancer, and some members have been proposed to act as tumor suppressors[59] and others as oncogenes.[60] CEACAM5 is the only member of the CEA

family accepted as a tumor marker of recurrence in cancer patients,[13] and different clinical trials of chimeric antigen T cells (CAR-T) have used CEACAM5 as the target.[11] CEACAM6 has emerged as the most specific marker for a number of aggressive cancers,[10] as its expression is greater than CEACAM5 in many tumors.[20] CEACAM6 has been identified as a target candidate for CAR-T therapy[61] and recent data show efficacy of an anti-tumor CEACAM6 vaccine combined with PD-1 or PD-L1 inhibitors.[62] In addition, expression of CEACAM6 on cancer cells has been suggested as inhibitory of the immune response, and antibodies against CEACAM6 can boost the T-cell response, thereby potentially possessing activity consistent with checkpoint blockade.[29] In this context, a phase I clinical trial is ongoing using the anti-CEACAM6 antibody BAY1834942 in patients with advanced solid tumors.[63] Although this approach is novel, it shows great potential for clinical development especially if this family of antibodies can be combined with anti-PD-1 or PD-L1 inhibitors.[64]

Our results show that expression of CEACAM6 was associated with worse OS and DFS with all studies reporting effect sizes consistent with adverse outcome (albeit not always with statistical significance in each study). This resulted in statistical heterogeneity for the OS, but not DFS analysis. For our OS analysis, pancreatic cancer studies less frequently observed statistically significant associations with worse outcome despite similar effect size as other disease sites. This is likely explained by low statistical power; two out of three of these studies analyzed survival data from very few CEACAM6 negative patients (<10% for study population). For our DFS analysis, studies in intrahepatic cholangiocarcinoma and acute lymphoblastic leukemia also did not identify statistically significant results for CEACAM6 and worse outcome. Of note, these studies did not use immunohistochemistry (IHC) for the evaluation of CEACAM6, but utilized reverse transcription polymerase chain reaction (RT-PCR) or flow cytometry, respectively. This may explain this observation. However, among other studies, no significant differences were observed between IHC and RT-PCR suggesting that method of evaluation is not a sensitive variable for outcome assessment. Similarly, we did not identify differences between tumor types, although the number of cancer subtypes included in the analysis was limited. In addition, we did not observe differences between the methods used for data extraction. Similar magnitude of effect was observed for studies in which data were extracted directly and those in which data were estimated from survival plots. Finally, when we evaluated the quality of the studies, we observed that most studies showed low risk for bias reinforcing the confidence in our results.

These analyses have several important implications. CEACAM6 is associated with worse OS and DFS, suggesting its potential use as a target for therapeutic intervention. As a membrane-bound protein, the application of different immunotherapy strategies is of great value, and development of effective antibodies against CEACAM6 for the treatment of different tumors is ongoing.[24–29,61] In addition, it could be used as a prognostic biomarker and some studies have evaluated the prognostic value of CEACAM6 serum levels in patients with cancer.[65–67] However, our study aimed to explore the prognostic role of CEACAM6 in human cancers, rather than in blood. With the potential role of CEACAM6 as immune checkpoint inhibitor in tumors, we focused this evaluation only on primary cancers. The prognostic effect of serum CEACAM6 may be an indirect measure of tumor volume and assessment of this independently of disease burden is not possible with study summary data. Future studies of blood CEACAM6 would be of value.

This analysis has limitations that are intrinsic to the type of data available and the analysis performed. First, as a literature-based analysis, individual patient data were not available. As such, the analysis would be subject to publication bias and relied on summary data. While inspection of the funnel plot did not suggest substantial publication bias, the risk of residual bias cannot be excluded. Second, there is no accepted and validated method for assessment of CEACAM6 expression. Therefore, there may be substantial heterogeneity, which may not be fully accounted for by our use of random-effects modeling. Finally, HR were not reported in every study and had to be estimated in a number of studies. While subgroup analysis did not suggest a difference between methods of HR extraction, this may have introduced further heterogeneity to the reported results.

In conclusion, our analyses show that overexpression of CEACAM6 is associated with a worse OS and DFS in different tumor types. Given its role

in immune modulation, our data suggest that the development of strategies targeting this membrane protein could have potential for therapeutic benefit.

Author contributions

Miguel Burgos: Conceptualization; Formal analysis; Investigation; Methodology; Software; Validation; Visualization; Writing – original draft; Writing – review & editing.

Iván Cavero-Redondo: Methodology; Software; Supervision.

Celia Álvarez-Bueno: Methodology; Software.

Eva María Galán-Moya: Formal analysis; Validation.

Atanasio Pandiella: Supervision.

Eitan Amir: Formal analysis; Investigation; Methodology; Supervision; Validation; Writing – original draft; Writing – review & editing.

Alberto Ocaña: Conceptualization; Resources; Supervision; Visualization; Writing – original draft; Writing – review & editing.

Conflict of interest statement

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: AO is currently an employee of Symphogen, Copenhagen, Denmark. There is no conflict of interest to declare in relation to this article. EA declares person fees from Sandoz, Novartis and Exact Sciences outside of this work.

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Supplemental material

Supplemental material for this article is available online.

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