

Original Article

Clinical significance of B7-H4 expression in ovarian cancer: a meta-analysis of proportions and time-to-event survival outcomes

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Abstract



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B7-H4 is an immune-regulatory molecule increasingly recognized for its role in tumor progression and immune evasion in epithelial ovarian cancer. To clarify its clinical relevance, we conducted a systematic review and meta-analysis evaluating the prevalence of B7-H4 expression and its association with survival outcomes. Nineteen eligible studies were included, of which sixteen provided data on expression proportions and eight reported progression-free or overall survival outcomes. The pooled prevalence of high or positive B7-H4 expression was 73%, though with considerable inter-study variability. High B7-H4 expression was associated with a significantly increased risk of disease progression (pooled unadjusted hazard ratio: 1.43), while its relationship with overall survival remained inconclusive due to limited data. Despite methodological differences among studies, the findings suggest B7-H4 is overexpressed and potentially prognostic in ovarian cancer. Additional studies are required to validate its clinical utility in patient risk assessment and as a therapeutic target.

Keywords: B7-H4, Ovarian cancer, Prognosis, Survival, Meta-analysis.

1. Introduction

Epithelial ovarian cancer (EOC) remains one of the deadliest malignancies affecting women, representing the most lethal form among gynecologic cancers and ranking as the fourth leading cause of cancer-related deaths globally. The high case-fatality rate is primarily attributed to its insidious onset and lack of early specific symptoms, resulting in the majority of cases being diagnosed at advanced stages when the disease has already metastasized within the peritoneal cavity. This late detection substantially limits treatment options and adversely affects patient outcomes. According to global cancer statistics, ovarian cancer contributed to approximately 3.7% of all cancer cases and 4.7% of total cancer deaths in 2020, although

significant geographic variability exists in both incidence and mortality rates. Alarming, the global burden of ovarian cancer continues to rise, with an estimated 206,956 deaths reported in 2022, and projections indicating that this number could surpass 350,000 by the year 2050. [1–2]

Recent research has increasingly focused on immunoregulatory molecules involved in tumor progression, among which B7-H4, also known as B7x, B7S1, or V-set domain-containing T cell activation inhibitor 1 (VCN1), has emerged as a promising biomarker and potential therapeutic target. As a co-inhibitory ligand within the B7 family, B7-H4 is primarily expressed on antigen-presenting cells and is known to inhibit T-cell proliferation, cytokine secretion, and cytotoxic activity, thereby contri-

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buting to immune evasion mechanisms in cancer. Elevated expression of B7-H4 has been consistently observed in ovarian cancer tissues compared to non-malignant ovarian samples, and its presence in circulating serum samples has also been reported, suggesting its potential utility as a minimally invasive diagnostic or prognostic indicator. [3,4]

Although the complete functional role of B7-H4 in ovarian carcinogenesis is still under investigation, mounting evidence suggests that its upregulation may contribute to tumor immune escape and progression. Clinically, high B7-H4 expression levels have been correlated with adverse outcomes, including shortened progression-free survival (PFS) and overall survival (OS), which are critical endpoints used to evaluate therapeutic efficacy and long-term prognosis in oncology studies. In light of these findings, the present meta-analysis is conducted to systematically assess the prevalence of B7-H4 expression in ovarian cancer and to elucidate its association with survival outcomes, aiming to better define its prognostic value and potential relevance in clinical practice. In addition to the meta-analysis of proportions for elevated B7-H4 expression, we also performed a meta-analysis of time-to-event outcomes by pooling both unadjusted and adjusted hazard ratios (HRs) for PFS and OS in patients with ovarian cancer.

2. Material and Methods

This systematic review and meta-analysis were conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). [5] This study was registered with the International Prospective Register of Systematic Reviews: PROSPERO 2025 CRD420251074807. Grading of Recommendations Assessment, Development, and Evaluation (GRADE) methodology based on domains including 'study design', 'risk of bias', 'inconsistency', 'indirectness', and 'imprecision' was used to assess the certainty of evidence (GRADEpro, Version 20. McMaster University, 2014). [6]

2.1. Literature search and study selection

A comprehensive literature search was conducted across PubMed/MEDLINE, Scopus, and the Cochrane Central Register of Controlled Trials (CENTRAL), with the final update on June 15, 2025. The search combined MeSH terms and free-text keywords related to B7-H4 and ovarian cancer. The PubMed search strategy included terms such as "B7-H4," "B7x," "B7S1," and "VTCN1" in combination with "ovarian cancer" or "ovarian neoplasms." Equivalent search strategies were adapted for other databases. Reference lists of relevant studies and reviews were also hand-searched. Records were imported into a reference manager, and duplicates were removed. Titles and abstracts were screened, followed by full-text reviews using predefined criteria.

Inclusion criteria: (1) observational prospective or retrospective studies on women with epithelial ovarian cancer; (2) B7-H4 tissue expression assessed by immunohistochemistry, protein quantification, or mRNA analysis; (3) comparison between high/positive and low/negative B7-H4 expression groups; and (4) reporting of B7-H4 expression proportions or survival outcomes (PFS, OS) as hazard ratios. Eligible designs included cohort, observational, and randomized or non-randomized clinical trials. Exclusion criteria were non-ovarian cancers, lack of B7-H4 or survival data, case reports, reviews, abstracts, and prelini-

cal studies. Two reviewers independently conducted study selection, resolving discrepancies through discussion or third-party consultation.

2.2. Data extraction and risk of bias assessment

Data extraction was performed using a standardized form to collect relevant study characteristics, including first author, year of publication, country, type and stage of ovarian cancer, method used to assess B7-H4 expression, number of patients with high B7-H4 expression relative to the total study population (proportion), and time-to-event outcomes reported as hazard ratios (HRs). Where applicable, details on covariates adjusted for in multivariate analyses were also recorded. The quality of included studies was assessed using the Newcastle–Ottawa Scale (NOS), which evaluates three domains: selection, comparability, and outcome. Studies receiving a total score between 6 and 7 were considered to be of good to high quality. The literature search, title/abstract screening, full-text review, data extraction, and quality assessment were conducted independently by two reviewers. Any discrepancies were resolved through discussion, and where clarification was required, corresponding authors were contacted via email.

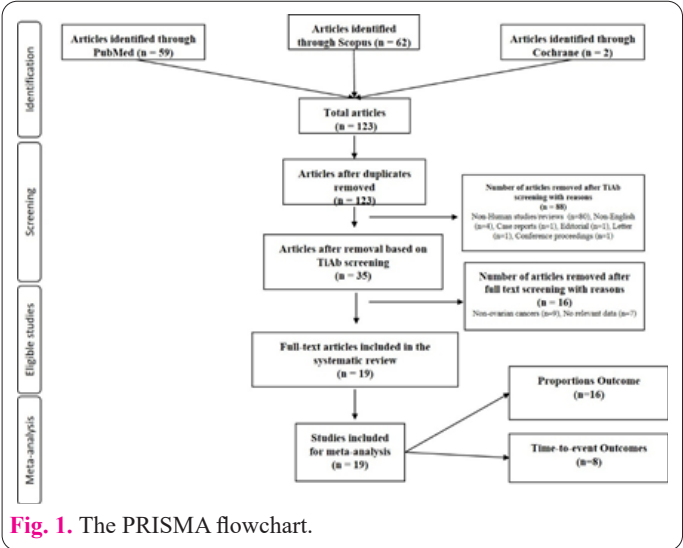
2.3. Data analysis

The meta-analysis comprised two components: pooled proportions of high B7-H4 expression and time-to-event outcomes reported as hazard ratios (HRs) for progression-free survival (PFS) and overall survival (OS). For proportion data, studies reporting the number of patients with high B7-H4 expression relative to the total were synthesized using a random-effects model by the DerSimonian–Laird method in OpenMeta[Analyst]. For time-to-event outcomes, both adjusted and unadjusted HRs for PFS and OS were pooled using a random-effects model with the inverse variance method in RevMan (version 5.4). Heterogeneity across studies was evaluated using the I^2 statistic and Cochran's Q test. Subgroup and sensitivity analyses were performed where applicable. To assess publication bias, funnel plot asymmetry was examined using rank correlation and regression tests. Statistical significance was set at a p-value < 0.05. To assess the certainty of the evidence, the GRADE (Grading of Recommendations, Assessment, Development, and Evaluation) approach was employed. This framework evaluates evidence quality based on several domains: risk of bias, inconsistency, indirectness, imprecision, and other considerations such as publication bias. Based on these criteria, the certainty of evidence was categorized as high, moderate, low, or very low.

3. Results

3.1. Study selection

A total of 123 records were identified through database searches (PubMed, Scopus, and Cochrane), out of which 19 studies met the eligibility criteria and were included in the systematic review. [7-25] The study selection process is illustrated in the PRISMA flow diagram (Fig. 1). Of these, 16 studies were included in the meta-analysis of B7-H4 expression proportions, while 8 studies were included in the meta-analysis of time-to-event outcomes. [11,12,17,20,23-25] With the overall NOS scores obtained for individual studies being between 5-8, the quality of the studies was assessed to be moderate to high. The characteristics of the studies included in this systematic review and



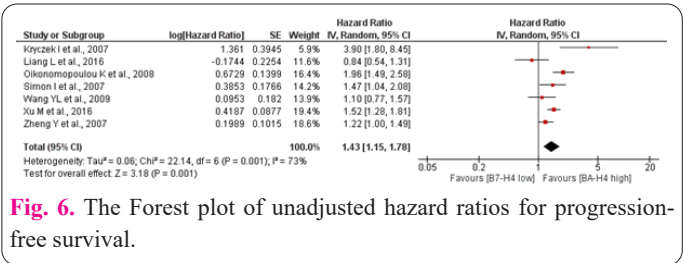
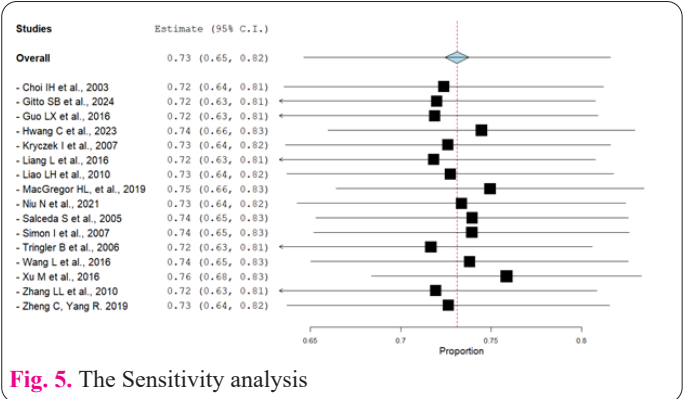
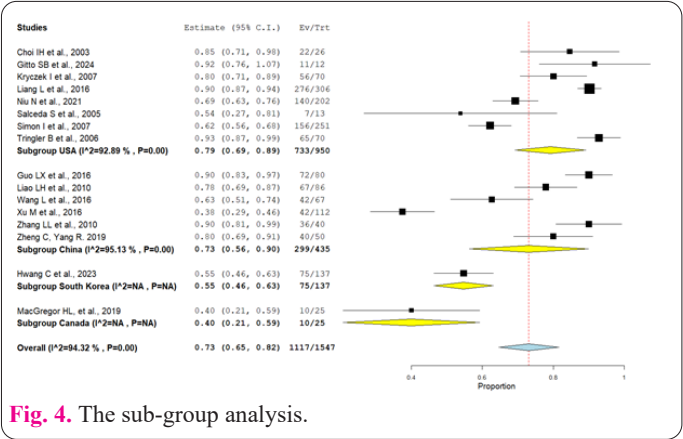
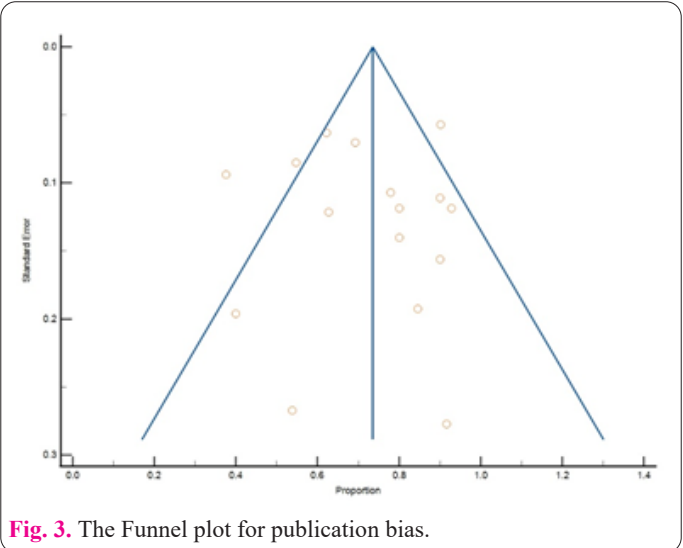
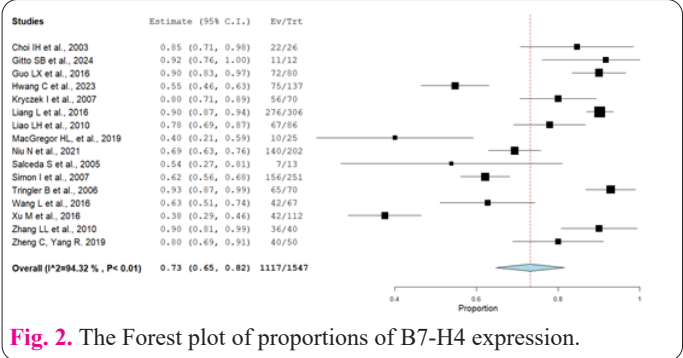
meta-analysis are summarized in Supplementary Table 1.

3.2. Proportion of B7-H4 expression in ovarian cancer

A meta-analysis of 16 studies comprising 1,547 patients demonstrated that the overall pooled proportion of high or positive B7-H4 expression in ovarian cancer tissues was 73% (95% CI: 0.65–0.82), with substantial heterogeneity across studies ($I^2 = 94.32\%$, $P < 0.01$) (Fig. 2). Funnel plot analysis, along with Egger’s test ($P = 0.92$) and Begg’s test ($P = 0.61$), indicated no significant evidence of publication bias or small-study effects (Fig. 3). Subgroup analysis based on geographical location showed the highest proportion of B7-H4 expression in studies from the United States (0.79; 95% CI: 0.69–0.89; $I^2 = 92.89\%$, $P < 0.01$), followed by studies from China (0.73; 95% CI: 0.56–0.90; $I^2 = 95.13\%$, $P < 0.01$), and South Korea (0.55; 95% CI: 0.46–0.63; single study). The lowest expression was observed in a Canadian cohort (0.40; 95% CI: 0.21–0.59; single study) (Fig. 4). Notably, both the U.S. and Chinese subgroups exhibited high heterogeneity, indicating that regional variation alone does not fully explain inter-study differences. Sensitivity analysis, conducted by omitting one study at a time, yielded consistent pooled estimates, confirming the robustness and reliability of the findings (Fig. 5).

3.3. Time-to-event survival outcomes

The impact of high B7-H4 expression on survival outcomes in epithelial ovarian cancer was evaluated through meta-analyses of both unadjusted and adjusted hazard ratios (HRs) for progression-free survival (PFS) and overall survival (OS). For PFS, six studies reporting unadjusted HRs showed a statistically significant association between high B7-H4 expression and worse PFS, with a pooled HR of 1.43 (95% CI: 1.15–1.78; $P = 0.001$; $I^2 = 73\%$) (Fig. 6).



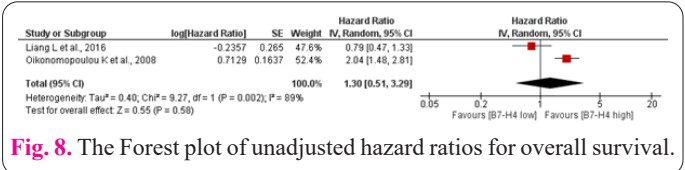
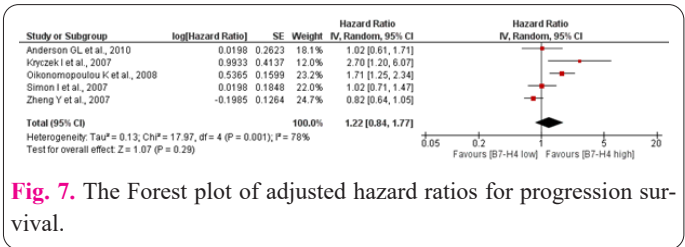
In contrast, the analysis of five studies reporting adjusted HRs for PFS revealed a non-significant association (HR = 1.22, 95% CI: 0.84–1.77; $P = 0.29$; $I^2 = 78\%$), suggesting attenuation of the effect after accounting for confounding variables (Fig. 7). For OS, two studies contributed unadjusted HRs, and the pooled estimate did not show statistical significance (HR = 1.30, 95% CI: 0.51–3.29; $P = 0.58$; $I^2 = 89\%$) (Fig. 8), reflecting high heterogeneity and uncertainty in the relationship between B7-H4 expression and overall survival outcomes.

3.4. Certainty of evidence

The certainty of evidence was assessed using the GRADE framework, which evaluates factors such as risk of bias, inconsistency, indirectness, imprecision, and publication bias. For the outcome related to the proportion of B7-H4 expression in epithelial ovarian cancer, the certainty was rated as moderate. This rating was based on the consistent direction of effect observed across studies, indicating a generally high prevalence of B7-H4 expression, despite considerable statistical heterogeneity. In contrast, the certainty of evidence for time-to-event survival outcomes, including both progression-free survival (PFS) and overall survival (OS), was rated as low to very low. This was primarily due to substantial inconsistency across studies, reflected by high heterogeneity (I^2 ranging from 73 to 89 percent), as well as imprecision in the pooled hazard ratio estimates, particularly in adjusted analyses and overall survival. Additionally, the limited number of studies reporting adjusted hazard ratios, along with variability in the covariates used for adjustment, further reduced confidence in the results. While the unadjusted analysis of PFS indicated a significant association with high B7-H4 expression, the lack of consistent and adequately adjusted data limited the strength of the overall conclusions regarding its prognostic significance. The GRADE summary of findings and the outcome-wise certainty of evidence assessment are presented in Table 1.

4. Discussion

In this meta-analysis, we found that B7-H4 is highly



expressed in ovarian cancer, with a pooled prevalence of approximately 73%. This is consistent with earlier reports of widespread tumor B7-H4: for example, Choi et al. found 85% (22/26) of ovarian adenocarcinomas were B7-H4-positive. [7] Zheng et al. reported that B7-H4 is highly overexpressed in primary ovarian tumors relative to normal tissue. [23] High-grade serous tumors in particular tend to be B7-H4-positive. Liang et al. found B7-H4 in 91% of high-grade serous cases. [12] In contrast, cohorts including lower-grade or mixed histologies sometimes show lower proportions: Xu et al. observed positive B7-H4 staining in only 37.5% of epithelial ovarian cancers. [20] This likely reflects differences in IHC scoring (e.g., tissue microarrays vs. full sections) and inclusion of benign or mucinous subtypes. Notably, B7-H4 expression is enriched in therapy-resistant disease. Niu et al. repor-

Table 1. The certainty of evidence assessment using GRADE approach for the dichotomous and time-to-event outcomes studies in this meta-analysis.

№ of studies	Certainty assessment						Effect			Certainty	Importance
	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	№ of events	№ of individuals	Rate (95% CI)		
B7-H4 Expression											
16	non-randomised studies	not serious	serious ^a	not serious	not serious	none	1117	1547	event rate 0.7% (0.65 to 0.82)	⊕⊕⊕○ Moderate ^a	CRITICAL
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Pooled HR	95% CI		Certainty	Importance
Progression-free survival (Unadjusted HR)											
7	non-randomised studies	not serious	serious ^b	not serious	not serious	publication bias strongly suspected ^c	1.43	1.15 to 1.78		⊕⊕○○ Low ^{b,c}	CRITICAL
Progression free survival (Adjusted HR)											
5	non-randomised studies	not serious	serious ^d	not serious	serious ^e	publication bias strongly suspected ^f	1.22	0.84 to 1.77		⊕○○○ Very low ^{d,e,f}	IMPOR-TANT
Overall Survival (Unadjusted HR)											
2	non-randomised studies	not serious	serious ^g	not serious	very serious ^h	publication bias strongly suspected ⁱ	1.30	0.51 to 3.29		⊕○○○ Very low ^{g,h,i}	NOT IMPOR-TANT

a. I-squared value of over 90%, b. I-squared value of 73%, c. As there are only 7 studies, funnel plot analysis for publication bias is inconclusive, d. I-squared value of 78%, e. the pooled estimate and its CI is clearly crossing the null line. In addition, 3 out of 5 individual studies are also touching the null line, f. Funnel plot analysis with 5 included studies was highly inconclusive, g. I-squared value of 89%, h. The pooled HR from 2 included studies is touching the null line and as the CI is very wide, it is Very Serious Imprecision, i. Only 2 included studies, not possible to assess for publication bias. However, based on the I-squared value, the publication bias is highly suspected.

ted B7-H4 in approximately 69% of platinum-resistant tumors. [15] Gitto et al. similarly found that B7-H4 was maintained in recurrent, drug-resistant high-grade serous ovarian cancer. [8] These findings suggest that patient selection (stage, grade, treatment setting) and diagnostic criteria (e.g., FIGO vs. WHO staging; IHC scoring systems) can influence the observed prevalence of B7-H4.

Our pooled unadjusted hazard ratio (HR) for progression-free survival (PFS) was 1.43, indicating that B7-H4-positive tumors carry a roughly 43% higher risk of progression than B7-H4-negative tumors. This estimate aligns with individual studies. For example, Oikonomopoulou et al. found that higher baseline serum B7-H4 predicted shorter PFS (adjusted HR 1.63). [25] Xu et al. reported a univariate HR for PFS of 1.52. [20] These results support the interpretation that B7-H4 expression portends modestly worse PFS. In contrast, our data for overall survival (OS) are limited. Only two studies reported OS hazard ratios: Oikonomopoulou et al. (adjusted HR 1.69) and Liang et al. (unadjusted HR 1.09). [12,25] Notably, only Oikonomopoulou adjusted for covariates such as age, FIGO stage, and chemotherapy response. [25] In practice, most studies used univariate or Kaplan–Meier survival analysis. For example, Hwang et al. observed no OS difference overall between B7-H4-positive and -negative cases, although they noted worse survival in patients with low tumor-infiltrating lymphocytes. [10] Kryczek et al. similarly found that tumor-cell B7-H4 did not significantly correlate with OS, but B7-H4 expression on tumor-associated macrophages was associated with markedly poorer survival. [11] These findings suggest that the prognostic impact of B7-H4 may depend on the tumor microenvironment and clinical setting.

Mechanistically and clinically, the B7-H4 literature underscores both the promise and complexity of this marker. Several studies associate B7-H4 positivity with advanced or aggressive disease. Liang et al. showed that higher B7-H4 scores were significantly associated with advanced tumor stage. [12] Niu et al. reported that B7-H4 co-expression with IDO1 correlated with chemotherapy resistance and worse survival in high-grade serous ovarian cancer. [15] Kryczek et al. highlighted the role of B7-H4-expressing macrophages in promoting regulatory T-cell expansion and immune suppression. [11] Importantly, B7-H4 also emerges as a viable therapeutic target. Gitto et al. demonstrated that a B7-H4-targeted antibody-drug conjugate induced tumor regression in a significant proportion of patient-derived xenograft models of ovarian cancer, including platinum- and PARPi-resistant tumors. [8] These findings support the continued exploration of B7-H4 as both a prognostic biomarker and a therapeutic target in ovarian cancer.

Zheng et al. showed that combining B7-H4 with kalikreins and CA125 improved prediction of recurrence and chemotherapy response in ovarian cancer, with prognostic utility after adjustment for clinical factors. [23] However, Anderson et al. found that serum B7-H4 levels did not rise prior to diagnosis in preclinical samples from the CARET trial, suggesting limited value for early detection. [24] Choi et al. identified B7-H4 as a tumor-specific immune checkpoint with minimal expression in normal tissue, supporting its role in immune evasion. [7] Gitto et al. further demonstrated that a B7-H4-targeted antibody-drug conjugate was effective in platinum-resistant ovarian cancer

models. [8] Together, these findings highlight B7-H4's potential as a prognostic biomarker and therapeutic target.

While this analysis consolidates critical findings, several limitations must be acknowledged. First, only a small number of studies reported multivariate-adjusted HRs, and these varied in covariates, including age, stage, chemotherapy response, regulatory T-cell infiltration, and tumor grade. Thus, our pooled HRs are largely unadjusted, and residual confounding is possible. Second, heterogeneity in B7-H4 quantification (e.g., different antibodies, scoring thresholds, and tissue vs. serum assays) likely contributed to variability in observed expression. Third, only two studies contributed OS HRs, limiting confidence in long-term outcome associations. [12,25] Additionally, most included studies were retrospective with modest sample sizes, and publication bias cannot be excluded. However, sensitivity analyses showed consistent pooled results after excluding individual studies, confirming the robustness of our findings. Importantly, this study has notable strengths. Sensitivity analyses confirmed the stability of results across subgroups and models. The use of the GRADE framework allowed for structured evaluation of evidence certainty. The outcome for B7-H4 expression prevalence was graded as moderate certainty, while the certainty for survival outcomes was rated as low to very low, primarily due to inconsistency and imprecision. These structured assessments increase the interpretability and reliability of our conclusions.

In conclusion, B7-H4 is overexpressed in ovarian cancer and is associated with a significantly increased risk of disease progression. While evidence on overall survival is limited, the consistent prevalence and its association with chemoresistance, immune evasion, and poor prognosis suggest that B7-H4 holds promise as both a prognostic marker and a therapeutic target. Future prospective studies should aim for standardized diagnostic criteria, consistent adjustment in survival analyses, and clinical validation of B7-H4-directed therapies to clarify its role in precision oncology.

Conflict of interest

The authors have no conflicts with any step of the article preparation.

Consent for publications

The authors read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were directly used in the present research.

Informed consent

The authors declare that no patients were directly used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

The conceptualization of the study was done by SRV, SS, PB, SV, and PG. Data curation, analysis, methodology, and software development were collaboratively undertaken by SRV, ML, SK, SKM, and PB. Supervision and

validation responsibilities were managed by SS, PG, SV and SRV. The original draft of the manuscript was written by SRV, PB, ML, and SS, while the review and editing process involved contributions from SV, PG, and SK. All the co-authors listed with the Department of Pathology affiliation, AIIMS, Deoghar, have equally contributed to updating this work, contributing valuable inputs and critical review comments during their participation in the “Internal Capacity Building Workshop (June 2025)” as part of the DHR-TRC-funded SMART Centre’s BRICS Initiative. All authors approved the final manuscript.

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