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Combined Cancer Stem Cell Signatures Improve Recurrence Stratification Compared with Single Biomarkers in Advanced Epithelial Ovarian Cancer

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Abstract
Background: The prognostic value of individual cancer stem cell (CSC) markers in epithelial ovarian cancer (EOC) remains inconsistent. The present study compared combined CSC signatures with individual markers for prognostic stratification in advanced EOC.
Method: This retrospective–prospective cohort study included 114 patients with stage II–IV EOC at a tertiary referral hospital in Indonesia. Progression-free survival (PFS) and recurrence were assessed over 24 months. Immunohistochemical expression of cluster of differentiation 24 (CD24), CD44, CD133, CD117, and aldehyde dehydrogenase (ALDH) was evaluated using semiquantitative histochemical scoring. Receiver operating characteristic (ROC) analysis, Kaplan–Meier analysis with log-rank testing, and Cox proportional hazards regression were performed using Statistical Package for the Social Sciences (SPSS) version 25, with $P < 0.05$ considered statistically significant.
Results: Individual markers showed poor discrimination for recurrence, with areas under the curve (AUCs) ranging from 0.469 to 0.578. The five-marker signature also showed poor ROC discrimination (AUC = 0.518; $P = 0.748$), but stratified 2-year PFS differed between the low- and high-expression groups (76.9 vs. 29.7%; $P = 0.009$). The five-marker signature was associated with recurrence (hazard ratio (HR) = 8.985; 95% confidence interval (CI) = 1.879–42.978; $P = 0.006$), whereas the CD117–ALDH combination was associated with a lower recurrence hazard (HR = 0.394; 95% CI = 0.198–0.784; $P = 0.008$).
Conclusion: Individual CSC markers had limited prognostic discrimination. The five-marker signature was associated with PFS and recurrence, but its discriminatory accuracy remained poor. These exploratory findings require validation in larger prospective cohorts.

Keywords: Biomarker, Neoplastic stem cells, Ovarian neoplasms; Progression-free survival, Tumor recurrence

Introduction

Epithelial ovarian cancer (EOC), the deadliest gynecologic malignancy worldwide, is still a major contributor to cancer-related mortality in women. According to Global Cancer Statistics

2024, EOC shows an aggressive biological behaviour and is mostly diagnosed at an advanced stage.[1] Recurrence is the main cause of treatment failure and death in advanced EOC. However, substantial progress has been made in cytoreductive surgery, platinum-based chemotherapy, targeted therapy, and maintenance treatment strategies. Most patients initially respond to platinum-based chemotherapy. However, the majority develop recurrent and chemoresistant disease, resulting in poor long-term survival outcomes.[1] Increasingly, the recurrence of ovarian cancer (OC) is appreciated as a result of extensive intratumoral heterogeneity and the presence of therapy-resistant cellular subpopulations. Conventional clinicopathologic prognostic factors, such as the International Federation of Gynecology and Obstetrics (FIGO) stage, residual tumor burden, histologic subtype, and serum biomarkers, often cannot account for differences in recurrence patterns and treatment responses among patients with similar disease features. Thus, there has been growing interest in identifying biologically relevant prognostic biomarkers that more accurately reflect tumor aggressiveness, therapeutic resistance, and recurrence risk.[2]

Cancer stem cells (CSCs) have emerged as one of the most interesting biological concepts in OC progression, as they are thought to be responsible for tumor initiation, metastasis, chemoresistance, and disease relapse. CSCs are a highly tumorigenic subpopulation with self-renewal capacity, multilineage differentiation potential, and resistance to chemotherapy-induced apoptosis. Growing evidence suggests that CSCs can survive conventional treatment, repopulate the tumor microenvironment, and contribute to recurrent disease after initial therapeutic response. In addition, CSC populations are characterized by high phenotypic plasticity and dynamic crosstalk with epithelial–mesenchymal transition (EMT) pathways, hypoxia signaling, metabolic reprogramming, and immune modulation, which contribute to therapeutic resistance and tumor progression.[3-6]

In OC, several CSC-associated biomarkers have been identified, including CD24, CD44, CD133, CD117, and aldehyde dehydrogenase (ALDH). These markers have been associated with several biological processes related to stemness, including tumor initiation, invasion, adhesion, oxidative stress resistance, detoxification, and platinum resistance. High expression of single CSC markers correlates with poor prognosis, shorter progression-free survival (PFS), and higher risk of recurrence in OC.[7] In particular, ALDH activity has been associated with maintenance of stemness and resistance to cytotoxic therapy, while CD117 expression has been associated with resistance to chemotherapy and aggressive tumor behaviour.[7-9] However, studies have shown inconsistent prognostic performance of a single CSC biomarker. OC stemness is increasingly recognized as a multidimensional and heterogeneous biological phenomenon that single biomarkers may not fully explain. Populations of CSCs exist in heterogeneous functional states shaped by treatment pressure and microenvironmental influences, resulting in wide variability in marker expression and prognostic relevance. Therefore, using a single CSC marker may not be sufficient to understand the complexities of tumor stemness and therapy resistance fully.[10-12]

Recent evidence indicates that combining CSC signatures with multiple biological pathways associated with recurrence and chemoresistance may yield better prognostic discrimination than single-marker approaches.[13] Multimarker CSC profiling may better represent the heterogeneity of OC stemness and improve identification of patients with highly aggressive and therapy-resistant disease phenotypes. However, evidence from comparative studies assessing the prognostic value of combined CSC

signatures versus single markers in advanced EOC remains inadequate. The present study aimed to evaluate the prognostic value of single versus combined CSC signatures for recurrence and PFS in patients with advanced EOC.

Methods

Study design and setting

The study was conducted at the Gynecologic Oncology Clinic, Department of Obstetrics and Gynecology, Prof. Dr. I.G.N.G. Ngoerah General Hospital. Immunohistochemical sample processing was performed in cooperation with the Departments of Anatomical Pathology, Prof. Dr. I.G.N.G. Ngoerah General Hospital and Dr. Cipto Mangunkusumo National Central General Hospital. The institutional Ethics Committee approved this study under approval number No. YR.02.01/D.IX.1.27/423/2024; approval date: December 11, 2024. The present study was conducted in accordance with the Declaration of Helsinki.

This retrospective–prospective cohort study was conducted from December 11, 2024 to April 30, 2026, following ethical approval obtained on December 11, 2024. The retrospective phase comprised patients diagnosed and treated during the preceding two-year period, from May 1, 2022 to April 30, 2024. Baseline demographic, clinicopathological, treatment, and tumor tissue data were extracted from medical records over a six-month period from December 2024 to May 2025. CSC marker expression was subsequently evaluated by immunohistochemistry on archived tumor specimens between June 1 and October 31, 2025. Following completion of retrospective data collection, patients were prospectively followed for clinical outcome subsequently updated disease progression and verified through April 30, 2026. For the present analysis, PFS and recurrence status were assessed over a predefined follow-up period of up to 24 months after cytoreductive surgery.

All 114 patients were drawn from this single cohort, with retrospectively collected baseline data linked prospectively to survival outcomes; no patients were enrolled as a separate group. No randomization was performed because this was an observational cohort study, and patient management was determined by standard clinical practice rather than by the investigators.

Patient demographics

Our study included 114 patients with EOC stage II or higher according to FIGO classification,[13] histopathologically confirmed at Prof. Dr. I.G.N.G. Ngoerah General Hospital. Participants: Eligible patients had cytoreductive laparotomy followed by standard first-line chemotherapy with carboplatin and paclitaxel. Postoperative residual tumour < 1cm. Before enrollment, each subject provided written informed consent. All patients underwent cytoreductive surgery followed by standard first-line carboplatin–paclitaxel chemotherapy. During the study period, there is no patients received maintenance therapy. The exclusion criteria were non-ovarian primary tumors, progressive disease during chemotherapy, and inadequate or damaged paraffin block specimens that were not suitable for evaluation.

Immunohistochemical study

Tumor tissue specimens were cut into 3–4 μm sections using a microtome and processed according to routine immunohistochemical staining protocols for CD117, CD133, CD24, CD44, and ALDH. For each block of paraffin processed with specimens from the study, positive and negative controls were performed. Positive controls were validated against reference images on antibody datasheets, and negative controls were performed without primary antibody staining. Microscopic analysis was performed using a light microscope and ImageJ software. Staining patterns were compared with antibody datasheets and Protein Atlas references for CD117,

CD133, CD24, CD44, and ALDH. All slides were examined by the anatomical pathologists from the Department of Anatomical Pathology, Dr. Cipto Mangunkusumo National Central General Hospital. Immunohistochemical (IHC) scoring was performed by pathologists blinded to patients' clinicopathological data and to recurrence/survival outcomes. CD117, CD133, CD24, CD44 and ALDH expression were graded semiquantitatively. The staining intensity was graded as 0 (absent), 1 (weak), 2 (moderate) and 3 (strong).

The following formula was used for the calculation of the histochemical scoring (H-Score):

H-Score = (% of cells with intensity 0 × 0) + (% of cells with intensity 1 × 1) + (% of cells with intensity 2 × 2) + (% of cells with intensity 3 × 3)

For each patient, the five-marker combined CSC score was calculated as the sum of the individual H-scores of the five biomarkers, according to the following formula:

Five-marker combined score = H-score(CD24) + H-score(CD44) + H-score(CD133) + H-score(CD117) + H-score(ALDH)

The resulting continuous combined score was subjected to receiver operating characteristic curve analysis. The receiver operating characteristic (ROC)-derived cut-off was subsequently used to classify patients into low- and high-combined-expression groups for Kaplan–Meier and Cox proportional hazards analyses. The primary antibodies used for immunohistochemical analysis, including their clones, manufacturers, countries of origin, catalogue numbers, and working dilutions, are summarized in Table 1.

Sample size

The minimum required sample size was calculated separately for four biomarkers with previously reported expression proportions in advanced-stage EOC (CD24, CD44, CD133, and CD117), using the formula for comparing two independent proportions with unequal group allocation:

$$N = [Z\alpha\sqrt{\{P(1-P)(1/q1 + 1/q0)\}} + Z\beta\sqrt{\{P1(1-P1)(1/q1) + P0(1-P0)(1/q0)\}}]^2 / (P1 - P0)^2$$

where $Z\alpha = 1.96$ (two-sided $\alpha = 0.05$), $Z\beta = 0.84$ (power = 80%), P0 = baseline (low-expression) recurrence proportion, P1 = recurrence proportion in the high-expression group, q1/q0 = the proportion of subjects allocated to the high- and low-expression groups, respectively.

Previously reported proportions were P0 = 0.30, P1 = 0.60, q1 = 0.60, and q0 = 0.40, giving an overall proportion P = 0.48 and a minimum required sample size of 103 subjects. A total of 114 eligible patients met the inclusion criteria and were included in the final analysis, exceeding the calculated minimum sample size.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 25 (IBM Corp., Armonk, NY, USA). Categorical variables are presented as frequencies and percentages, and continuous variables as mean ± standard deviation or median values with interquartile ranges, as appropriate. ROC curve analysis was used to evaluate the discriminatory ability of individual, dual-marker, and five-marker CSC scores for recurrence. For each biomarker panel, the area under the ccurve (AUC), sensitivity, specificity, cut-off value, and Youden index were calculated. Kaplan–Meier survival analysis with log-rank testing was used to compare 2-year PFS, and Cox proportional hazards regression was used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs). Statistical significance was defined as $P < 0.05$. No formal adjustment for multiple comparisons was applied because the biomarker-combination analyses were

exploratory; therefore, statistically significant findings from these analyses should be interpreted as hypothesis-generating.

The optimal cut-off value for each individual and combined CSC score was determined using receiver operating characteristic curve analysis based on the Youden index. The same ROC-derived cut-off values were then consistently applied to dichotomize patients into low- and high-expression groups for Kaplan–Meier survival analysis and Cox proportional hazards regression. Internal validation of the biomarker models using bootstrapping or cross-validation was not performed in the present analysis. Accordingly, the reported model performance estimates were derived from the original study cohort without resampling-based internal validation.

Results

The AUC values of the individual markers ranged from 0.469 to 0.578, indicating poor discriminatory ability, with values close to 0.5. All *P* values were > 0.05, indicating that none of the individual markers significantly discriminated recurrence status. CD24 had the numerically highest AUC among individual markers (AUC = 0.578; Youden index = 0.137; cut-off = 84.17; sensitivity = 66.2%; specificity = 47.5%), but its discriminatory performance remained limited.

Among dual-marker combinations, CD133–CD117 had the numerically highest AUC (0.582; Youden index = 0.155; cut-off = 124.00), with high sensitivity (90.5%) but low specificity (25.0%). The five-marker combined score had high sensitivity (95.9%) but low specificity (25.0%), with an AUC of 0.518 (P = 0.748). Thus, neither the dual-marker combinations nor the five-marker panel demonstrated strong discriminatory accuracy for recurrence by ROC analysis. In summary, ROC analysis did not demonstrate significant discriminatory ability for recurrence for either individual or combined CSC markers. The combined signatures should therefore not be interpreted as validated predictive classifiers on the basis of ROC performance alone.

As shown in Table 2, patients with advanced stages of disease, particularly stage IV (100%), had higher recurrence rates than stage III tumors (78.3%) and stage II disease (34.2%). Mucinous ovarian carcinoma and high-grade serous ovarian carcinoma had the highest recurrence rates among histopathological subtypes (81.8% and 78.7%, respectively), and low-grade serous ovarian carcinoma had the lowest (33.3%). The recurrence rate was highest in grade 2 tumors (80%), followed by grade 3 (65%) and grade 1 (55.6%) based on tumor differentiation. The recurrence rate was similar in patients with positive and negative lymphovascular space invasion.

In general, low expression of CSC markers correlated with lower recurrence rates than high expression. Recurrence occurred in 71.7% of patients with high CD24 expression compared with 57.4% in the low CD24 expression group. Similar trends were observed for CD44 (68.0 vs. 47.1%), CD133 (71.1 vs. 61.8%), CD117 (71.4 vs. 60.0%), and ALDH (70.0 vs. 63.8%). Taken together, these findings indicate a general trend toward increased recurrence in tumors with high expression of CSC markers, but the extent of difference was marker-dependent.

In time-to-event analysis, the five-marker combined signature significantly stratified 2-year PFS. The low combined-expression group had a higher 2-year PFS than the high-expression group (76.9% vs. 29.7%; log-rank *P* = 0.009). This significant survival stratification should be distinguished from the poor discriminatory performance observed in ROC analysis.

Among the 114 patients, 13 (11.4%) were classified into the low five-marker expression group and 101 (88.6%) into the high-expression group. During the 24-month follow-up, recurrence/progression occurred in 3 of 13 patients (23.1%) in the

low-expression group compared with 71 of 101 patients (70.3%) in the high-expression group. The corresponding 2-year PFS rates were 76.9% and 29.7%, respectively (log-rank $P = 0.009$). None of the 114 patients received maintenance therapy during the study period. Therefore, maintenance treatment was unlikely to confound the observed association between CSC expression and PFS. The overall AUC values of the markers from Table 3 ranged from 0.469 to 0.582, indicating poor discrimination performance, with most values close to 0.5. All *P*-values were > 0.05 indicating that no markers significantly discriminated between outcome groups. Among all single biomarkers, CD24 showed the best performance, with an AUC of 0.578 and a Youden index of 0.137 at a cut-off value of 84.17, yielding a sensitivity of 66.2% and a specificity of 47.5%. The results showed that CD24 had slightly better predictive power than the other single markers, but its diagnostic performance remained limited. The AUC value for CD133 was 0.543, with sensitivity (53.2%) and specificity (60.0%). CD44 had high sensitivity (89.6%) with a cut-off of 65.10, but low specificity (22.5%), performing better at identifying positive cases but with limited ability to exclude negative cases. ALDH was highly specific (85.0%) and had very low sensitivity (18.2%), and was more useful in identifying non-recurrent patients than in detecting recurrence.

The best performance was obtained by the dual combination of CD133–CD117 with an AUC of 0.582 and a Youden index of 0.155 at a cut-off value of 124.00. The combination showed high sensitivity (90.5%) and low specificity (25.0%), suggesting relatively good screening performance but poor overall accuracy. The combinations of CD24–CD44 and ALDH–CD133 showed moderate performance with AUCs of 0.549 and 0.496, respectively. However, the ALDH–CD117 and ALDH–CD44 combinations had low discrimination power with the Youden indices close to zero. The combined variable of all CSC markers had the highest sensitivity (95.9%) at a cut-off value of 321.43, but the specificity was low (25.0%). The Youden index was the highest (0.209), but the overall AUC was only 0.518 ($P = 0.748$). This indicates that the predictive performance was poor and statistically insignificant.

This study demonstrated that individual CSC markers had limited prognostic use for recurrence in advanced EOC. Although the five-marker combined signature showed poor discriminatory ability in ROC analysis (AUC = 0.518; $P = 0.748$), it significantly stratified 2-year PFS, with better outcomes in patients with low combined expression than in those with high combined expression (76.9% vs. 29.7%; $P = 0.009$). The five-marker signature was also associated with recurrence in Cox regression analysis (HR = 8.985; 95% CI = 1.879–42.978; $P = 0.006$), although the wide CI indicates substantial uncertainty in the magnitude of this association. No internal validation using bootstrapping or cross-validation was performed; therefore, the reported estimates represent apparent performance within the original cohort and should not be interpreted as internally validated estimates. The CD117–ALDH combination was associated with a lower recurrence hazard (HR = 0.394; 95% CI = 0.198–0.784; $P = 0.008$); however, this should not be interpreted as evidence of a biologically protective effect. Taken together, these findings suggest that combined CSC expression may provide biological and survival-related stratification, but its ability to discriminate recurrence at the individual-patient level remains limited.

Table 4 presents the Kaplan–Meier survival analysis of single, dual, and multipanel combined CSC markers in relation to 2-year PFS in advanced EOC. Among the single CSC markers, Patients with low CD44 expression had higher 2-year PFS than those with high expression (52.9% vs. 32%), although the difference

was not statistically significant ($P = 0.179$). Similar trends were observed for CD24, CD133, and CD117, in which low-expression groups consistently showed better 2-year PFS rates than high-expression groups; however, none reached statistical significance (all $P > 0.05$). ALDH expression showed minimal difference between low and high groups (36.2% vs. 30.0%; $P = 0.804$).

The inverse association observed for the CD117–ALDH combination should be interpreted cautiously. The apparently discordant direction of association compared with the five-marker signature may reflect differences in the tumor subpopulations captured by specific marker combinations, data-driven dichotomization, and statistical instability related to the limited sample size and multiple exploratory comparisons. Therefore, the CD117–ALDH finding should be regarded as hypothesis-generating until replicated in an independent cohort.

The dual combination CSC marker analyses discriminated survival better than single-marker analyses alone. The low-expression group of CD133–CD117 had significantly better 2-year PFS than the high-expression group (58.8% vs. 30.9%) with values approaching statistical significance ($P = 0.076$). Likewise, ALDH–CD133, CD24–CD44, and ALDH–CD44 combinations showed a trend toward better survival in low-expression groups, but these associations remained nonsignificant ($P > 0.05$).

Significantly, the combination model with the five markers demonstrated the best prognostic performance. The 2-year PFS was markedly greater in the low combined-expression group than in those in the high-expression group (76.9% vs. 29.7%), and this difference was significant in the Kaplan–Meier analysis ($P = 0.009$). These findings provide evidence that the prognostic value of single CSC markers is limited. In contrast, combined CSC marker profiling may better stratify the risk of recurrence in advanced EOC.

As shown in Figure 1, most individual CSC markers were not significantly associated with recurrence, as indicated by CIs crossing 1 and *P*-values > 0.05. CD24, CD133, CD117, and ALDH trended toward an increased risk of recurrence, and CD44 trended toward a reduced recurrence risk, but none of these differences were statistically significant. Among the dual-marker combinations, only CD117–ALDH was significantly associated with a decreased risk of recurrence (HR = 0.394; 95% CI = 0.198–0.784; $P = 0.008$). By contrast, the five-panel marker combination model was significantly associated with the likelihood of recurrence (HR = 8.985; 95% CI = 1.879–42.978; $P = 0.006$). In general, the results indicate limited prognostic value for individual CSC markers but higher prognostic relevance for selected combined signatures in EOC.

Discussion

In this cohort of 114 patients with advanced epithelial ovarian cancer, individual cancer stem cell markers showed limited discrimination for recurrence, with area under the curve values of 0.469–0.578. High expression of CD24, CD44, CD133, CD117, and aldehyde dehydrogenase generally corresponded to higher recurrence rates and shorter progression-free survival, although most individual-marker associations were not statistically significant. In contrast, the five-marker combined signature significantly stratified 2-year progression-free survival, which was 76.9% in the low-expression group and 29.7% in the high-expression group ($P = 0.009$), and the high-expression signature was associated with a higher recurrence hazard (HR = 8.985; 95% CI = 1.879–42.978; $P = 0.006$). The CD117–aldehyde dehydrogenase combination was associated with a lower recurrence hazard (HR = 0.394; 95% CI = 0.198–0.784; $P = 0.008$), although this discordant finding should be

considered exploratory. Overall, combined cancer stem cell expression provided stronger survival-related stratification than individual biomarkers in this cohort.

The limited prognostic performance of individual CSC markers is biologically consistent with the plasticity and intratumoral heterogeneity of ovarian CSCs. CSC phenotypes are dynamically modulated by chemotherapy exposure, hypoxia, metabolic reprogramming, and immune modulation.[7,16,17] Individual biomarkers may therefore be insufficient to represent the interdependent stemness-related pathways driving tumor relapse. Functional overlap has been described among CSC-related pathways, including epithelial–mesenchymal transition, oxidative phosphorylation, Wnt/β-catenin signaling, and transcriptional regulation of stemness.[18-20] CD117 has been associated with tumor initiation, aggressive behavior, and chemoresistance,[9] whereas ALDH contributes to oxidative stress adaptation and platinum resistance.[8] CD44 and CD24 are involved in adhesion, migration, epithelial–mesenchymal transition, and metastatic dissemination, while CD133 has been associated with tumor-initiating capacity and recurrence potential.[10,11,21] These observations support our finding that no single marker adequately captured recurrence risk.

The significant survival stratification observed with the five-marker signature suggests that integrated CSC profiling may better reflect the multidimensional biology of recurrence than single-marker assessment. The conceptual framework in Figure 2 emphasizes that recurrence in advanced epithelial ovarian cancer is influenced not only by tumor burden and disease stage but also by intrinsic stemness biology and intratumoral heterogeneity. This interpretation is consistent with the concept that simultaneous activation of several stemness-related pathways may characterize more aggressive and therapy-resistant tumor phenotypes. Thus, the five-marker signature may serve as a broader biological summary of CSC-related heterogeneity, although its poor ROC discrimination precludes interpretation as a validated individual-patient classifier.

The association between high combined CSC expression and shorter progression-free survival is also consistent with emerging precision-oncology models that recognize CSC-associated pathways as potential therapeutic targets, particularly in recurrent and platinum-resistant disease.[21-23] ALDH signaling, Wnt/β-catenin activation, mitochondrial oxidative phosphorylation, and epithelial–mesenchymal transition-related pathways are being investigated as strategies to suppress CSC survival and overcome chemoresistance.[6,17,24] Interactions between ovarian CSCs and immune cells, cancer-associated fibroblasts, extracellular vesicles, and hypoxic signaling networks further support recurrence as a biologically multidimensional process.[19,24] Collectively, these data provide biological context for the survival separation observed with the combined CSC signature in our cohort.

The potential clinical relevance of CSC-based stratification should nevertheless be interpreted cautiously. Patients with high-risk CSC signatures could, in future studies, be evaluated for intensified surveillance, closer radiologic monitoring, or CSC-directed therapeutic approaches, whereas lower-risk groups might avoid unnecessary intervention. However, because the five-marker panel showed poor ROC discrimination and has not undergone internal or external validation, it should not currently guide clinical management. Thus, it is primarily an exploratory prognostic signature for further validation.

Our study has several strengths. ROC, Kaplan–Meier, and Cox regression analyses were applied to multiple CSC biomarkers using single-, dual-, and multimarker approaches, and standardized semiquantitative immunohistochemical H-score evaluation was used consistently for

clinically relevant outcomes, including recurrence and 2-year progression-free survival. Several limitations should also be acknowledged. The single-center design and relatively small sample size may limit statistical power and generalizability. The wide confidence interval around the hazard ratio of the five-marker panel indicates considerable imprecision, likely related to the limited sample size and uneven group and event distribution. Multiple individual and combined biomarker comparisons were evaluated without formal multiplicity adjustment, increasing the possibility of type I error. Immunohistochemistry is semiquantitative and may not fully capture the functional activity or spatial heterogeneity of CSC populations. Molecular profiling, transcriptomic analysis, BRCA mutation status, and homologous recombination deficiency status were not evaluated. In addition, only baseline CSC expression was assessed, without longitudinal evaluation during chemotherapy or at recurrence. Accordingly, the findings should be considered exploratory and hypothesis-generating.

The Cox regression analyses were also not comprehensively adjusted for conventional clinicopathological prognostic factors. Given the limited sample size and number of events, inclusion of numerous clinicopathological covariates together with multiple biomarker variables could have resulted in model instability and overfitting. Therefore, the observed associations should not be interpreted as demonstrating independent prognostic effects. Pairwise correlations among the five CSC markers were not evaluated in the present analysis. Therefore, potential biological overlap and statistical redundancy among the markers cannot be excluded, and the five-marker panel should be interpreted as an exploratory integrated signature rather than evidence that each individual marker provides independent prognostic information.

Future studies should validate combined CSC signatures in larger prospective multicenter cohorts with longer follow-up and should incorporate multivariable adjustment for established clinicopathological prognostic factors. Integration with transcriptomics, BRCA mutation status, homologous recombination deficiency, immune-related biomarkers, and other multi-omics data may improve prognostic stratification. Internal validation using bootstrapping or cross-validation, followed by external validation in independent cohorts, is also needed. Single-cell sequencing, spatial transcriptomics, extracellular vesicle analysis, and liquid biopsy approaches may help characterize dynamic changes in CSC phenotypes and their interaction with the tumor microenvironment. Ultimately, translational studies and clinical trials will be required to determine whether CSC-directed strategies can improve outcomes in advanced epithelial ovarian cancer.

Conclusion

Individual CSC markers showed limited prognostic use in advanced EOC. Although the five-marker combined signature demonstrated poor discriminatory accuracy for recurrence by ROC analysis, it significantly stratified 2-year PFS and associated with recurrence risk in Cox regression. These findings suggest that integrated CSC expression may capture biologically relevant heterogeneity associated with disease progression; however, the panel should currently be considered exploratory rather than a validated predictive tool. Larger prospective studies with multivariable adjustment, internal and external validation, and established clinicopathological predictors are required before clinical application.

Data Availability Statement

The present study used datasets derived from clinical records and patient interviews at the Gynecologic Oncology Clinic, Department of Obstetrics and Gynecology,

Prof. I.G.N.G. Ngoerah, General Hospital. Due to ethical policies and confidentiality requirements, public access to the data is restricted but can be accessed through the corresponding author upon reasonable request and institutional approval.

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Authors' Contribution
Conceptualization, B.D.A. and K.Y.S.; methodology, B.D.A. and K.Y.S.; formal analysis, B.D.A. and K.Y.S.; investigation, B.D.A. and K.Y.S.; data curation, B.D.A. and K.Y.S.; writing—original draft preparation, B.D.A. and K.Y.S.; writing, review and editing, B.D.A. and K.Y.S.; visualization, B.D.A. and K.Y.S.; supervision, K.Y.S. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest
None declared.

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Table 1. Characteristics of primary antibodies used for immunohistochemical detection of cancer stem cell markers (CD24, CD44, CD133, CD117, and ALDH)

Antibody	**Clone**	**Host/Clonality**	**Manufacturer**	**Catalogue No.**	**Working dilution (manual protocol)**	**Country of origin**
CD24	Polyclonal	Rabbit	Invitrogen (Thermo Fisher Scientific)	PA5-141185	1:50–1:200	USA
CD44	IHC144	Rabbit monoclonal	GeneAb™ (GenomeMe)	IHC144	1:50–1:100	Canada
CD133	C1C2	Rabbit polyclonal	GeneTex	GTX100567	1:100–1:1000	USA
ALDH	Polyclonal	Rabbit	GeneTex	GTX123973	1:100–1:1000	USA
CD117 (c-Kit)	IHC526	Rabbit monoclonal	GeneAb™ (GenomeMe)	IHC526	1:25–1:50	Canada

ALDH: Aldehyde dehydrogenase; CD: Cluster of differentiation; GTX: GeneTex; IHC: Immunohistochemistry; No.: Number; USA: United States of America

Table 2. Recurrence distribution according to clinicopathological characteristics and cancer stem cell marker expression in 114 patients with advanced (FIGO Stage II–IV) epithelial ovarian cancer

Variable	Recurrence frequency (%)	
	Yes	No
Stage		
II	13 (34.20)	25 (65.80)
III	54 (78.30)	15 (21.70)
IV	7 (100.00)	0 (0.00)
Histopathology		
Clear cell	11 (50.00)	11 (50.00)
Endometrioid	15 (53.60)	13 (46.40)
LGSOC	2 (33.30)	4 (66.70)
HGSOC	37 (78.70)	10 (21.30)
Mucinous	9 (81.80)	2 (18.20)
Cell differentiation		
G1	5 (55.60)	4 (44.40)
G2	4 (80.00)	1 (20.00)
G3	65 (65.00)	35 (35.00)
LVSI		
Negative	54 (65.10)	29 (34.90)
Positive	20 (64.50)	11 (35.50)
CD24		
Low	31 (57.40)	23 (42.60)
High	43 (71.70)	17 (28.30)
CD44		
Low	8 (47.10)	9 (52.90)
High	66 (68.00)	31 (32.00)
CD133		
Low	47 (61.80)	29 (38.20)
High	27 (71.10)	11 (28.90)
CD117		
Low	39 (60.00)	26 (40.00)
High	35 (71.40)	14 (28.60)
ALDH		
Low	60 (63.80)	34 (36.20)
High	14 (70.00)	6 (30.00)
Five-marker combination		
Low		
High	3 (23.10)	10 (76.90)
	71 (70.30)	30 (29.70)

ALDH: Aldehyde dehydrogenase; CD: Cluster of differentiation; FIGO: International Federation of Gynecology and Obstetrics; HGSOC: High-grade serous ovarian carcinoma; LGSOC: Low-grade serous ovarian carcinoma; LVSI: Lymphovascular space invasion. Low- and high-expression groups were defined using the receiver operating characteristic-derived H-score cut-offs presented in table 3.

Table 3. ROC analysis of individual, dual, and five-marker cancer stem cell scores for recurrence/progression in 114 patients with advanced EOC

Marker	**AUC**	**Std-error**	**Cut-off**	***P* value**	**Sensitivity**	**Specificity**	**Youden index**
CD24	0.578	0.057	84.17	0.170	0.662	0.475	0.137
CD133	0.543	0.058	89.65	0.451	0.532	0.600	0.132
CD44	0.531	0.059	65.10	0.589	0.896	0.225	0.121
CD117	0.469	0.057	111.50	0.589	0.468	0.650	0.118
ALDH	0.545	0.056	130.60	0.430	0.182	0.850	0.032
ALDH–CD133	0.496	0.059	131.00	0.948	0.784	0.325	0.109
CD24–CD44	0.549	0.057	156.59	0.389	0.824	0.300	0.124
CD133–CD117	0.582	0.056	124.00	0.147	0.905	0.250	0.155
ALDH–CD117	0.509	0.057	137.70	0.875	0.703	0.300	0.003
ALDH–CD44	0.479	0.061	121.56	0.717	0.811	0.275	0.086
Combination	0.518	0.060	321.43	0.748	0.959	0.250	0.209

ALDH: Aldehyde dehydrogenase; AUC: Area under the curve; CD: Cluster of differentiation; EOC: Epithelial ovarian cancer; ROC: Receiver operating characteristic; Std-error: Standard error. ROC-derived cut-off values were determined using the Youden index and subsequently applied to classify patients into low- and high-expression groups for survival analyses.

Table 4. Two-year PFS according to low- and high-expression groups of individual and combined cancer stem cell markers in 114 patients with advanced EOC

Variable	2-year PFS (%)	Median (95% CI)	*P* value (Log rank)
CD24			0.094
Low	42.60	12 (7.885 - 16.115)	
High	28.30	10 (9.056 - 10.944)	
CD44			0.179
Low	52.90	*	
High	32.00	11 (9.837 - 12.163)	
CD133			0.215
Low	38.20	12 (8.801 - 15.199)	
High	28.90	10 (9.156 - 10.844)	
CD117			0.205
Low	40.00	12 (7.499 - 16.501)	
High	28.60	10 (8.040 - 11.960)	
ALDH			0.804
Low	36.20	11 (9.761 - 12.239)	
High	30.00	12 (7.639 - 16.361)	
ALDH–CD133			0.294
Low	44.80	15 (6.209 - 23.791)	
High	31.80	11 (9.769 - 12.231)	
CD24–CD44			0.263
Low	45.80	12	
High	32.20	10 (8.732 - 11.268)	
CD133–CD117			0.076
Low	58.80	*	
High	30.90	10 (8.886 - 11.114)	
ALDH–117			0.610
Low	35.30	10 (7.718 - 12.282)	
High	35.00	12 (10.267 - 13.733)	
ALDH–CD44			0.349
Low	44.00	15 (6.840 - 23.160)	
High	32.60	11 (9.740 - 12.260)	
Combination			**0.009**
Low	76.90	*	
High	29.70	10 (8.906 - 11.094)	

ALDH: Aldehyde dehydrogenase; CD: Cluster of differentiation; CI: Confidence interval; EOC: Epithelial ovarian cancer; PFS: Progression-free survival; ROC: Receiver operating characteristic. *Median PFS was not reached because more than 50% of patients remained progression-free at 2 years. Low- and high-expression groups were defined using the ROC-derived H-score cut-off for each individual or combined CSC marker, as presented in table 3.

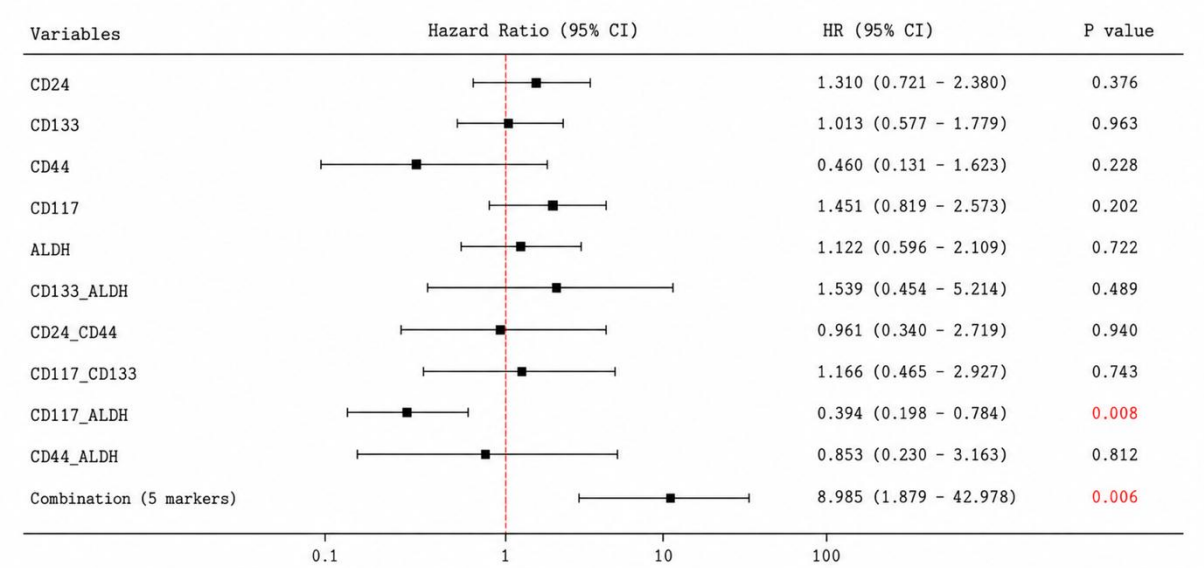


Figure 1. Forest plot showing HRs and 95% CIs for recurrence according to individual cancer stem cell markers, dual-marker combinations, and the five-marker combined signature. HRs greater than 1 indicate a higher recurrence hazard, whereas values below 1 indicate a lower recurrence hazard. The CD117–ALDH combination was associated with a lower recurrence hazard, whereas the five-marker combined signature was associated with a higher recurrence hazard. The magnitude of the latter association should be interpreted cautiously because of the wide CI.

ALDH: Aldehyde dehydrogenase; CD: Cluster of differentiation; CI: Confidence interval; HR: Hazard ratio

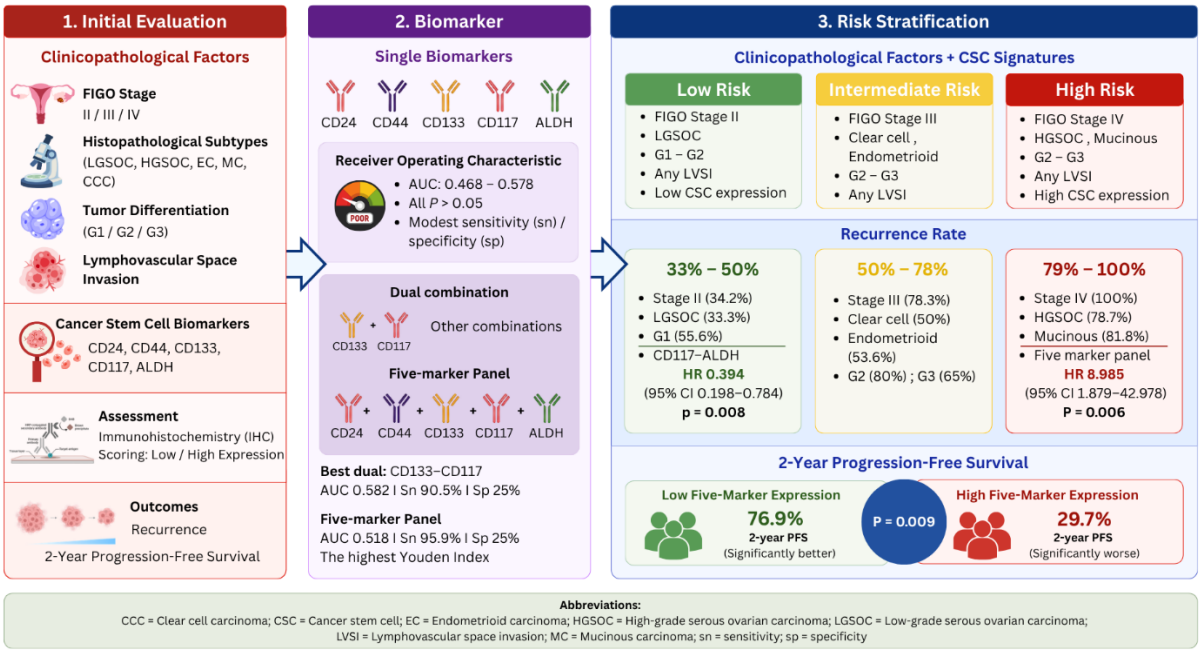


Figure 2. Conceptual framework illustrating the prognostic relevance of combined CSC expression in advanced EOC. Low combined CSC expression is associated with longer PFS, whereas high combined expression is associated with shorter PFS and a more aggressive, therapy-resistant tumor phenotype. The model illustrates how integration of multiple CSC markers may capture intratumoral heterogeneity and stemness-related biological pathways more comprehensively than individual biomarkers. The five-marker signature should be interpreted as an exploratory prognostic signature rather than a validated predictive classifier.

ALDH: Aldehyde dehydrogenase; AUC: Area under the curve; CCC: Clear cell carcinoma; CD: Cluster of differentiation; CI: Confidence interval; CSC: Cancer stem cell; EC: Endometrioid carcinoma; FIGO: International Federation of Gynecology and Obstetrics; HGSOC: High-grade serous ovarian carcinoma; HR: Hazard ratio; IHC: Immunohistochemistry; LGSOC: Low-grade serous ovarian carcinoma; LVSI: Lymphovascular space invasion; MC: Mucinous carcinoma; PFS: Progression-free survival; Sn: Sensitivity; Sp: Specificity.