Title

Actionable Biomarker Co-Expression in Advanced Ovarian Cancer: Folate Receptor alpha Expression and Related Markers

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Authors

Shachar, Eliya K
Silverstein, Jordyn
Gornbein, Jeffrey
et al.

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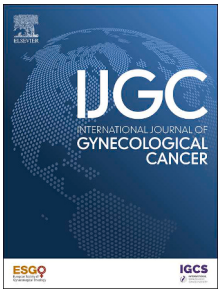
ORIGINAL RESEARCH

Actionable biomarker co-expression in advanced ovarian cancer: folate receptor alpha expression and related markers



Eliya K. Shachar[a,b], Jordyn Silverstein[c], Jeffrey Gornbein[d], Ritu Salani[a], Beth Y. Karlan[a], Dana M. Chase[a,*]

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Highlights

- Ovarian cancer actionable biomarkers showed heterogeneous co-expression patterns.
- Folate receptor-alpha (FRα), estrogen receptor, (ER), progesterone receptor, and CLDN6 formed a hormone receptor—associated cluster.
- FRα and ER demonstrated the strongest biomarker co-expression.
- *BRCA* and homologous recombination deficiency showed distinct co-expression clustering.
- Findings support exploring graded rather than binary FRα and ER classification.

ABSTRACT

Background: Folate receptor-α is an established therapeutic target in ovarian cancer and identifies candidates for mirvetuximab soravtansine. However, co-expression patterns between folate receptor-α and other actionable biomarkers, and their therapeutic implications, remain incompletely characterized.

Methods: We conducted a single-institution cohort study of 110 patients with platinum-resistant ovarian cancer treated with mirvetuximab soravtansine (2019-2025). Tumor biomarkers were assessed using immunohistochemistry, fluorescence in situ hybridization, and next-generation sequencing. Co-expression among 10 biomarkers, including folate receptor-alpha, estrogen receptor, progesterone receptor, human epidermal growth factor receptor-2, *BRCA*, homologous recombination/loss of heterozygosity, programmed death-ligand 1, claudin-6, and tumor mutational burden, were evaluated using Jaccard similarity indices, Cohen's kappa statistics, and correlation analyses. Exploratory progression-free survival and overall survival analyses were performed by folate receptor-α and estrogen receptor sub-groups.

Results: Folate receptor-α expression was high in 80% of tumors and intermediate/low in 20%. Co-expression analyses demonstrated largely independent biomarker patterns, reflecting biological heterogeneity. A hormone receptor—associated cluster emerged, including strong co-expression between folate receptor-α and estrogen receptor (Jaccard = 0.76), and moderate overlap between estrogen receptor and claudin-6 (0.62), programmed death-ligand-1 and estrogen receptor (0.56), and programmed death-ligand-1 and folate receptor-α (0.50). Survival outcomes were comparable between folate receptor-α-high and folate receptor-α-intermediate/low tumors. Among folate receptor-α-high tumors, estrogen receptor expression ($\geq$10%) did not meaningfully stratify progression-free or overall survival outcomes.

Conclusions: Biomarker co-expression in advanced ovarian cancer demonstrates substantial heterogeneity, with patterns that may inform future biomarker-guided treatment combinations and sequencing strategies. Prospective validation is needed to refine multi-

WHAT IS ALREADY KNOWN ON THIS TOPIC

Folate receptor alpha (FRα) is an established therapeutic target in ovarian cancer and is used to select eligible patients for mirvetuximab soravtansine. However, FRα expression is currently treated as a binary biomarker for monotherapy, and the extent to which it is co-expressed with other actionable alterations remains incompletely defined. Biomarker co-expression patterns informing treatment selection in ovarian cancer are not well characterized.

WHAT THIS STUDY ADDS

In this real-world cohort of patients with platinum-resistant ovarian cancer treated with mirvetuximab soravtansine, FRα expression demonstrated largely independent co-expression patterns with other actionable biomarkers, highlighting substantial biological heterogeneity. A hormone receptor—associated cluster was identified, including strong overlap between FRα and estrogen receptor (ER). Survival outcomes were similar across FRα expression levels and were not meaningfully stratified by ER status within FRα-high tumors.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

These findings highlight the biological diversity of FRα-expressing ovarian cancers and support further investigation of FRα as a potentially graded rather than strictly binary biomarker. The observed co-expression patterns may help inform future biomarker-guided combination and sequencing strategies, including evaluation of endocrine modulation alongside FRα-targeted therapy approaches. However, given the exploratory and retrospective nature of this study, these findings should be considered hypothesis-generating. Prospective studies are needed to validate multi-marker approaches and determine their clinical utility in ovarian cancer.

* **Correspondence to** Dr Dana M Chase, Department of Urology, and Obstetrics & Gynecology, University of California, Los Angeles, Los Angeles, CA, USA; DMChase@mednet.ucla.edu (D.M. Chase)

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marker frameworks and determine whether biologically driven treatment approaches based on co-expression biomarker patterns may have clinical utility. Exploratory analyses support further investigation of folate receptor-α as a potentially graded rather than strictly binary biomarker; however, these findings are hypothesis-generating.

Keywords:

Biomarkers; Ovarian Cancer; Folate Receptor Alpha; Estrogen Receptor; Co-Expression

ORIGINAL RESEARCH

INTRODUCTION

Ovarian cancer represents a biologically heterogeneous group of diseases with distinct molecular drivers and therapeutic vulnerabilities.[1,2] Biomarker-directed therapies are increasingly shaping ovarian cancer management, highlighting the importance of understanding biomarker prevalence and co-expression patterns.

Folate receptor alpha (FRα) is detected in approximately 80% of epithelial ovarian cancers, with the highest expression observed in high-grade serous carcinomas and substantially lower expression in endometrioid and clear cell sub-types.[3] FRα is an established therapeutic target that identifies candidates for treatment with mirvetuximab soravtansine, an antibody—drug conjugate (ADC).[4]

Pre-clinical studies suggest estradiol signaling may transcriptionally represses FRα expression through estrogen receptor (ER)-mediated regulation of *FOLR1*, whereas anti-estrogen exposure results in rapid de-repression and upregulation of FRα expression in gynecologic cancer cell lines.[5,6] These findings raise the possibility that hormonal pathways influence FRα-targeted therapeutic vulnerability in ovarian cancer.

ER and progesterone receptor (PR) expression are common in high-grade serous and endometrioid carcinomas.[7] In addition to estrogen-mediated repression, FRα expression has also been reported to be indirectly upregulated by progesterone and glucocorticoids and directly activated by androgen signaling.[8]

Additional biomarkers with emerging or established therapeutic relevance and may inform targeted and immune-based treatment modalities (Table S1). Human Epidermal Growth Factor Receptor-2 (HER2) amplification or over-expression is frequently observed in mucinous and clear cell carcinomas, although lower but clinically meaningful expression rates are also reported in high-grade serous tumors.[9] A subset of ovarian cancers co-express HER2 and FRα, potentially creating opportunities for sequential or combinatorial targeted therapies.[9] Another emerging biomarker is CLDN6, a tight junction protein highly expressed during embryonic development, with aberrant re-expression reported in approximately 29% to 55% of ovarian cancers, particularly high-grade serous carcinomas, and currently under investigation in CLDN6-targeted ADC clinical trials.[10-12]

Immune-related biomarkers, including programmed death-ligand 1 (PD-L1) and tumor mutational burden, may further inform immune checkpoint inhibitor (ICI)-based treatment strategies in ovarian cancer.[2] Additionally, *BRCA* mutations and homologous recombination deficiency (HRD) remain important biomarkers identifying eligibility for poly(adenosine diphosphate-ribose) Polymerase inhibition.[2]

Despite the expanding use of biomarker-guided therapies, patterns of co-expression among established and emerging biomarkers in epithelial ovarian cancer remain poorly characterized, particularly with respect to FRα. Defining these relationships is clinically relevant, as biomarker co-expression may inform treatment selection, optimal therapeutic sequencing of FRα-targeted ADCs, and the rational design of trials of combination or subsequent targeted therapies as multi-marker treatment algorithms continue to evolve.

The primary objective of this study was to characterize co-expression patterns among established and emerging biomarkers in epithelial ovarian cancer. Given evidence suggesting that estrogen signaling may influence FRα expression through shared transcriptional and differentiation pathways, a secondary objective was to evaluate FRα expression levels and other actionable biomarkers, particularly ER status, in patients with advanced ovarian cancer treated with mirvetuximab soravtansine, with the goal of informing future trial design.

Exploratory outcomes included progression-free survival and overall survival analyses across biomarker-defined sub-groups. Comparisons focused on FRα-high versus FRα-intermediate/low tumors and ER-defined sub-groups within FRα-high disease.

METHODS

Study Design

We conducted a single-institution retrospective cohort study of patients with platinum-resistant epithelial ovarian cancer treated with mirvetuximab soravtansine between June 2019 and September 2025 at University of California, Los Angeles. Patients were identified through the institutional electronic health record system. The study was approved by the institutional review board (IRB-24-6039). Eligible patients were 18 years or older, had pathologically confirmed ovarian cancer, and received at least 1 dose of mirvetuximab soravtansine.

Data Collection

Clinical and treatment-related variables were extracted from the electronic health record and included histopathology, platinum-resistant disease status, prior systemic therapies, line of therapy at mirvetuximab soravtansine initiation, Eastern Cooperative Oncology Group performance status, mirvetuximab soravtansine treatment duration, reasons for treatment discontinuation, clinical outcomes, and available biomarker testing results, including genomic profiling and germline genetic testing.[13] Patients received mirvetuximab soravtansine in routine clinical practice across varying lines of therapy.

Biomarker Assessment

Tumor biomarkers were evaluated using institutional and commercial clinical assay platforms, including immunohistochemistry (IHC), fluorescence in situ hybridization, and next-generation sequencing.

FRα, HER2, ER, PR, PD-L1, and CLDN6 were assessed primarily by IHC using established and study-specific thresholds (Table S2). FRα, ER, and PR were additionally analyzed as continuous variables. HER2 status was determined using gastric cancer—based IHC scoring criteria, with confirmatory *ERBB2* amplification testing when indicated.[14] Tumor mutational burden, HRD, loss of heterozygosity, and somatic/germline *BRCA*1/2 variants were recorded. Micro-satellite instability and mismatch repair status were not included (all tumors were micro-satellite instability-stable, and 1 case was mismatch repair-deficient).

ER expression was recorded as continuous and categorical variables with $\geq$1% nuclear staining defining ER positivity, consistent with breast cancer guidelines and common gynecologic pathology reporting practices. Additional analyses utilized a >10% ER threshold to distinguish ER-low-positive (1%-10%) from higher ER-expressing tumors, given the absence of standardized ER cutoffs in ovarian cancer.

Biomarker data were retrospectively abstracted from pathology and molecular reports derived from primary and recurrent tumor specimens. Biomarker assessment reflected routine clinical practice, including testing at diagnosis, recurrence, and/or prior to ADC therapy. Serial biomarker assessments were available, specifically cases of repeated FRα and HER2 testing performed in patients with initially low or borderline expression; the highest reported expression level guiding treatment selection was recorded. Other biomarkers were derived from single available reports. Biomarker availability varied across patients because testing was performed according to routine clinical practice rather than a pre-defined study protocol. Analyses were therefore conducted using available-case data without imputation (Tables S3A and B). Co-expression analyses should therefore be interpreted as exploratory and hypothesis-generating.

Statistical Analysis

Descriptive statistics included medians and interquartile ranges (IQRs) for continuous variables and frequencies and percentages for categorical variables. Pairwise co-expression patterns among the 10 biomarkers were assessed using both binary and continuous measures. Pairwise biomarker co-expression was evaluated using Jaccard similarity coefficients and Cohen's kappa statistics.[15,16] For binary biomarkers, similarity was quantified using the Jaccard index to identify clusters of biologically meaningful overlap. All Jaccard analyses were conducted using pairwise complete observations. Cohen's kappa accounts for both positive and negative biomarker expression when evaluating agreement, whereas the Jaccard index measures overlap only among positively expressed biomarkers. For continuous or ordinal—continuous comparisons, associations were quantified using Spearman rank correlation coefficients (ρ) with corresponding *P*-values.

Exploratory analyses evaluating progression-free and overall survival across biomarker expression and co-expression profiles. Survival curves were estimated using the Kaplan—Meier method, and comparisons between groups were performed using Cox proportional hazards models.

Table Platinum Resistant Ovarian Cancer Population Demographics and Biomarker Expression Profile

Variable *n*, (%)	**Total population *n* = 110**
Age at Diagnosis, median (IQR)	60.8 (54.0-71.3**)**
Ethnicity *n*, (%)	
Not Hispanic/Latina	96 (87.3%)
Hispanic/Latina	9 (8.2%)
Other	5 (4.5%)
Race *n*, (%)	
Asian	13 (11.8%)
Black	6 (5.5%)
White	76 (69.1%)
NOS	15 (13.6%)
Histology *n,* (%)	
High-grade Serous	95 (86.4%)
Low-grade Serous	4 (3.6%)
Clear Cell	3 (2.7%)
Endometrioid	1 (0.9%)
Other	7 (6.4%)
Prior lines, median (range)	4 (2-10)
Folate receptor α *n*, (%)	
High FRα ($\geq$75%)	88 (80%)
Intermediate FRα (25%-74%)	12 (10.9%)
Low FRα (<25%)	8 (7.3%)
Unknown FRα	2 (1.8%)
HER2[a] *n*, (%)	
HER2 positive	22 (20%)
HER2 negative	57 (51.8%)
Unknown	31 (28.2%)
HER2 IHC *n*, (%)	
IHC 0	37 (33.6%)
IHC 1+	23 (20.9%)
IHC 2+	10 (9.1%)
IHC 3+	3 (2.7%)
Unknown	37 (33.6%)
Amplification HER2/neu oncogene[b] *n*, (%)	
Amplified	14 (12.7%)
Non-amplified	96 (87.3%)
ER *n*, (%)	
Positive	77 (70%)
Negative	6 (5.5%)
Unknown	27 (24.5%)
PR *n*, (%)	
Positive	41 (37.3%)
Negative	26 (23.6%)
Unknown	43 (39.1%)
HRR alteration *n*, (%)	
HRD/Loss of heterozygosity-high	48 (43.6%)
HRP	52 (47.3%)
Unknown	10 (9.15)
***BRCA* somatic/germline** *n*, (%)	
s*BRCA* alteration	24 (21.8%)
Negative	85 (77.3%)
Unknown	1 (0.9%)
***BRCA* germline** *n*, (%)	
gBRCA alteration	16 (14.5%)
BRCA1	13 (11.8%)
BRCA2	3 (2.7%)
Negative	94 (85.5%)
PD-L1[c] *n*, (%)	
Positive	29 (26.4%)
Negative	22 (20.0%)
Unknown	59 (53.6%)
CLDN6 *n*, (%)	
Positive	22 (20.0%)
Negative	21 (19.1%)
Unknown	67 (60.9%)
Tumor mutation burden *n*, (%)	
Tumor mutation burden-low	75 (68.2%)
Tumor mutation burden-intermediate	18 (16.4%)
Tumor mutation burden-high	0
Unknown	17 (15.5%)

(*continued on next page*)

Abbreviations: ECOG-Eastern Cooperation Oncology Group; FRα -Folate receptor α; HER2, human epidermal growth factor receptor 2; Immunohistochemistry- IHC; ER-Estrogen receptor, PR-progesterone receptor; HRR- homologous recombination repair; gBRCa-germline BRCA alteration, sBRCA-somatic *BRCA* alteration; PD-L1 (Combined Positive Score [CPS]; CLDN6- Claudin 6; NOS-not otherwise specified

[a] HER2 expression for eligibility was based on local assessment where available based on either IHC and/or amplification.

[b] HER2 is the protein encoded by the ERBB2 gene; therefore, HER2 amplification and ERBB2 amplification refer to the same underlying genomic event—amplification of ERBB2

[c] PD-L1 (Combined Positive Score [CPS] $\geq$1) positive, and CPS<1 negative score.

Progression-free and overall survival of patients treated with mirvetuximab soravtansine were evaluated according to FRα expression (FRα-high $\geq$75% vs FRα-intermediate/low <75%). Moreover, analyses stratified outcomes according to HRD/loss-of-heterozygosity-high and *BRCA* mutation status (somatic/germline). Among patients with FRα-high tumors, survival outcomes were further assessed according to ER co-expression. Survival outcomes were compared between FRα-high/ER-positive tumors (>10% ER expression) and FRα-high/ER-low-positive/ER-negative tumors ($\leq$10% ER expression) within a cohort of 90 patients. Associations across continuous FRα expression levels were also assessed. *P*-values for associations with ordinal FRα categories were calculated using the Kruskal–Wallis test. All survival analyses were exploratory, two-sided (α = 0.05), and unadjusted for multiple comparisons.

In accordance with the journal's guidelines, the study data will be made available for independent analysis by a team selected by the Editorial Office for purposes of reproducibility assessment or external validation, if requested.

RESULTS

Among 110 women with ovarian cancer treated with mirvetuximab soravtansine, the median age at diagnosis was 60.8 years (IQR 54.0-71.3; Table). Most patients were non-Hispanic/Latina White (69%), with high-grade serous (86%) being the most common histologic subtype. Among evaluable patients (*n* = 103), 44.7% received

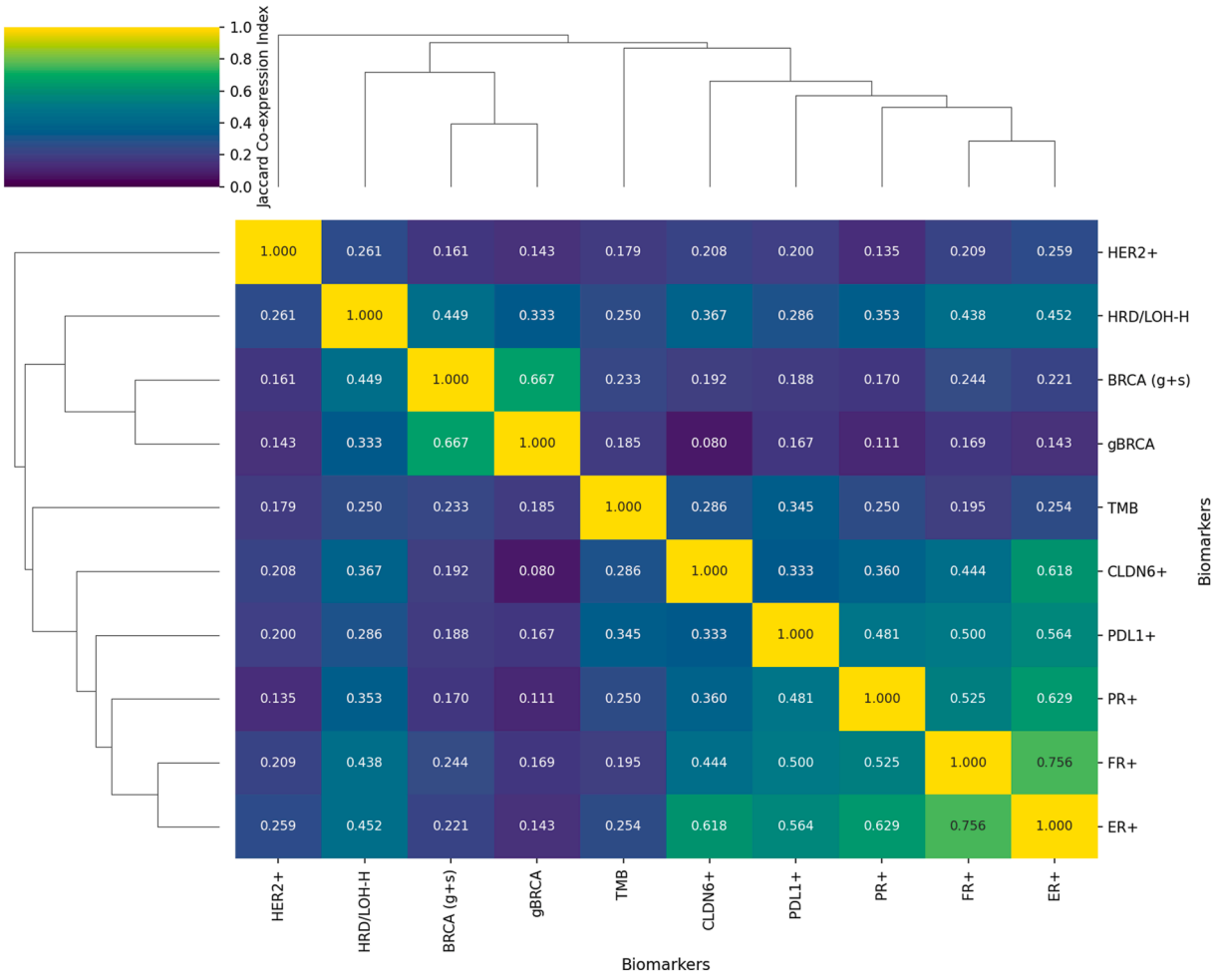

Figure 1 Hierarchical clustering of co-expressed ovarian cancer biomarkers using Jaccard similarity index.
Values range from 0 (no co-occurrence) to 1 (complete overlap), with warmer colors (yellow/green) indicating stronger co-expression and cooler colors (blue/purple) indicating weaker associations. Jaccard coefficients were interpreted as minimal/no association (<0.10), weak (0.10-0.29), moderate (0.30-0.49), strong (0.50-0.69), and very strong (≥0.70). The Jaccard index was calculated as the ratio of the intersection to the union of positive cases for each biomarker pair ($J = |A \cap B|/|A \cup B|$).
Strong co-expression was observed between FRα and ER (0.756) and between combined somatic/germline BRCA and germline BRCA alterations (0.667), suggesting biologically related subgroups. Lower co-expression values may reflect distinct molecular pathways or independent biomarker expression patterns.
Pairwise co-expression analyses used available-case data only. The number of evaluable cases varied by biomarker availability, and each heatmap cell reflects only patients with valid results for both biomarkers being compared. Evaluable pairwise comparisons ranged from 15 cases (PD-L1 vs CLDN6) to 109 cases (somatic BRCA vs germline BRCA). Missing data were not imputed. Tables S3A and B summarize evaluable biomarker frequencies and pairwise comparison counts. FRα, folate receptor alpha; HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; HRD, homologous recombination deficiency; LOH-H, loss of heterozygosity-high; sBRCA, somatic BRCA mutation; gBRCA, germline BRCA mutation; PD-L1, programmed death-ligand 1; CLDN6, claudin-6; TMB, tumor mutational burden.

mirvetuximab soravtansine by the third line of therapy, 35.9% during the fourth or fifth line, and 19.4% during the sixth line or beyond. Most patients had preserved performance status at treatment initiation, with an Eastern Cooperative Oncology Group score of 0 to 1 observed in 85.5% of evaluable patients (Table S4). The median duration of mirvetuximab soravtansine therapy was 5.1 months (IQR 2.5-8.5; range; 0-55.8 months; $n = 102$). Mirvetuximab soravtansine discontinuation occurred primarily because of disease progression (65.5%), whereas discontinuation due to toxicity occurred in 14.5% of patients.

FRα expression was high in 80% of the tumors ($n = 88$), 10.9% ($n = 12$) intermediate, and low in 7.3% ($n = 8$; Table S5). HER2 positivity was observed in 20% ($n = 22$), and amplification was identified in 13% ($n = 14$). Hormone-receptor expression varied: ER was positive (≥1%) in 70% ($n = 70$%) and PR was positive in 37% ($n = 41$) of evaluable tumors. HRD/loss of heterozygosity-high status was identified in 44% ($n = 48$), *BRCA* alterations (germline/somatic) in 21.8% ($n = 24$), PD-L1 positivity in 26.4% ($n = 29$), low tumor mutational burden in 68% ($n = 75$), and CLDN6 positivity in 20% ($n = 22$).

Across the 10 binary biomarkers, several co-expression patterns emerged (Fig. 1). The strongest associations were between the hormone receptor markers, with FRα and ER demonstrating a high degree of co-expression (Jaccard = 0.76), with moderate co-expression between FRα and PR (Jaccard = 0.53), reflecting biological co-positivity. HER2 showed weak co-expression with FRα, ER and PR (Jaccard = 0.21, 0.26, and 0.14, respectively). ER and PR clustered with moderate co-expression (Jaccard = 0.63). CLDN6 showed moderate overlap with ER (Jaccard = 0.62), positioning it closer to the hormone receptor cluster.

Markers associated with DNA repair were clustered: HRD/loss of heterozygosity-high co-expressed moderately with *BRCA* alterations (somatic/germline) and *BRCA* germline variants (Jaccard = 0.45, and 0.33, respectively). *BRCA* somatic variants and *BRCA* germline variants showed strong overlap (Jaccard = 0.67) reflecting a biologically similar subgroup.

Among immune biomarkers, PD-L1 exhibited moderate to strong overlap with ER (Jaccard = 0.56) and FRα (Jaccard = 0.50), and more modest overlap with CLDN6 (Jaccard = 0.33), and tumor mutational burden (Jaccard = 0.35). Tumor mutation

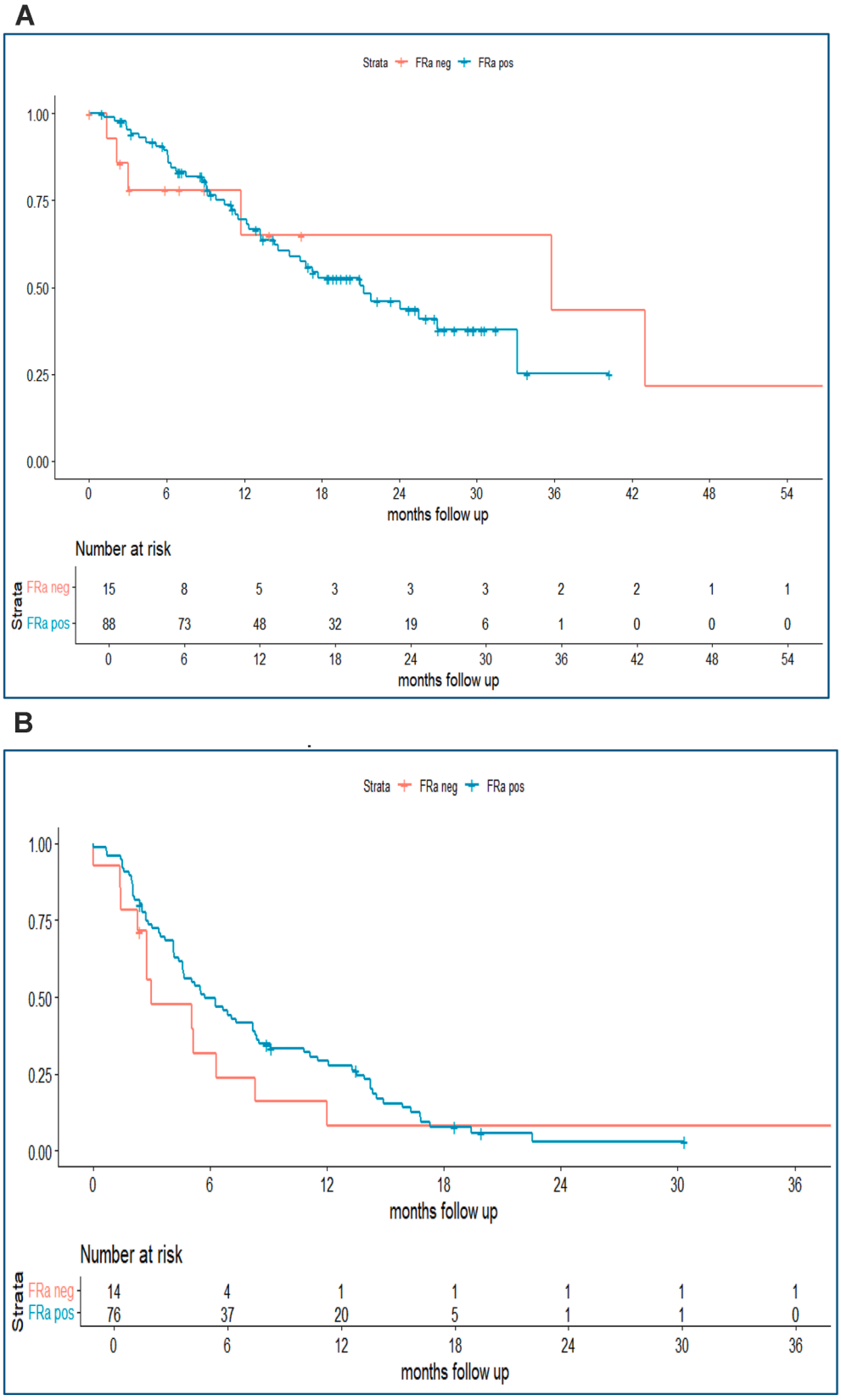

Figure 2 **(A)** Overall survival by FRα expression FRα high expression ($\geq$75%) versus FRα low/intermediate expression level. Median overall survival 12.3 months, HR 1.29, 95% CI 0.48 to 3.45, $p = .60$
(B) Progression-free survival by FRα expression FRα high expression ($\geq$75%) versus FRα low/intermediate expression level. Median progression-free survival 5.7, HR 0.72, 95% CI 0.39 to 1.34, $p = .31$.
CI, confidence interval; ER, estrogen receptor; FRα, folate receptor alpha; HR, hazard ratio.

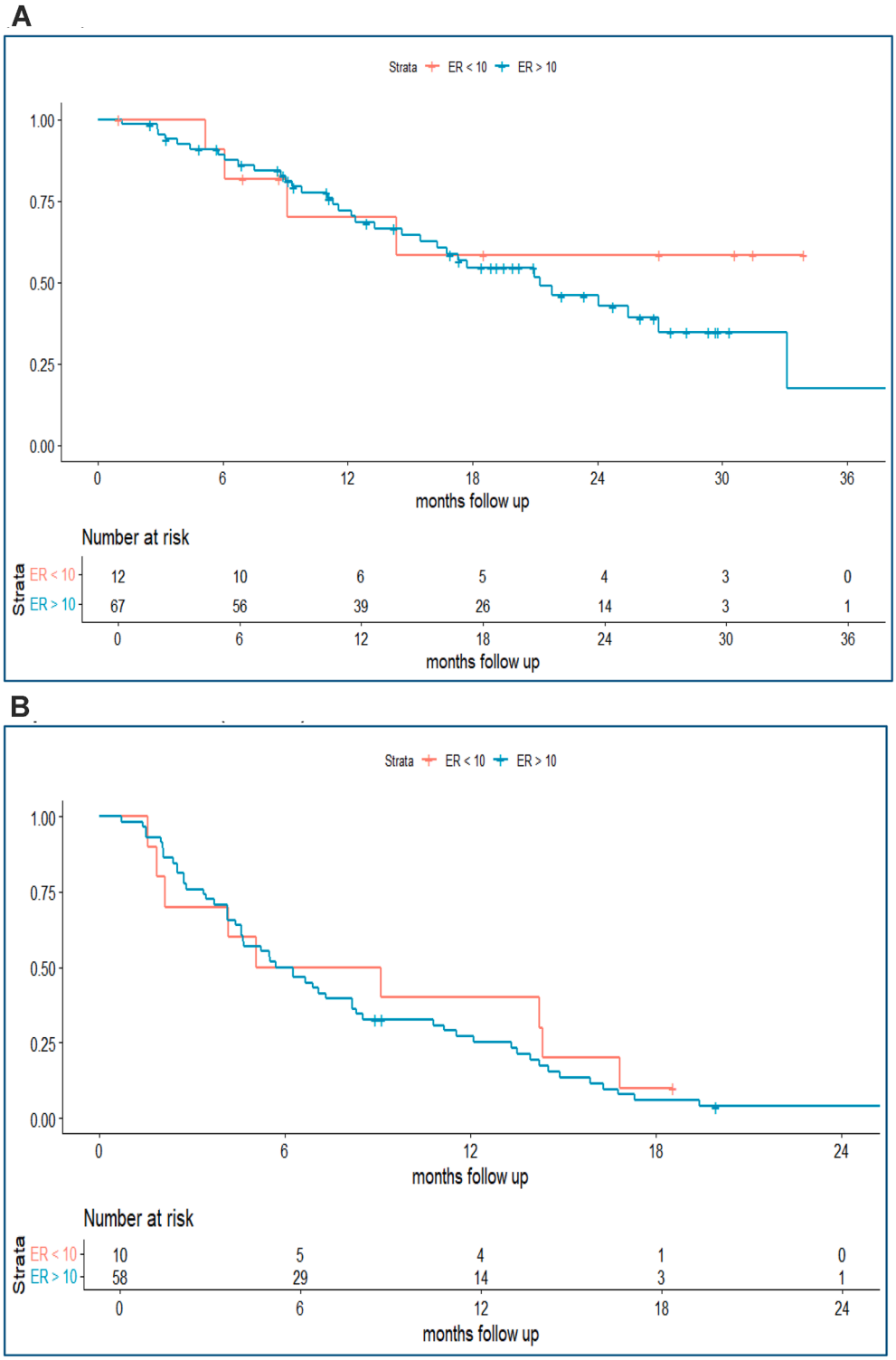

Figure 3 **(A)** Overall survival among FRα-high and ER co-expression level. FRα-high expression ($\geq$75%) ER-positive (>10%) versus FRα-high and ER-negative/low-positive expression level ($\leq$10%).
HR 1.55, 95% CI 0.54 to 4.40, $p = .41$
(B) Progression-free survival among FRα-high and ER co-expression level. FRα-high expression ($\geq$75%) ER-positive (>10%) versus FRα-high and ER-negative/low-positive expression level ($\leq$10%).
HR 1.24, 95% CI 0.61 to 2.53, $p = .55$.
Footnote: In the subset of tumors with FRα expression $\geq$75%, no patients had ER expression between >1% and 10%. All 12 tumors with FRα-high and ER expression $\leq$10% also had ER expression $\leq$1%, of these, 3 had ER expression of 0% and 9 had ER expression of 1%.
CI, confidence interval; ER, estrogen receptor; FRα, folate receptor alpha; HR, hazard ratio.

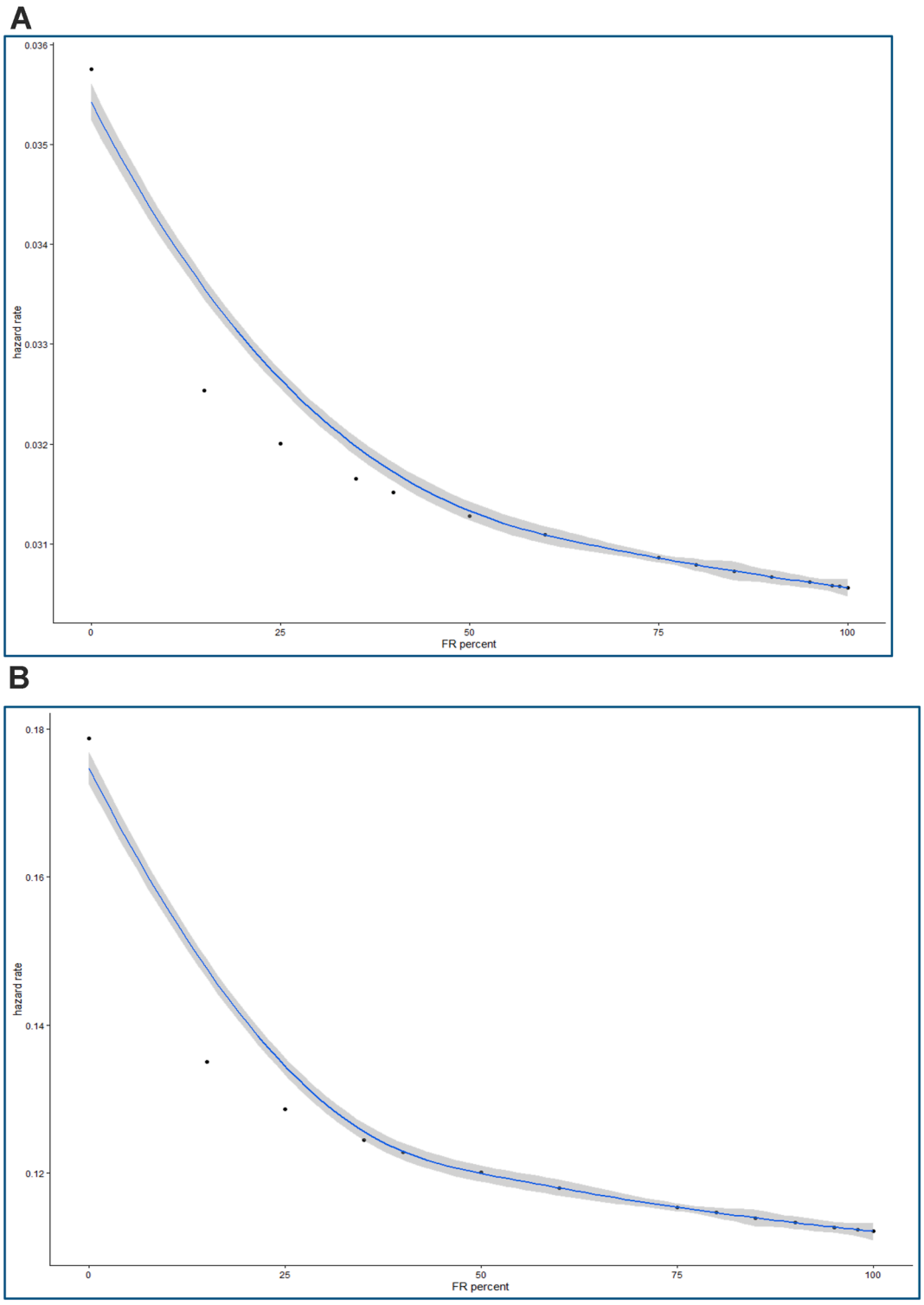

Figure 4 **(A)** Association between FRα percent expression and death hazard. Relationship between FRα percent expression and death hazard. **(B)** Association between FRα percent expression and progression-free survival hazard (progression or death). Relationship between FRα percent expression and progression hazard.
CI, confidence interval; ER, estrogen receptor; FRα, folate receptor alpha; HR, hazard ratio.

burden demonstrated low co-expression with other biomarkers (Jaccard range; 0.18-0.35), indicating that tumor mutational burden is largely a non-overlapping biomarker.

Biomarkers co-expression patterns were evaluated across histologic subtypes; however, subgroup analyses were limited by small sample sizes ($p > .05$).

Pairwise kappa analyses demonstrated minimal agreement across most biomarkers, consistent with largely independent biomarker expression (Fig. S1). Moderate concordance was observed between HRD/Loss of heterozygosity-high and *BRCA* (somatic/germline) alterations ($\kappa = 0.45$, $p < .001$), as well as between HRD/loss of heterozygosity-high and *BRCA* germline alterations alone ($\kappa = 0.34$. $p < .001$), suggesting partial biologic overlap between these related biomarkers. In contrast, most remaining biomarker pairs demonstrated minimal to no meaningful agreement.

Continuous analyses demonstrated a modest positive correlation between FRα% expression and ER% expression ($\rho = 0.22$, $p = .049$), with ER expression increasing across higher FRα expression levels, while FRα% expression and PR% expression

showed no meaningful association ($\rho = 0.06$, $p = .62$). The strongest relationship was observed between ER% and PR%, demonstrating a moderate positive correlation ($\rho = 0.35$, $p = .004$).

Across ordered FRα expression levels (low, intermediate, high), no statistically significant association was observed between categorical FRα expression groups and binary positivity for co-expressed biomarkers; FRα values remained widely distributed within biomarker-positive and biomarker-negative subgroups, and *p*-values were uniformly non-significant. However, exploratory analyses utilizing continuous biomarker measurements suggested a modest biologic gradient between FRα and ER expression levels that was not fully captured using categorical threshold-based analyses.

Specifically, binary and categorical co-expression analyses demonstrated minimal overall concordance between FRα and ER, whereas continuous analyses demonstrated a modest positive correlation between FRα and ER expression levels. Median ER expression increased progressively across higher FRα expression categories. Tumors with low FRα expression demonstrated a median ER level of 10%, whereas those with intermediate FRα expression showed a higher median ER level of 40%, and tumors with high FRα demonstrated a median ER expression level of 80%. These findings suggest a modest continuous relationship between FRα abundance and hormone receptor signaling despite limited agreement using dichotomized biomarker classifications.

Progression-free and overall survival, were compared between patients with FRα-high tumors (≥75%) and those with FRα-intermediate/low tumors (<75%). Kaplan—Meier curves demonstrating no significant differences in survival outcomes between patients with FRα-high and FRα-intermediate/low tumors (Fig. 2 A-B).

Among patients with FRα-high tumors, ER expression (>10% vs ≤10%) was not associated with significant differences in progression-free or overall survival (Fig. 3A-B). Among patients with HRD/loss of heterozygosity-high tumors, high FRα expression (vs intermediate/low) was associated with significantly improved progression-free survival (hazard ratio [HR] 0.42, 95% confidence interval [CI] 0.18 to 0.99, $p = .047$). Similarly, within the *BRCA*-positive subgroup, high FRα expression was associated with improved progression-free survival (HR 0.05, 95% CI 0.003 to 0.86, $p = .039$). No consistent associations were observed for overall survival or within biomarker-negative subgroups (homologous recombination proficient/loss of heterozygosity-low and *BRCA*-negative).

Cox proportional hazards models were used to evaluate associations between FRα% expression with overall and progression-free survival. FRα% expression consistently showed an inverse association with risk of death, with higher FRα% corresponding to a lower estimated HR, although CIs were wide and crossed unity (. With every tenfold increase in FRα%, the hazard of death decreased by 7.5% (HR 0.93, 95% CI 0.43 to 1.97, $p = .84$; Fig. 4A). Although not statistically significant, there appeared to be improved progression-free survival outcomes with increasing FRα% expression (Fig. 4B). With every tenfold increase in FRα% the progression hazard decreases by 20.8% (HR 0.79, 95% CI 0.44 to 1.44, $p = .45$).

DISCUSSION

Summary of Main Results

This exploratory analysis evaluated biomarker co-expression patterns in ovarian cancer to identify potential biologic synergies that may inform therapeutic strategies. Most biomarkers demonstrated limited clustering, supporting the concept that ovarian cancer comprises biologically heterogeneous tumors with largely independent molecular patterns. Distinct biomarker groupings included a hormone receptor—associated cluster (FRα, ER, PR, CLDN6) and an HRD-related-cluster (BRCA/HRD). Moderate associations were observed between FRα, PD-L1, and ER, whereas tumor mutation burden showed minimal overlap.

Among binary biomarkers, the strongest overlap was observed between FRα and ER. Continuous biomarkers analyses demonstrated modest co-expression, with a moderate correlation between ER% and PR%. Although ER% increased across higher FRα expression levels, this association did not reach statistical significance, suggesting that continuous biomarker measures may capture biologic relationships that are obscured by categorical thresholds.

Patients with tumors showing low/intermediate FRα expression had survival outcomes comparable with those with tumors showing high FRα expression. Increasing FRα expression showed a consistent but non-significant trend toward reduced progression and death.

Results in the Context of Published Literature

The limited biomarker clustering observed in this study aligns with the recognized molecular diversity of ovarian cancer. The HRD/BRCA cluster reflects established DNA repair biology, while the moderate concordance between HRD/loss of heterozygosity-high status and *BRCA* alterations emphasizes that HRD represents a broader genomic instability phenotype extending beyond *BRCA1*/2 mutations alone.

Pre-clinical studies demonstrating ERα-mediated repression of *FOLR1* support a biologic interplay between hormonal signaling and FRα expression, with anti-estrogens (tamoxifen and fulvestrant) shown to increase FRα expression by up to 36-fold.[5] These observations raise the hypothesis that endocrine modulation could enhance FRα expression and potentially expand eligibility for FRα-directed therapies among patients with low or intermediate expression. However, this remains speculative and was not directly evaluated in the current study.

Previous works have described an inverse relationship between ER signaling and PD-L1 expression through immune regulatory pathways.[17] ER-mediated suppression of interleukin 17 signaling may contribute to a less inflamed tumor micro-environment, characterized by attenuated PD-1/PD-L1 expression and reduce cluster of differentiation 8-positive T-cell infiltration.[18,19] The observed co-expression of ER and PD-L1 therefore identifies a biologically distinct subgroup that may warrant further study of combined endocrine—ICI strategies to convert "cold" tumors into immunologically targetable disease.[19,20]

Our findings are broadly consistent with prior biomarker co-expression studies demonstrating that FRα expression exists within a diverse molecular landscape with incomplete overlap among actionable biomarkers.[9,21] Collectively, these results support the value of comprehensive multi-marker profiling to guide therapeutic sequencing, rational combinations, and future biomarker-driven trial design.

Interestingly, a recent study identified a positive correlation between FRα and ER expression ($p = .040$), seemingly contrasting with the pre-clinical mechanistic studies suggesting inverse ER-mediated regulation of *FOLR1* expression.[5] This discrepancy may reflect differences between transcriptional regulation observed under experimental endocrine manipulation and static biomarker co-expression patterns measured in tumor specimens. Histologic composition may also confound these associations, as patients with high-grade serous carcinoma, which commonly expresses both ER and FRα, comprised the majority of both cohorts.[22] Importantly, the study was observational and designed to characterize biomarker co-expression patterns rather than evaluate endocrine intervention strategies. Therefore, although pre-clinical data raise the hypothesis that anti-estrogen exposure could modulate FRα expression and potentially influence therapeutic responsiveness, this concept was not directly tested in the current study. Furthermore, the current sample size was not adequately powered to robustly evaluate differential survival outcomes according to combined ER/FRα co-expression subgroups, and these exploratory findings should therefore be interpreted cautiously. The same prior study also reported an association between FRα over-expression and PD-L1 positivity, which was similarly observed in our cohort.[22] Together, these findings support the biologic heterogeneity of FRα-associated tumor phenotypes and reinforce the need for prospective validation incorporating standardized multi-marker profiling.

CLDN6 and ER co-expression has been reported in breast and endometrial cancers, where ER-dependent regulatory mechanisms influence CLDN6 expression and downstream signaling.[23,24] However, data regarding the interplay between these biomarkers in ovarian cancer remain limited, highlighting an important gap in ovarian cancer-specific understanding.

The absence of clear survival differences across FRα thresholds suggests that FRα expression may exist along a continuum rather than as a strictly binary therapeutic classification, exerting a graded effect analogous to the graded response observed with trastuzumab deruxtecan.[25] The comparable outcomes across FRα expression levels contrast with FORWARD-I, which supported a threshold-approach to FRα-targeted therapy.[26] Importantly, the current study was not designed to challenge mirvetuximab soravtansine indications or efficacy as defined in the prospective MIRASOL registrational trial.[4] Rather, it aimed to characterize real-world actionable biomarker co-expression patterns in recurrent platinum-resistant ovarian cancer. Accordingly, all biomarker and survival associations should be considered exploratory.

Stratified analyses suggested that FRα-high expression may be associated with improved progression-free survival among patients with HRD/loss of heterozygosity-high or *BRCA*-mutant tumors. However, given the retrospective design, these findings should be interpreted cautiously and should not be directly compared with MIRASOL. Differences in study design, patient selection, prior therapies, biomarker testing methodologies, and sample size limit cross-study comparability.

Among patients with FRα-high tumors, ER-negative/low-positive expression was associated with progression-free and overall survival outcomes comparable to those of patients with ER-positive tumors using a >10% threshold. This suggests that, within FRα-high disease, ER expression defined by a 10% cutoff may not meaningfully stratify survival risk. Current practice largely extrapolates from breast cancer, where ≥1% nuclear staining is commonly used to define ER positivity; however, the prognostic and predictive significance of varying ER expression levels in ovarian cancer remains poorly defined. In breast cancer, ER-low-positive disease is now recognized

as a distinct category in updated American Society of Clinical Oncology/College of American Pathologists guidelines, reflecting the biologic heterogeneity of this subgroup and its similarities to ER-negative tumors in endocrine sensitivity, molecular profile, and prognosis.[27,28]" In contrast, there remains no consensus regarding the optimal threshold for defining clinically relevant ER positivity in ovarian cancer.

An under-appreciated consideration in interpreting biomarker co-expression patterns is the distinct cellular and micro-environmental localization of clinically relevant targets. ER and PR are nuclear transcription factors, whereas FRα, HER2, and CLDN6 are cell-surface membrane proteins, commonly targeted by monoclonal antibody-based therapies and ADCs.[4,10,29,30] PD-L1 /PD-1 are expressed on tumor and immune cells within the tumor micro-environment.[31] These spatial differences may partially explain observed co-expression patterns and suggest that discordant biomarkers may represent biologically orthogonal vulnerabilities or potential therapeutic intersections.

Strengths and Weaknesses

Limitations include the retrospective, single-institution design, modest sample size, incomplete clinical annotation, and limited power for subgroup analyses. These factors may introduce selection bias, unmeasured confounding, and limited generalizability while precluding robust assessment of simultaneous associations between multiple biomarkers and survival outcomes. Biomarker testing reflected routine clinical practice rather than standardized prospective sampling, introducing variability in assay platform, tissue source, and timing of assessment. Assays were ordered at clinician discretion, which may have further contributed to selection bias. Since analyses were exploratory and unadjusted for multiple comparisons, findings should be considered hypothesis-generating and require prospective validation.

Despite these limitations, this study provides insights into biomarker co-expression in advanced ovarian cancer and identifies potential mechanistic interactions that may inform future biomarker-driven investigations using adequately powered trial designs (Supplementary). Prospective studies incorporating standardized tissue acquisition, harmonized biomarker testing methodologies, and comprehensive clinical annotation will be necessary before these findings can be translated into clinical decision-making.

Implications for Practice and Future Research

These findings highlight biomarker heterogeneity in ovarian cancer and the limited overlap among common actionable therapeutic targets. Collectively, the data support the development of ovarian cancer–specific multi-biomarker treatment frameworks and further evaluation of endocrine modulation as a potential strategy to enhance FRα expression and inform rational sequential and combinatorial approaches integrating endocrine therapy, ADCs, and ICIs.

Moreover, the results suggest that biomarker expression, particularly FRα and ER, may not conform to simple binary classifications but instead exist along a clinically meaningful continuum. These findings underscore the need to refine the clinical utility and therapeutic relevance of current ER positivity thresholds in ovarian cancer.

Future prospective studies incorporating standardized multi-marker profiling, longitudinal sampling, spatial transcriptomics, and multiplex imaging will be important to better characterize biomarker interactions, define temporal changes in expression, and optimize biomarker-guided therapeutic strategies.

CONCLUSIONS

Ovarian cancer demonstrates largely non-overlapping biomarker expression profiles across actionable therapeutic targets. Although co-expression between FRα, ER, PR, and CLDN6 was observed, most biomarkers exhibited predominantly independent expression patterns. FRα expression alone was not associated with clear survival differences across predefined categorical thresholds, and ER status did not further stratify outcomes within FRα-high tumors. These findings highlight the limitations of current single-marker, threshold-based approaches and support the need for integrated biomarker approaches incorporating molecular context, longitudinal assessment, and prospective validation in uniformly characterized cohorts.

Author Affiliations

[a]University of California Los Angeles, Department of Obstetrics and Gynecology, Los Angeles, CA, USA
[b]Tel Aviv University, Grey Faculty of Medicine & Health Sciences, Tel Aviv, Israel
[c]University of California, Los Angeles, Department of Medicine, Division of Hematology/Oncology, Los Angeles, CA, USA
[d]University of California, Los Angeles, Department of Medicine, Statistics Core, Los Angeles, CA, USA

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JG: Formal analysis, Validation, Methodology, Writing — review and editing
RS: Writing — review and editing
BYK: Supervision, Conceptualization, Resources, Writing — review and editing
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Data Availability The data underlying this study are available from the corresponding author upon reasonable request, subject to institutional review board approval and applicable data use agreements to ensure patient confidentiality.

Appendix A. Supplementary data Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijgc.2026.104893.

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