Characterization of *ESR1* mutations in Endometrial and Ovarian Cancers

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BACKGROUND

- Low-grade ovarian (OC) and endometrial (EC) cancers frequently express estrogen receptor (ERα, encoded by *ESR1*), and are considered hormonally responsive tumors.
- The use of endocrine therapy in the advanced and recurrent disease setting is common.
- Mechanisms for endocrine therapy failure in gynecologic cancers are not well understood.
- In breast cancer, *ESR1* mutations (*ESR1mt*) confer resistance to endocrine therapy.
- In this study, we aim to evaluate the prevalence of *ESRmt*, and associated characteristics in OC and EC.

METHODS

- 17,666 EC and 21,879 OC samples were analyzed by NGS of DNA (NextSeq, 592 genes, and NovaSeq, WES) and RNA (NovaSeq, WTS) (Caris Life Sciences, Phx, AZ).
- Tumor mutation burden (TMB) totaled all somatic mutations (mt) per tumor (TMB-H ≥ 10mt/MB).
- Statistical significance was determined using chi-square and Mann-Whitney U test adjusted for multiple comparisons (q<0.05).

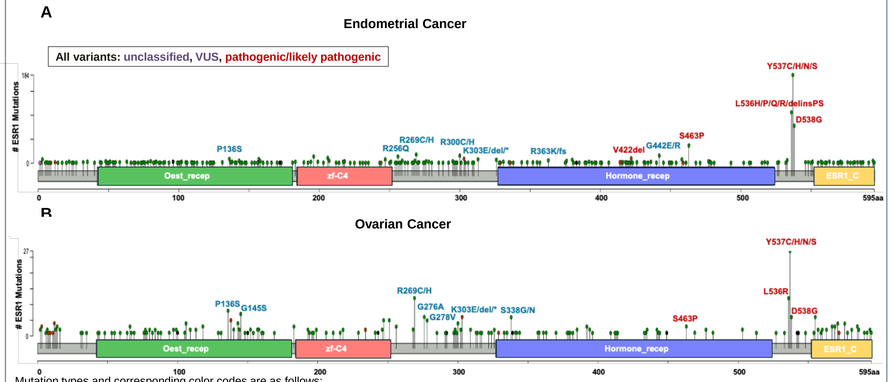
Table 1. Patient Characteristics

Endometrial		
Characteristics	***ESR1* MT**	***ESR1* WT**
N	421 (2.38%)	17245 (97.6%)
Age, median (range)	66 (28->89)	65 (0->89)
Histology, N (%)		
Carcinosarcoma	4 (0.45%)	888 (99.6%)
Clear Cell	0 (0%)	482 (100%)
Endometrioid	211 (4.18%)	4834 (95.8%)
Serous	7 (0.19%)	3680 (99.8%)
Low-grade Serous	0 (0%)	3 (100%)
Other/Mixed	199 (2.63%)	7358 (97.4%)
Site, N (%)		
Primary	240 (57%)	11825 (68.6%)
Metastatic	180 (42.8%)	5254 (30.5%)
Unclear	1 (0.42%)	166 (0.96%)

Ovarian		
Characteristics	***ESR1* MT**	***ESR1* WT**
N	49 (0.22%)	21879 (99.8%)
Age, median (range)	65 (29-88)	64 (56->89)
Histology, N (%)		
Carcinosarcoma	0 (0%)	709 (100%)
Clear Cell	0 (0%)	983 (100%)
Endometrioid	14 (1.48%)	933 (98.5%)
HGSOC	10 (0.07%)	13693 (99.9%)
Low-grade Serous	6 (1.08%)	550 (98.9%)
Mixed	0 (0%)	116 (100%)
Mucinous	1 (0.25%)	407 (99.8%)
Other	18 (0.40%)	4488 (99.6%)
Site, N (%)		
Primary	11 (22.5%)	9804 (44.8%)
Metastatic	38 (77.6%)	11826 (54.1%)
Unclear	0 (0%)	249 (1.14%)

RESULTS

Figure 1. Distribution of *ESR1* hotspot mutations in endometrial and ovarian cancer

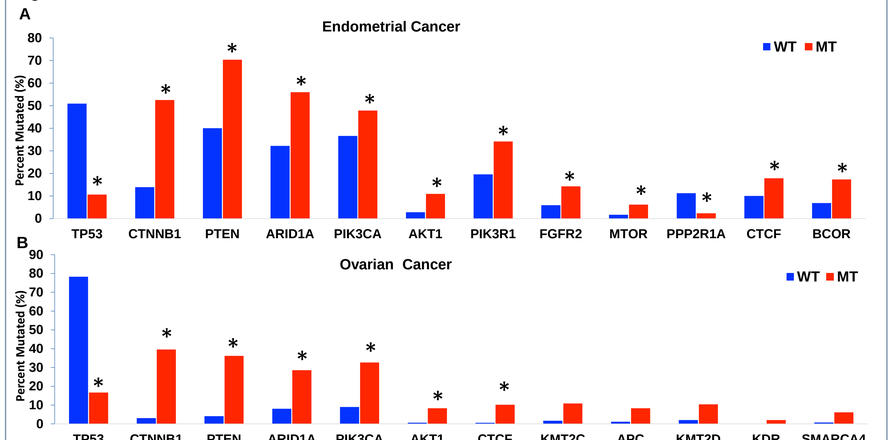


Mutation types and corresponding color codes are as follows:

- Missense Mutations
- **Truncating Mutations**: Nonsense, Nonstop, Frameshift deletion, Frameshift insertion, Splice site
- **Inframe Mutations**: Inframe deletion, Inframe insertion
- Splice Mutations
- Fusion Mutations
- Other Mutations: All other types of mutations

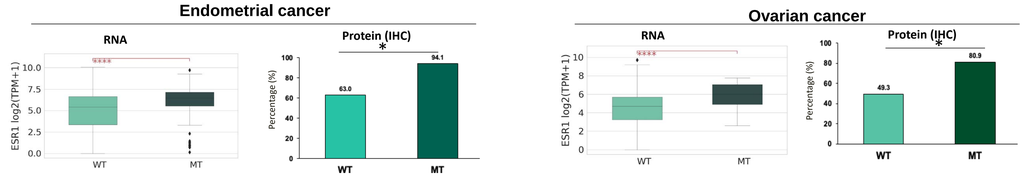
L536, Y537, and D538 were the most common *ESR1* hotspot mutations in endometrial and ovarian cancer.

Figure 2. Cooccurrence of other mutations in *ESR1* WT and mutant in endometrial and ovarian cancer



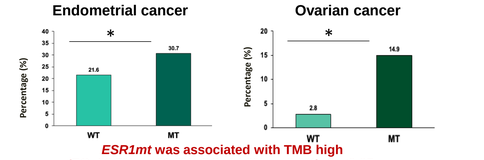
ESR1mt* were associated with decreased *TP53mt (EC: 10.7% vs 50.9%, OC: 16.7% vs 78.3%), **but increased PI3K pathway alterations** (EC: *PTENmt*: 70.4% vs 40%, *PIK3R1mt*: 34.1% vs 19.6%, *PIK3CAmt*: 47.9% vs 36.6%, *AKT1mt*: 11% vs 2.8%; OC: *PTENmt*: 36.2% vs 4.%, *PIK3CAmt*: 32.7% vs 8.97%, *AKT1mt*: 8.33% vs 0.62%), *CTNNB1mt* (EC: 52.5% vs 13.9%; OC: 39.6% vs 3.09%), *ARID1Amt* (EC: 56% vs 32.2%; OC:28.6% vs 8.04%). *q<0.05

Figure 3: Estrogen receptor (ER) expression in *ESR1* mutation samples



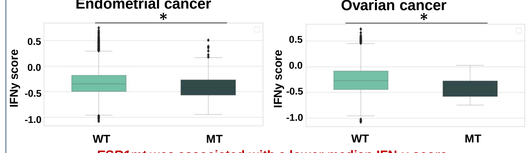
***ESR1mt* had higher ESR1 RNA expression (EC: 1.19-fc, OC: 1-.28-fc), ER+ (EC: 94.1% vs 63%, OC: 80.9% vs 49.3%). *q<0.05**

Figure 4: TMB-high analysis of *ESR1* WT and mutation



***ESR1mt* was associated with TMB high (EC: 30.7% vs 21.6%, OC: 14.9% vs 2.8%). *q<0.05.**

Figure 5: IFN-γ score analysis



***ESR1mt* was associated with a lower median IFN-γ score (EC: -0.42 vs -0.35, OC: -0.48 vs -0.27). * q<0.05.**

Table 2. *ESR1* mutation frequency in patients who received prior endocrine therapy

Endometrial cancer

AIs

Histology	Prior AIs			No Prior AIs			p-value	q-value
	%	N pos	Total N	%	N pos	Total N		
All Endometrial	6.3%	24	376	2.6%	36	1356	5.00E-04	0.02
Carcinosarcoma	-	-	-	-	-	-	-	-
Clear Cell	-	-	-	-	-	-	-	-
Endometrioid	14.7%	10	68	3.3%	14	415	1.00E-04	0.046
Serous	-	-	-	-	-	-	-	-

SERMs

Histology	Prior SERMs			No Prior SERMs			p-value	q-value
	%	N pos	Total N	%	N pos	Total N		
All Endometrial	4.1%	9	217	4.02%	21	522	0.93	0.96
Carcinosarcoma	-	-	-	-	-	-	-	-
Endometrioid	7.1%	2	28	5.03%	9	179	0.64	0.89
Serous	-	-	-	-	-	-	-	-

LHRH

	Prior LHRH			No Prior LHRH			p-value	q-value
	%	N pos	Total N	%	N pos	Total N		
All EC	6.4%	2	31	9.09%	1	11	0.77	0.95

Ovarian cancer

AIs

Histology	Prior AIs			No Prior AIs			p-value	q-value
	%	N pos	Total N	%	N pos	Total N		
All Ovarian	1.2%	6	478	0.18%	3	1666	0.0014	0.016
Carcinosarcoma	-	-	-	-	-	-	-	-
Clear Cell	-	-	-	-	-	-	-	-
Endometrioid	0.0%	0	13	1.03%	1	97	0.71	0.94
High-grade serous	1.07%	3	280	0%	0	1083	6.00E-04	0.21
Low-grade serous	4.3%	1	23	0%	0	136	0.01	0.18
Mucinous	-	-	-	-	-	-	-	-

SERMs

Histology	Prior SERMs			No Prior SERMs			p-value	q-value
	%	N pos	Total N	%	N pos	Total N		
All Ovarian	0.6%	2	290	0%	0	450	0.05	0.42
Carcinosarcoma	-	-	-	-	-	-	-	-
Clear Cell	-	-	-	-	-	-	-	-
Endometrioid	-	-	-	-	-	-	-	-
High-grade serous	0.5%	1	180	0%	0	390	0.14	0.61
Low-grade serous	-	-	-	-	-	-	-	-

Aromatase Inhibitors (AIs): Anastrozole, Exemestane, Letrozole; **Selective estrogen receptor modulators (SERMs):** Tamoxifen, Raloxifene; **LHRH:** Goserelin, Leuprolide

Endometrial and ovarian cancer had increased frequency of *ESR1* mutation post aromatase inhibitor therapy (EC: 6.3% vs 2.6%, OC: 1.2% vs 0.18%).

CONCLUSIONS

ESR1mt were more common in EC and were enriched in the endometrioid subtype, and in general associated with increased molecular alterations but a more cold immune microenvironment. Hotspot mutations known to confer endocrine resistance in breast cancer were most common and enrichment was observed in cases previously exposed to AIs, suggesting this may be a mechanism of resistance to endocrine therapy for some gynecologic malignancies.