Molecular analysis of non-epithelial ovarian cancer by histologic subtype

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Abstract

Background: Non-epithelial malignancies of the ovary account for <2%–4% of all ovarian tumors. The classification of non-epithelial ovarian cancer can be broadly divided into two histological subtypes, ovarian germ cell tumors (OGCTs) and ovarian sex cord stromal tumors (OSCSTs). Molecular phenotypes of these groupings may identify genetic susceptibilities to existing therapies and thus improve patient care.

Methods: 275 non-epithelial ovarian cancers (non-EOC) were profiled by Caris Life Sciences between 2009 and 2013 using a multiplatform approach. Within this cohort, patients could be further grouped according to histopathological subtypes, particularly OGCTs (n=42) and OSCSTs (n=217). Testing of FFPE tissues included a combination of sequencing (Sanger, NGS and pyrosequencing), protein expression (IHC), gene amplification (CISH or FISH), and/or RNA fragment analysis.

Results: Of interest, the frequency of positive hormone receptor over-expression was higher in OSCSTs than OGCTs(AR 76%v 0%, PR 90% v 12%, ER 12% v 0%). Granulosa Cell tumors (n=173) were particularly strongly expressing for AR (81.7%), PR (95.9%) but not for ER (9.3%), with Alk, APC, and ATM mutations occurring rarely. Sertoli-leydig tumors (n=25) were characterized by high Topo2A (66.7%), TUBB3 (65%), BRAF (14.3%) and kras (20%) mutation, with lower AR (38%) and PR (60%) and higher ER (28%) expression than granulosa cell tumors. OGCT (n=42) cohorts exhibited RRM1 (51%), TLE3 (55%), TOP2A (87%), TUBB3 (51%), PTEN loss (67%) with mutations of P53 (56%) and PI3K (22%) also occurring. Kras mutation was observed in 1 of 9 OGCTs tested.

Conclusions: Tumor profiling has identified molecular differences between non-EOC histological subtypes, by both expression and NGS mutational approaches in FFPE tissues. Identification of these changes can provide a rationale for treatment options not routinely considered or those associated with targeted therapies and warrant future clinical trials in this cancer type.

Background

- Cancers arising from the stromal and germ cell layers of the ovary are rare, heterogeneous, and require specialized management. [1, 2]
- OGCTs are derived from primitive germ cells of embryonic gonads and account for 2-10% of all ovarian tumors. They are most common in women under the age of 35.
- OSCSTs are derived from connective tissue cells and account for <5% of all ovarian tumors.
- Platinum-based chemotherapy is usually used to treat advanced/recurrent OGCTs. There is very little data available on the treatment of advanced/recurrent OSCSTs meaning that there is no defined standard of care.
- It is hoped that comprehensive tumor profiling can provide insights into molecular mechanisms and potential treatment options for these difficult-to-treat patients.

Methods

- 275 non-epithelial ovarian cancers (non-EOC) were profiled by Caris Life Sciences between 2009 and 2013 were initially evaluated. Selection of tests which were performed was based on physician requests. The diagnoses were based on reported pathology which reflects community practice. Review of the pathology reports after abstract submission found a number of patients should not be classified within this non-epithelial ovarian cohort and in total, 28 patients were discarded from further evaluation.
- IHC analysis was performed on formalin-fixed paraffin-embedded tumor samples using commercially available detection kits, automated staining techniques (Benchmark XT, Ventana, and AutostainerLink 48, Dako), and commercially available antibodies.
- Fluorescent in-situ hybridization (FISH) was used for evaluation of the HER-2/neu [HER-2/CEP17 probe], EGFR [EGFR/CEP7 probe], and cMET [cMET/CEP7 probe] (Abbott Molecular/Vysis). HER-2/neu and cMET status were evaluated by chromogenic in-situ hybridization (INFORM HER-2 Dual ISH DNA Probe Cocktail; commercially available cMET and chromosome 7 DIG probe; Ventana). The same scoring system was applied as for FISH.
- Direct sequence analysis was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor samples using the Illumina MiSeq platform. Specific regions of 45 genes of the genome were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel.
- Mutation analysis by Sanger sequencing included selected regions of BRAF, KRAS, c-KIT, EGFR, and PIK3CA genes and was performed by using M13-linked PCR primers designed to amplify targeted sequences.
- In Caris Molecular Intelligence™ (CMI) reports provided to the ordering physician after comprehensive tumor profiling, treatments associated with benefit were found in 99.5% of patients.
- Immunohistochemistry provided 99.5% of patients (246/247) with a potential treatment associated with benefit (with a median of 6 biomarkers linked positive predictive associations per patient) compared to biomarkers measured by ISH providing a treatment associated with benefit in 2.1 % of cases tested (5/241). NGS found a mutation in 30.8% of tumors tested (20/65) compared to Sanger sequencing with a mutation found in 3.7% of tumors tested (2/54).
- Statistical analysis (unpaired t-tests used to compare biomarker expression across histologic subtypes) performed using GraphPad™.

Demographics

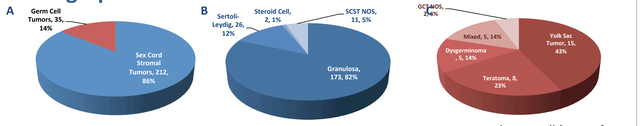


Figure 1: Distribution of patients within non-epithelial ovarian cancer cohort (A) ,OSCT (B) and OGCT (C) subgroups with age by subgroup (D).

The overall number of OGCTs analyzed compared to OSCSTs is lower than would be expected in the normal distribution of disease. This may reflect the ordering practices of the medical community and may be explained by OSCSTs having less treatment options along with higher recurrence rate. As expected in the adjacent table, OGCTs occurred in younger patients compared to OSCSTs.

D

	n	Average	Median	Range
Sex Cord Stromal Tumors	212	53	54	8-86
Granulosa Cell Tumors	*173*	*57*	*56*	*19-86*
Sertoli-Leydig	*26*	*34*	*24*	*8-75*
Steroid Cell	*2*	*39*	*39*	*34-45*
SCST NOS	*11*	*41*	*41*	*14-63*
Germ Cell Tumors	35	31	26	4-89
Yolk Sac Tumor	*15*	*33*	*27*	*4-89*
Teratoma	*8*	*40*	*36*	*19-59*
Dysgerminoma	*5*	*26*	*22*	*18-43*
Mixed	*5*	*24*	*23*	*16-35*
GCT NOS	*2*	*20*	*20*	*19-21*
TOTAL	247	50	52	4-89

Results – Tumor Profiling in Non-epithelial Ovarian Cancer

- Comprehensive tumor profiling identified significant differences between OSCSTs and OGCTs cohorts.
- Significantly higher number of OSCSTs overexpressed hormone receptors compared to OGCTs (AR: 76.4% vs 0%, p<0.0001, ER: 12.3% vs 0%, p=0.0281; PR 90.5% vs 14.3% (p<0.001).
- In addition, OSCSTS had significantly more RRM1 loss (81.1% vs 68.6%, p<0.0001) and TUBB3 loss (73.1% vs 46.7%, p=0.0038) than OGCTs.
- OGCTs had significantly more PTEN loss (68.6% vs 45%, p=0.0097) with significantly more tumors having overexpression of TOP2A (84% vs 31.1%, p<0.001) compared to OSCSTs.
- OGCTs had significantly more EGFR gene amplification (14.3% vs 1%, p=0.0023) compared to OSCSTs.

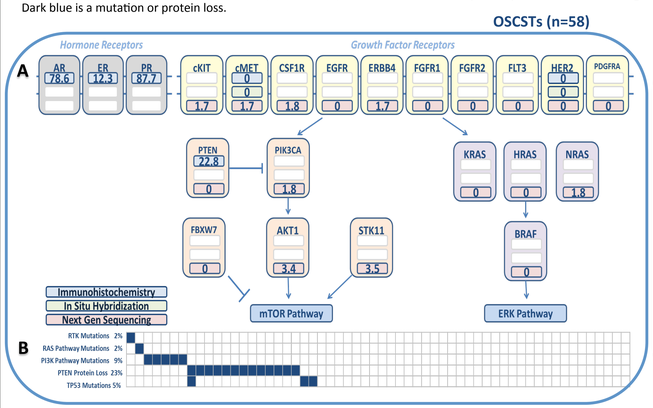
Table 1: Comparison of biomarker changes in patients with OSCSTs (n=212) and OGCTs (n=35)

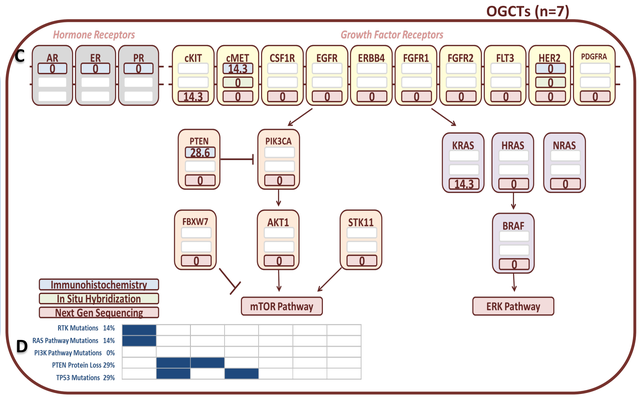
Biomarker	Platform	Sex Cord Stromal Tumors	Germ Cell Tumors	Thresholds/ Amino Acids Covered	p-value
AR	IHC	76.4% (81/106)	0% (0/12)	=0+ or <10% or ≥1+ and ≥10%	**<0.0001**
cMET	IHC	2.4% (5/209)	5.7% (2/35)	<50% or <2+ or ≥2+ and ≥50%	0.2778
ER	IHC	12.3% (26/211)	0% (0/35)	<2+ or ≤3+ and <50% or =2+ and <75% or ≥3+ and ≥50% or ≥2+ and ≥75%	**0.0281**
ERCC1 Loss	IHC	74.6% (97/130)	87.5% (21/24)	<2+ or ≤3+ and <10% or =2+ and <50% or ≥3+ and ≥10% or ≥2+ and ≥50%	0.1728
HER2	IHC	0% (0/211)	0% (0/35)	≤1+ or =2+ and ≤10% or ≥3+ and >10%	-
MGMT Loss	IHC	64.9% (137/211)	68.6% (24/35)	=0+ or ≤35% or ≥1+ and >35%	0.6762
PGP	IHC	4.8% (8/166)	20% (6/30)	=0+ or <10% or ≥1+ and ≥10%	**0.0028**
PR	IHC	90.5% (191/211)	14.3% (5/35)	=0+ or <10% or ≥1+ and ≥10%	**<0.0001**
PTEN Loss	IHC	45% (95/211)	68.6% (24/35)	=0+ or ≤50% or ≥1+ and >50%	**0.0097**
RRM1 Loss	IHC	81.1% (146/180)	45.2% (14/331)	=0+ or <50% or <2+ or ≥2+ and ≥50%	**<0.0001**
SPARC	IHC	24.6% (52/211)	22.9% (8/35)	<30% or <2+ or ≥2+ and ≥30%	0.8205
TOPO1	IHC	43.9% (79/180)	41.9% (13/31)	=0+ or <30% or <2+ or ≥2+ and ≥30%	0.8404
TOP2A	IHC	31.1% (47/151)	84% (21/25)	=0+ or <10% or ≥1+ and ≥10%	**<0.0001**
TS Loss	IHC	47.4% (36/76)	37.5% (3/8)	=0+ or ≤3+ and <10% or ≥1+ and ≥10%	0.5997
TUBB3 Loss	IHC	73.1% (122/167)	46.7% (14/30)	<30% or <2+ or ≥2+ and ≥30%	**0.0038**
cMET	FISH	0% (0/96)	0% (0/14)	*Positivity for increased gene copy number by FISH has been defined as ≥ 5 copies in lung tumor cells. The gene copy number threshold for other tumor types has not been determined.*	-
EGFR	FISH	1% (1/96)	14.3% (3/21)	*Positivity for increased gene copy number by FISH has been defined as ≥ 4 copies in 40% or more tumor cells. Gene amplification is defined by the presence of a gene/chromosome per cell ratio of ≥2, or ≥1 copies of the genes per cell in ≥10% of analyzed cells*	**0.0023**
HER2	FISH	0% (0/204)	0% (0/34)	*HER2/Neu:CEP17 signal ratio of ≥ 2.0; and non-amplification as <2.0 as per Ventana INFORM HER2 CISH Package Insert*	-
ATK1	NGS	3.4% (2/58)	0% (0/7)	*Amino acids 16-47*	0.6243
APC	NGS	5.3% (3/57)	0% (0/7)	*Amino acids 866-927, 1105-1161, 124801583*	0.5416
ATM	NGS	5.4% (3/56)	0% (0/7)	*Amino acids 343-355, 400-412, 601-633, 837-880, 1300-1331, 1670-1773, 1784-1832, 1931-1973, 2436-2488, 2656-2670, 2685-2756, 2880-2891, 2938-3056*	0.538
cKIT	NGS	1.7% (1/58)	14.3% (0/7)	*Amino acids 42-101, 4930592, 632-745, 806-866*	**0.0709**
cMET	NGS	1.7% (1/58)	0% (0/7)	*Amino acids 168-218, 366-400, 1105-1132, 1238-1284*	0.7312
KRAS	NGS	0% (0/58)	14.3% (1/7)	*Amino acids 1-31, 38-71 and 97-150*	**0.0032**
NRAS	NGS	1.8% (1/57)	0% (0/7)	*Amino acids 1-27 and 38-71*	0.729
PIK3CA	NGS	1.8% (1/56)	0% (0/7)	*Amino acids 75-118, 336-353, 418-555, 692-729 and 979-1068*	0.7268
STK11	NGS	2.5% (2/58)	0% (0/7)	*Amino acids 27-86, 184-219, 252-288, 324-370*	0.6212
TP53	NGS	5.2% (3/58)	28.6% (2/7)	*Amino acids 1-20, 60-121, 126-307 and 322-346*	**0.0283**

Results – Pathway Alteration Analysis in OSCSTs and OGCTs – NGS

Figure 2: Receptor Overexpressions and Pathway Alterations Observed in OSCSTs and OGCTs – Data only from tumors analyzed by NGS

Figures 2A and 2C show the relative changes in hormone receptor, growth factor receptor and pathway alterations found in OSCSTs and OGCTs respectively. Figures 2B and 2D shows all patients in whom a mutation in an RTK, RAS pathway or PIK3CA pathway or PTEN loss occurred and the overlap in these alterations within these patients. Data from all 59 OSCST patients and 7 OGCTs in whom NGS was performed is shown. RTK mutations include mutations in either cKIT, cMET, CSF1R, EGFR, ERBB4, FGFR1, FGFR2, FLT3, HER2 and PDGFRA. RAS pathway mutations have been grouped as KRAS, NRAS, HRAS and BRAF. PI3K pathway alterations include PIK3CA, PTEN, FBXW7, AKT1 and STK11. Dark blue is a mutation or protein loss.

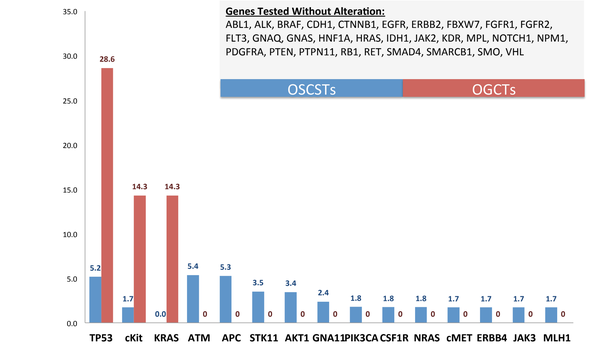




Results – Mutation Prevalence in OSCSTs and OGCTs

- Although low number of patients in the OGCT cohort had NGS performed, significantly higher rates of *TP53* (28.6% vs 5.2%, p=0.00283) and *KRAS* (14.3% vs 0%, P=0.0032) mutations were found in OGCTs. A trend for *cKIT* mutation rate to be higher in OGCTs did not reach significance (p=0.0709).
- In OSCSTs, overlapping mutations in the *APC* and *ATM* gene were observed in 2 patients, with co-existing mutations in *cKIT* and *AKT1*, *cMET* and *ATM*, and *STK11* and *MLH1* observed in one patient each (all overlapping mutations were found in granulosa patients)
- One OGCT tumor (yolk sac) had mutations in both the *cKIT* and *KRAS* genes.
- Mutations in activating oncogenic pathways such as RTK/RAS/PI3K occurred independently of the loss of PTEN.

Figure 2: Mutation Prevalence Observed in OSCSTs and OGCTs



Conclusions

- Comprehensive tumor profiling using IHC, ISH, and gene sequencing detected mutations in 99.6% of patients.
- Next generation sequencing detected approximately 10x as many mutations as Sanger sequencing (30.8% vs 3.7%).
- SCSTs appears to be a disease associated with frequent loss of the PTEN, RRM1 and TUBB3 tumor suppressors, as well as endocrine receptor over expression.
- OGCTs may have a higher percentage of TP53 mutations than SCSTs, suggesting that genomic chaos is an important mechanism in the pathogenesis of these tumors; this interesting hypothesis will require further validation.

References

1. Kouloris CR and Penson RT. Ovarian Stromal and Germ Cell Tumors. Semin Oncol (2009) 36:126-136. doi:10.1053/j.seminoncol.2008.12.004
2. Reed N, Millan D, Verheijen R and Castiglione M on behalf of the ESMO Guidelines Working Group. Non-epithelial ovarian cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology (2010) 21(Supplement 5):v31-v36