Molecular profiling of clear cell ovarian carcinoma

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Abstract

Background: Clear cell ovarian carcinomas (CCOCs) are a distinct histopathological subtype and account for 5-10% of epithelial ovarian cancers (EOCs). They have a poor prognosis in advanced stages and at recurrence. They are commonly resistant to platinum-based chemotherapy and treatment options are limited in patients with progressive disease. Molecular profiling may identify patient subsets who could benefit from targeted therapies when standard treatment has failed and also provide an insight into the genomic heterogeneity of CCOC's that share a similar phenotype.

Methods: Over 435 CCOC's referred to Caris Life Sciences (from 2009 - 2014) were evaluated; diagnoses were based on reported pathology. Specific testing was performed per physician request and included a combination of sequencing (Sanger, NGS or pyrosequencing), protein expression (IHC), gene amplification (CISH or FISH), and/or RNA fragment analysis.

Results: Patients were further grouped into pure CCOCs (n=363) and mixed CCOCs (n=72). The most common findings in CCOC's were overexpression of TOP2A (61%), TS (52%), TLE3 (48%), loss of TUBB3 (49%) and MGMT (56%). cMET was overexpressed in 19% of CCOCs tested and in 6% of mixed CCOCs. CCOCs had lower expression of AR, ER, and PR (7%, 9%, and 15%) than mixed CCOCs (21%, 39%, and 31%) and EOCs (24%, 45%, and 30%). In 69 CCOC's analyzed by NGS, PIK3CA was the most common mutation (52% - vs. 8% in all EOCs and 14% in mixed CCOCs) followed by TP53 (16%) and KRAS (11%). Mutations in FBXW7 (10%), APC (7%) and ATM (6%) were observed at a higher rate than in all EOCs. No BRAF mutations were seen. In the 33 CCOCs with PIK3CA mutations, 4 (12%) had co-existing mutations in KRAS and 2 (6%) had TP53 mutations while 70% (23/33) overexpressed cMET and 12% had a loss of PTEN.

Conclusions: Molecular profiling of proteins, gene expression, and mutations underscores the heterogeneity of CCOC and the potential role in better selecting patients for clinical trials. Drugs, which target the mTOR pathway or cMET may have therapeutic potential in selected subsets. Mutations in FBXW7, APC and ATM may also help direct patients to trials of targeted therapies.

Background

- Clear cell ovarian carcinomas (CCOCs) are a relatively uncommon but a distinct histopathological subtype that and account for 5-10% of epithelial ovarian cancers (EOCs) in the Europe and North America, but constitute 25% of EOCs in Asia. Although they usually present at an early stage and are often cured with surgery, they have a poor prognosis when beyond FIGO Stage 1 as well as in patients with recurrent disease who have particularly bad outcomes.
- They have poor prognosis in advanced stages and at recurrence and are typically resistant to platinum-based chemotherapy and treatment options are limited and with minimal benefit reported with most chemotherapeutic agents. There are isolated case reports of responses to tyrosine kinase inhibitors.
- There has been increased interest in identifying molecular targets which has led to an improved understanding of the unique features of this group of EOC's[1, 2] which are characterized by frequent activating mutations in the PIK3CA pathway which is a complex signaling network coordinating upstream inputs from growth factors, tyrosine kinase receptors and other receptors such as Met as well as cross talk with the Ras/Raf/Mek/Erk pathway via direct input from Ras.

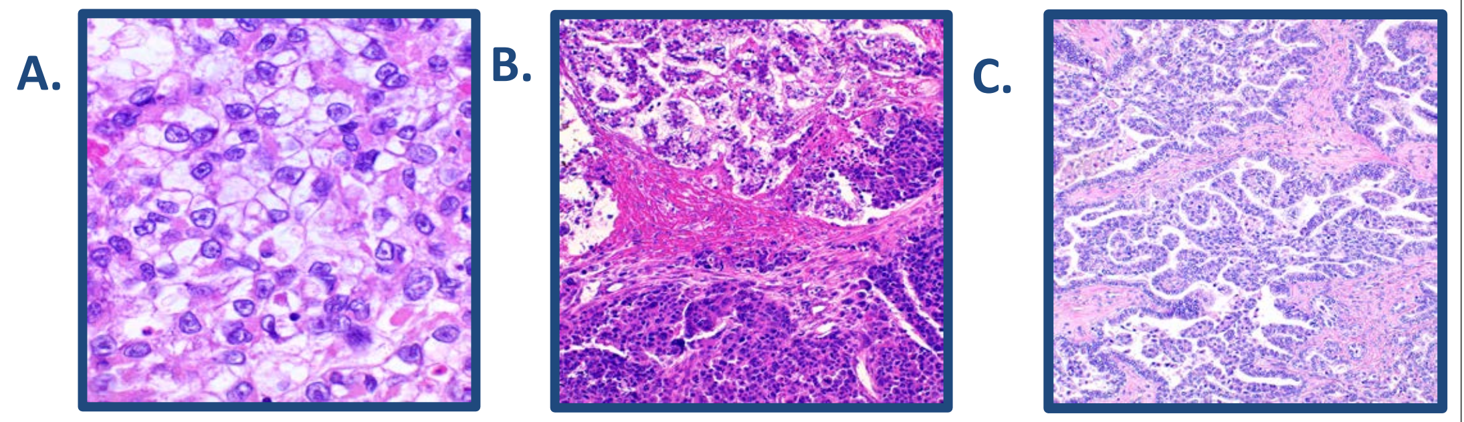


Figure 1: Histopathology of CCOC and differential diagnoses
(A) classic clear cell carcinoma
(B) mixed clear cell (top) and serous (bottom) carcinoma
(C) serous carcinoma with secretory change (could be confused with clear cell carcinoma).
Images kingly provided by Professor Peter Russell, Douglass Hanly Moir Pathology

Methods

- 435 CCOCs referred to Caris Life Sciences were profiled by Caris Life Sciences between 2009 and 2013 and considered for inclusion in this cohort. Specific testing was performed per physician request and included a combination of sequencing (Sanger, NGS or pyrosequencing), protein expression (IHC), gene amplification (CISH or FISH), and/or RNA fragment analysis.
- 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 96.6% of patients tested (420/435).
- Immunohistochemistry provided 95.8% of patients (415/433) with at least one treatment associated with benefit (with a median of 3 biomarkers linked to positive predictive associations per patient) compared to biomarkers measured by ISH providing a treatment associated with benefit in 13.1 % of cases tested (49/374). NGS found a mutation in 72.2% of tumors tested (60/83) compared to Sanger sequencing with a mutation found in 32% of tumors tested (24/75).
- Statistical analysis (unpaired t-tests used to compare biomarker expression across histologic subtypes) performed using GraphPad™.

Demographics

- No clinical data on disease stage, recurrence or prior treatment history was collected for these samples.
- Based on review of the original pathology reports, patients subdivided into "pure" or "mixed" CCOC based on pathology report and analyzed separately.
- Age and site of biopsy (primary versus other metastatic sites) was balanced across pure and mixed CCOC.

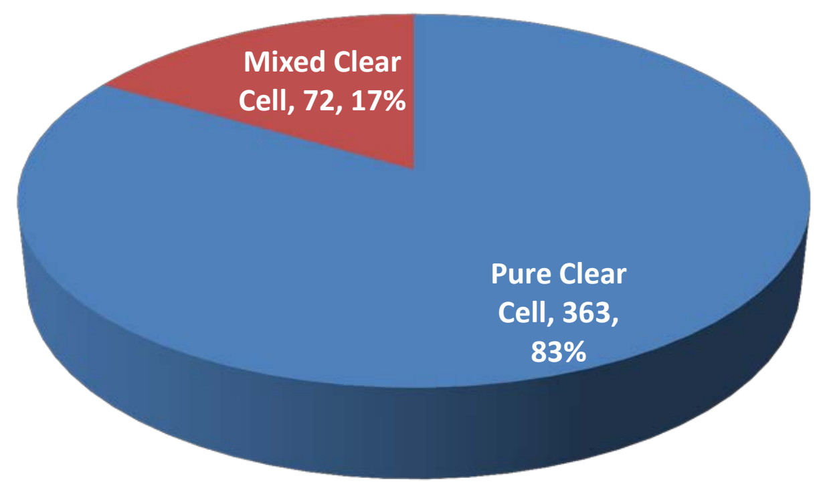


Figure 2: Distribution of patients in "Pure" and "Mixed" CCOC Cohorts

Results – Comprehensive Tumor Profiling of CCOCs

- Comparison of the cohorts found that the pure CCOC cohort are likely to have less hormone receptor expression with significantly less AR (p=0.0112), ER (p<0.0001) and PR (p=0.0015) expression compared to mixed CCOC.
- Pure CCOC tumors were also found to express significantly more cMET (p=0.0152) and TOPO1 (p=0.0407) and had less MGMT loss (p=0.0009) than mixed CCOCs.

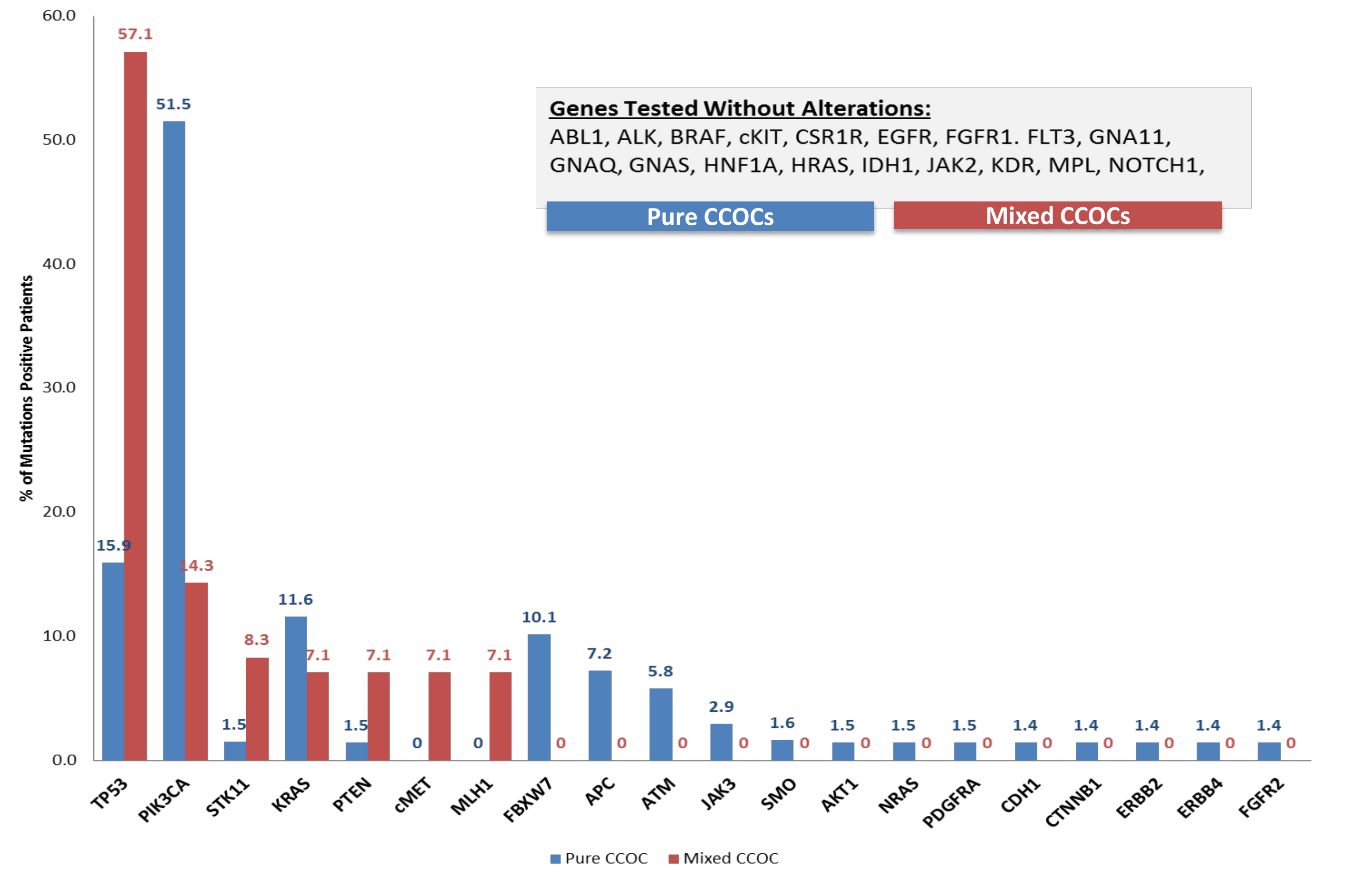
TABLE 1: Percentage (tests performed/number of patients) of biomarker alterations which may be actionable based on predictive associations with treatments in cohorts of pure clear cell (n=363) and mixed clear cell (n=72) ovarian cancer patients.

PURE CLEAR CELL CARCINOMA (n=363)	MIXED CLEAR CELL CARCINOMA (n=72)	Biomarker	Platform	Thresholds
7.3% (12/164)	**21.1%** (8/38)	**AR**	**IHC**	*=0+ or <10% or ≥1+ and ≥10%*
19.1% (61/319)	**6.5%** (4/62)	**cMET**	**IHC**	*<50% or <2+ or ≥2+ and ≥50%*
8.7% (31/357)	**38.9%** (28/72)	**ER**	**IHC**	*<2+ or ≤3+ and <50% or =2+ and <75% or ≥3+ and ≥50% or ≥2+ and ≥75%*
78.8% (186/236)	**89.8%** (44/49)	**ERCC1 Loss**	**IHC**	*<2+ or ≤3+ and <10% or =2+ and <50% or ≥3+ and ≥10% or ≥2+ and ≥50%*
2.3% (8/355)	**0%** (0/72)	**HER2**	**IHC**	*≤1+ or =2+ and ≤10% or ≥3+ and >10%*
43.5% (147/338)	**65.2%** (45/69)	**MGMT Loss**	**IHC**	*=0+ or ≤35% or ≥1+ and >35%*
16.9% (52/308)	**9.8%** (6/61)	**PGP**	**IHC**	*=0+ or <10% or ≥1+ and ≥10%*
15.0% (53/354)	**30.6%** (22/72)	**PR**	**IHC**	*=0+ or <10% or ≥1+ and ≥10%*
45.4% (162/357)	**54.2%** (39/72)	**PTEN Loss**	**IHC**	*=0+ or ≤50% or ≥1+ and >50%*
80.6% (253/314)	**83.9%** (52/62)	**RRM1 Loss**	**IHC**	*=0+ or <50% or <2+ or ≥2+ and ≥50%*
18.7% (64/342)	**15.9%** (11/69)	**SPARC**	**IHC**	*<30% or <2+ or ≥2+ and ≥30%*
37.8% (118/312)	**24.2%** (15/62)	**TOPO1**	**IHC**	*=0+ or <30% or <2+ or ≥2+ and ≥30%*
60.6% (171/282)	**68.4%** (39/57)	**TOP2A**	**IHC**	*=0+ or <10% or ≥1+ and ≥10%*
47.9% (67/140)	**45.2%** (14/31)	**TS Loss**	**IHC**	*=0+ or ≤3+ and <10% or ≥1+ and ≥10%*
49.2% (131/266)	**54.9%** (28/51)	**TUBB3 Loss**	**IHC**	*<30% or <2+ or ≥2+ and ≥30%*
4.3% (5/116)	**0%** (0/21)	**cMET**	**FISH**	*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.*
9.7% (30/309)	**5.3%** (3/57)	**HER2**	**FISH**	*HER2/Neu:CEP 17 signal ratio of ≥ 2.0; and non-amplification as <2.0 as per Ventana INFORM HER2 CISH Package insert*
0% (0/69)	**7.1%** (1/14)	**cMET**	**NGS**	*Amino acids 168-218, 366-400, 1105-1132 and 1238-1284*
1.4% (1/69)	**0%** (0/14)	**HER2**	**NGS**	*Amino acids 746-827 and 832-883*
11.6% (8/69)	**7.1%** (1/14)	**KRAS**	**NGS**	*Amino acids 1-31, 38-71 and 97-150*
1.5% (1/68)	**0%** (0/14)	**NRAS**	**NGS**	*Amino acids 1-27 and 38-71*
1.5% (1/68)	**0%** (0/14)	**PDGFRA**	**NGS**	*Amino acids 552-596 and 650-719*
51.5% (35/68)	**14.3%** (2/14)	**PIK3CA**	**NGS**	*Amino acids 75-118, 336-353, 418-555, 692-729 and 979-1068*
1.5% (1/68)	**7.1%** (1/14)	**PTEN**	**NGS**	*Amino acids 1-27, 165-267 and 280-342*
15.9% (11/69)	**57.1%** (8/14)	**TP53**	**NGS**	*Amino acids 1-20, 60-121, 126-307 and 322-346*

Results - Mutation Prevalence in CCOC

- The prevalence of PIK3CA mutations was significantly higher in pure CCOC compared to mixed CCOC (51.5% vs 14.3%, p=0.0198).
- TP53 mutations were present in significantly higher numbers of mixed CCOC compared to pure CCOCs (57.1% vs 15.9%, p=0.0006).
- Mutations in cMET and MLH1 were found in mixed CCOCs at a significantly higher rate than in pure CCOCs (p=0.0255 for both).

Figure 3: Mutation prevalence in patients with pure CCOC (n=69) and mixed CCOC (n=14) tested with NGS



Genes Tested Without Alterations:
ABL1, ALK, BRAF, cKIT, CSR1R, EGFR, FGFR1. FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, KDR, MPL, NOTCH1,

- As can be seen in Table 2, there are a number of co-existing mutations in pure CCOC.
- Taking these into consideration may help to identify potential synergistic and resistance mechanisms for drugs in early clinical investigation.

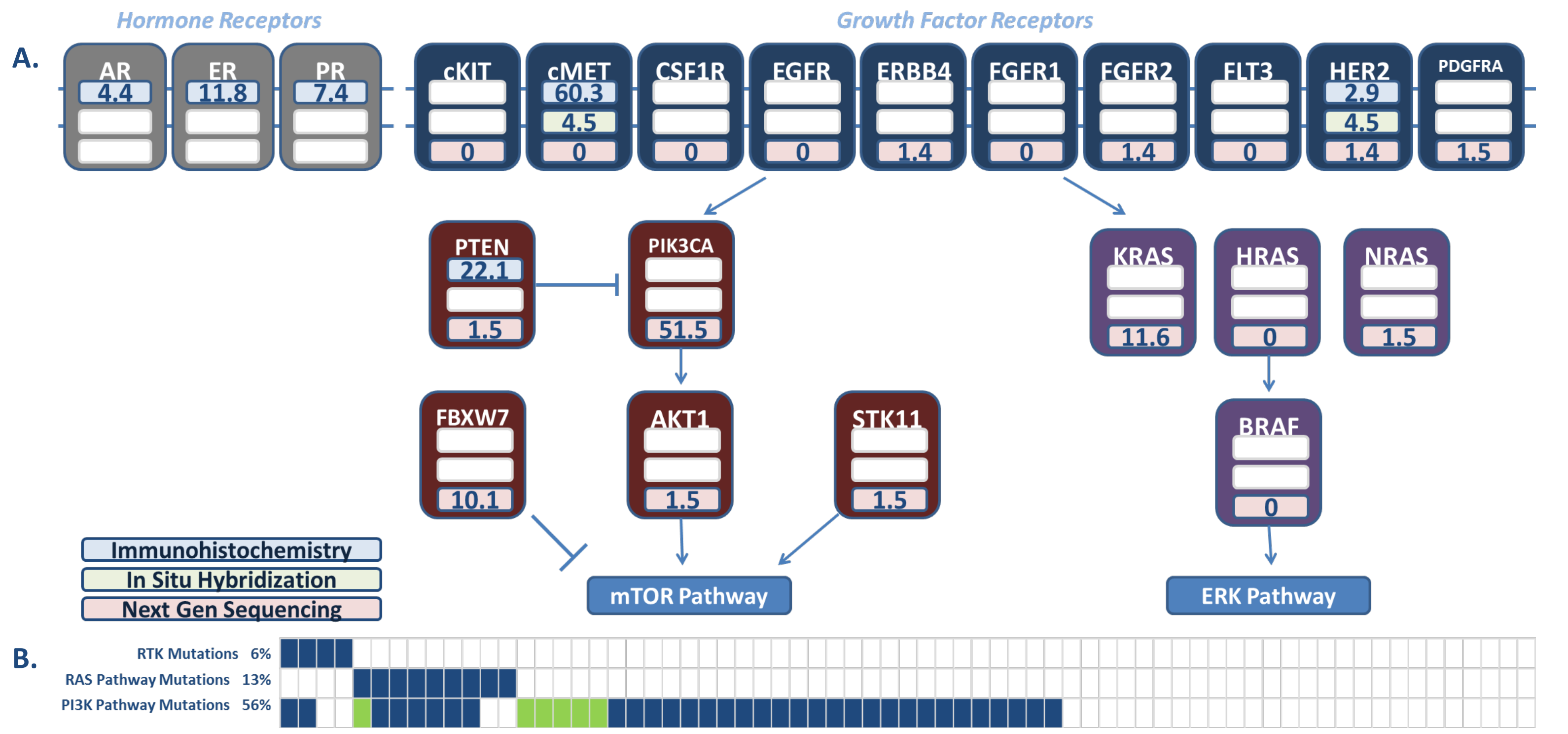
TABLE 2: The prevalence and overlap of all mutations observed by NGS in a cohort of 69 pure CCOC patients.

	AKT1	APC	ATM	CDH1	CTNNB1	ERBB2	ERBB4	FBXW7	FGFR2	JAK3	KRAS	NRAS	PDGFRA	PIK3CA	PTEN	SMO	STK11	TP53	
AKT1	**1.5% (1/68)**																		*Akt Inhibitors*
APC	0	**7.2% (5/68)**																	*WNT pathway inhibitors*
ATM	0	1	**5.8% (4/69)**																*PARP inhibitors, HDAC inhibitors*
CDH1	0	0	0	**1.4% (1/69)**															*No clinical trials*
CTNNB1	0	1	1	0	**1.4% (1/69)**														*WNT pathway inhibitors*
ERBB2	0	1	1	0	1	**1.4% (1/69)**													*Anti-Her2 monoclonal antibody or conjugate, HER2-targeted TKIs*
ERBB4	0	0	0	0	0	0	**1.4% (1/69)**												*EGFR TKIs, pan-HER inhibitors*
FBXW7	0	1	2	1	1	1	1	**10.1% (7/69)**											*No clinical trials*
FGFR2	0	0	0	0	0	0	0	0	**1.4% (1/69)**										*FGFR Antibodies, FGFR ligand traps*
JAK3	0	0	0	0	0	0	0	0	0	**2.9% (2/69)**									*No clinical trials*
KRAS	1	0	0	0	0	0	0	1	0	1	**11.6 (8/69)**								*MEK inhibitors, ERK inhibitors, regorafenib*
NRAS	0	0	0	0	0	0	0	0	0	0	0	**1.5% (1/68)**							*MEK inhibitors, ERK inhibitors*
PDGFRA	0	0	0	0	0	0	0	0	0	0	0	0	**1.5% (1/68)**						*PDGFRA inhibitors*
PIK3CA	0	3	3	0	0	0	0	2	0	1	4	1	0	**51.5% (35/68)**					*PI3K/AKT/mTOR inhibitors, aspirin*
PTEN	0	0	0	1	0	0	0	1	0	0	0	0	0	0	**1.5% (1/68)**				*PI3K/AKT/mTOR inhibitors, PARP inhibitors*
SMO	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	**1.6% (1/61)**			*SMO inhibitors*
STK11	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	**1.5% (1/66)**		*No clinical trials*
TP53	0	0	0	1	0	0	0	1	0	1	3	0	1	2	1	0	0	**15.9% (11/69)**	*WEE1 inhibitors, CHK1 inhibitor, biologicals, P53 activators*

Results - Pathway Alterations in Pure CCOC

- Data from all 69 patients in whom NGS was performed is shown.
- Table B shows all patients in who a mutation in an RTK, RAS pathway or PIK3CA pathway occurred and the overlap in mutations within these patients. Dark blue is a mutation, green indicates 2 mutations present.
- 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.

Figure 4: Pathway Alterations in Pure CCOC (A) with overlap of mutations (B)



- Nearly three quarters of pure CCOC are apparently driven by alterations in PI3K pathway
- Of 69 pure CCOC patients profiled using NGS, only 19 (28%) had no PI3K pathway alterations (PIK3CA, PTEN, FBXW7, AKT1, STK11 genes wildtype and normal PTEN expression)

Table 3: Differences in PIK3CA mutated and PIK3CA wildtype patients

PIK3CA Mutated (52% of pure CCOC tumors profiled)			
cMET overexpressed	PTEN Loss	KRAS Mutated	TP53 Mutated
70%	12%	12%	6%
PIK3CA Wildtype (48% of pure CCOC tumors profiled)			
cMET overexpressed	PTEN Loss	KRAS Mutated	TP53 Mutated
51%	31%	11%	26%

Conclusions

- Demonstrates the complexity and molecular heterogeneity of CCOC and confirms the findings of other groups with respect to common mutations
- Highlights the differences between pure and mixed CCOC. Traditionally, these are grouped together but this study shows that molecular analysis is so crucial to help better define CCOC subsets.
- Pure CCOCs exhibit significant genetic heterogeneity, but majority characterised by mutations in PIK3CA pathway or PTEN loss – should direct future trials
- cMET overexpression high in subset with PIK3CA mutations suggesting a possible role for a combinatorial approach with cMET and mTOR or angiogenesis inhibitors
- Other potential targets in subsets- e.g HER2
- TP53 mutations are known to be very uncommon in CCOC and presence in a tumour should raise suspicion and a high grade serous / endometrioid cancer should be excluded.
- Most mixed clear cell cancers are very different from pure should not be included in clear cell trials (but currently are)

References

1. Kobayashi H, Kajiwara H, Kanayama S et al. Molecular pathogenesis of endometriosis-associated clear cell carcinoma of the ovary. Oncol Rep. 2009 Aug;22(2):233-40.
2. Yamamoto S, Tsuda H, Miyai K et al. Gene amplification and protein overexpression of MET are common events in ovarian clear-cell adenocarcinoma: their roles in tumor progression and prognostication of the patient. Mod Pathol. 2011 Aug;24(8):1146-55.