Genomic profiles inform treatment decisions and enable future drug and biomarker discovery

D. A. Zajchowski, PhD[1], M. Whitlow, PhD[2], K. M. Zajchowski[1], L. K. Shawver, PhD[1]

[1]The Clearity Foundation, San Diego, CA and [2]Colabrativ, Inc., Orinda, CA.

INTRODUCTION

Despite major progress in the molecular characterization of ovarian cancers (OC), women with recurrent, advanced stage OC continue to be treated with cytotoxic chemotherapy agents that achieve poor overall response rates. This contrasts with the treatment paradigm for other cancers, where outcomes have been improved by selecting treatment based upon "actionable" genomic alteration(s) that are in drug-targetable pathways. There is a need to determine if molecular profiling for OC patients can improve treatment outcomes by informing therapy choices. Such a profile should comprehensively identify "actionable" genetic aberrations as well as measure expression levels of proteins that are drug targets/response biomarkers.

As part of molecular profiling analyses performed for recurrent ovarian cancer patients to inform selection of their next treatment, the presence of mutations or alterations (e.g., copy number (CN) changes) in ~200 genes that encode proteins that are key mediators in oncogenic and tumor suppressive pathways were determined using a validated exon-capture next-gen sequencing platform provided in a CLIA-certified laboratory setting. The expression of target proteins for some FDA-approved therapies was also measured. Comparisons of high grade serous (HGS) to non-HGS histology tumors are described.

Figure 1. Frequency of Genetic Alterations in 99 Epithelial Ovarian Cancers

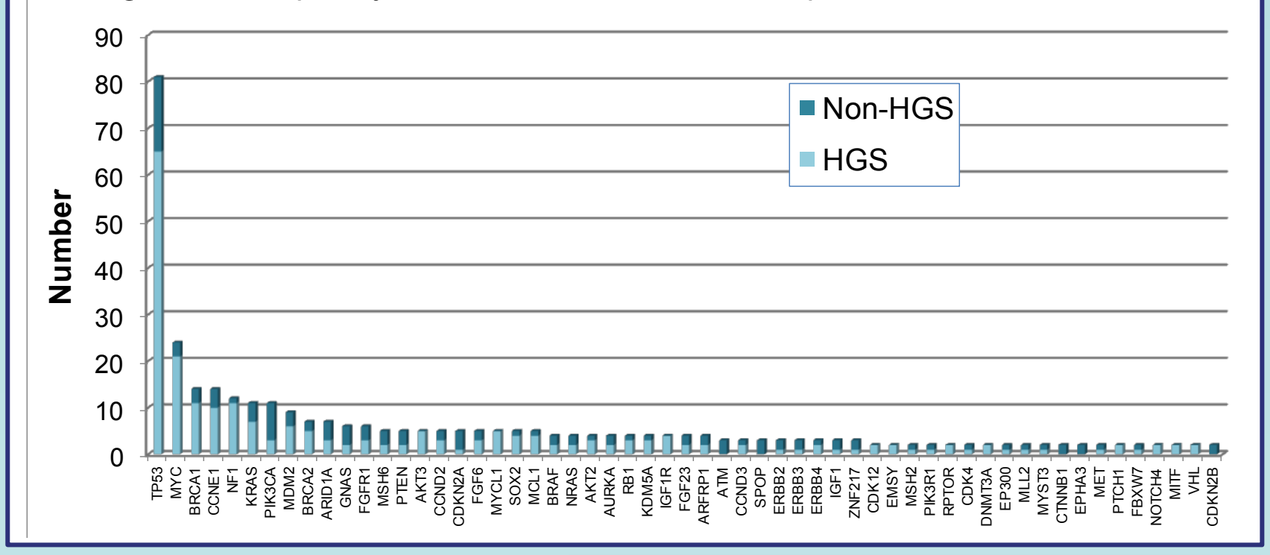


Figure 2. "Actionable" Genetic Alterations in each Specimen

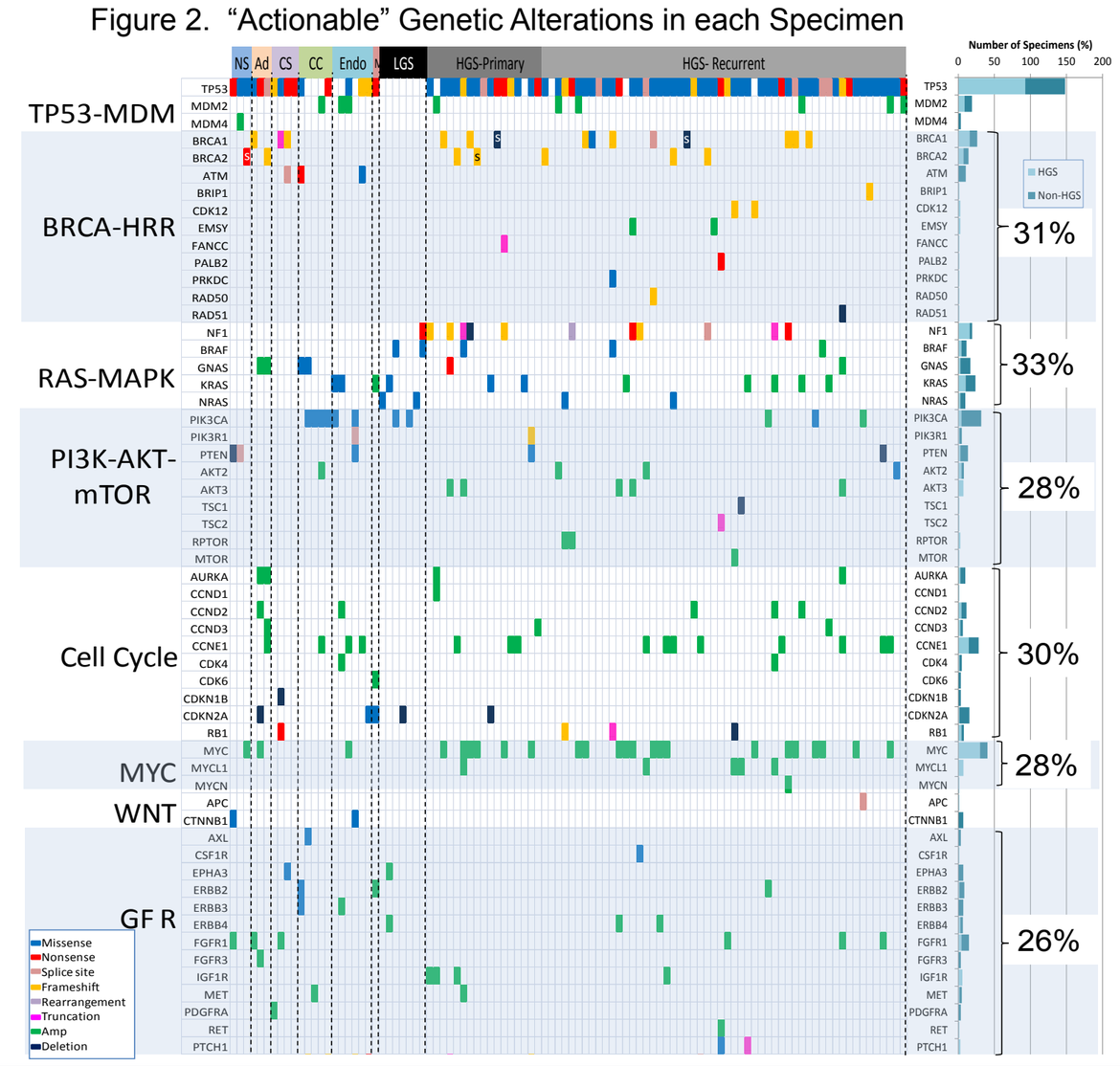


Table 1. Patient and Specimen Characteristics

	ALL	Non-HGS	HGS
Total	**99**	**29**	**70**
Cancer Dx			
Ovary	80 (81%)	24 (83%)	56 (79%)
Peritoneal	7 (7%)	1 (3%)	6 (8%)
Fallopian Tube	8 (8%)	-	8 (9%)
OLMP	3 (3%)	3 (10%)	-
UPSG	1 (1%)	1 (3%)	-
Stage			
I	5 (5%)	5 (17%)	-
II	5 (5%)	2 (7%)	3 (4%)
III	79 (80%)	22 (76%)	57 (80%)
IV	10 (10%)	-	10 (14%)
Histology			
HG Serous	70 (71%)	-	100%
Non-HGS	29 (29%)	100%	-
LG Serous	7 (7%)	24%	-
Endometrioid	6 (6%)	21%	-
Clear Cell	5 (5%)	14%	-
Ad	3 (3%)	10%	-
NS	3 (3%)	10%	-
Carcinosarcoma	4 (4%)	3%	-
Mucinous	1 (1%)	3%	-
Specimen Site			
Ovary/Fallopian Tube	10	5 (17%)	5 (8%)
Peritoneal	13	2 (7%)	11 (15%)
Peritoneal Recurrence	66	19 (66%)	47 (66%)
Distant Recurrence	10	3 (10%)	7 (10%)

Figure 3. Protein over-expression or loss is not only associated with genetic alteration

Gene/ Protein	n	Gene Alteration	High Exp	Low/No Exp
ERBB2	76	3 (3.9%)		
HER2	73		1 (1.4%)	
EGFR	67	0	9 (13%)	
c-Met	71	0	4 (5.6%)	
RB1	76	3 (3.9%)		
	54			6 (11.1%)
CDKN2A	76	3 (3.9%)		
p16	53			12 (17%)
PTEN	76	4 (5.3%)		
	66			6 (9.1%)

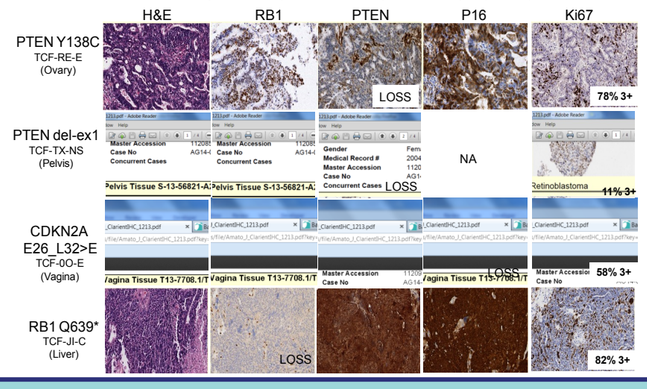
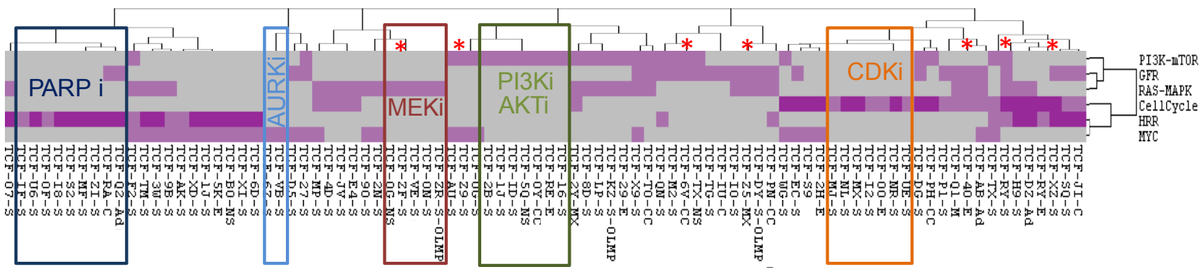


Figure 4. Cluster analysis of specimens based on pathway alterations. Therapy with potential clinical benefit is shown for specimens with only 1 altered pathway.



Specimens are in columns. Purple squares: presence of alteration in pathway designated in row. Dark purple: For Cell Cycle: CCNE1 AMP; For HRR, BRCA1/2 MUT. *, patient in Figure 5.

MATERIALS and METHODS

Patients and tumor specimens. The patients are an unselected population who sought molecular profiling assistance from The Clearity Foundation from 11/2011 to 7/2014. FFPE tissue specimens, pathology reports, and patient treatment histories were obtained under written informed consent.

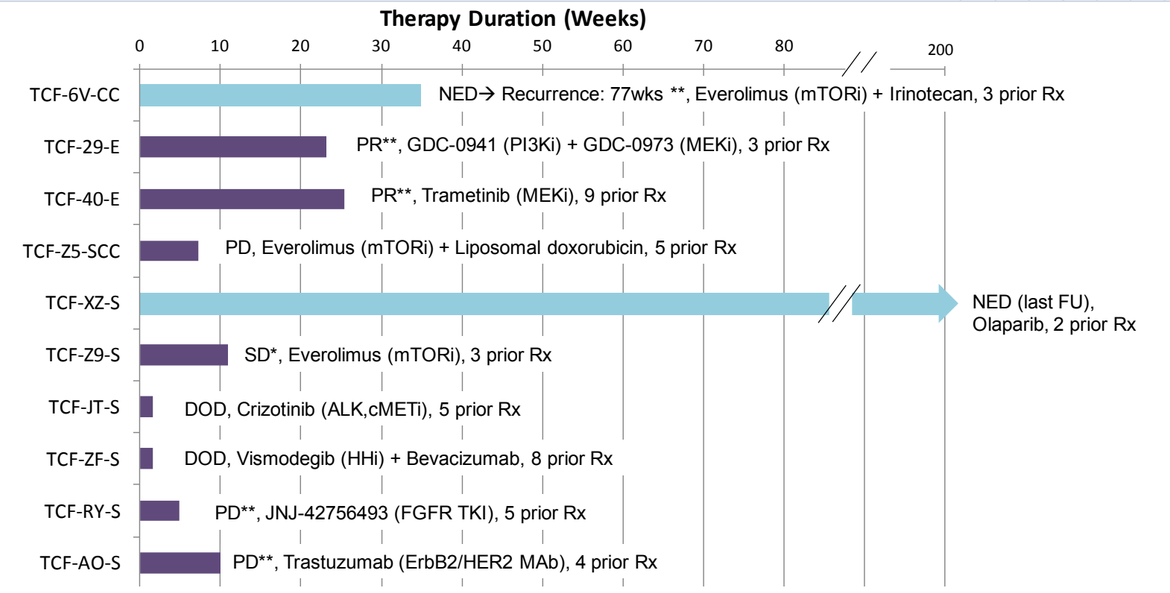
Treatment Outcomes. The clinical responses to targeted therapies were derived from radiology reports of diagnostic imaging scans and/or blood levels of CA125.

Genomic and protein analyses. Next-gen DNA sequencing was performed by Foundation Medicine, Inc. Protein levels were measured by IHC for ERBB2, cMET, and PTEN at Caris Life Sciences and EGFR, RB1, PTEN, and p16 at Clarient, Inc. High levels: ≥10% positive cells at 3+ intensity for ERBB2 and EGFR; H score ≥ 150 for cMET

Figure 5. Genetic alterations / clinical responses for patients who received matched Rx

Patient ID	Cancer Dx	Genetic Alteration	Matched Treatment	TP53	PI3K-mTOR	RAS-MAPK	HRR	GFR	CellCycle	MYC
TCF-6V-CC	Clear Cell IIIC G3	PIK3CA H1047R	Everolimus (mTORi) + Irinotecan		■			■		
TCF-29-E	Endometrioid 1A G1	PIK3CA H1047R + KRAS G12V	GDC-0941 (PI3Ki) + GDC-0973 (MEKi)		■	■				
TCF-40-E	Endometrioid 1A G2	KRAS G12D	Trametinib (MEKi)			■		■	■	
TCF-Z5-SCC	Mixed Serous-Clear Cell IV G3	NF1 L993fs*15	Everolimus (mTORi) + Liposomal doxorubicin	■		■		■		
TCF-XZ-S	Serous IIIC G3	somatic BRCA2 D2005fs*34	Olaparib	■			■	■	■	
TCF-Z9-S	Serous IIIC G3	TSC1 loss	Everolimus (mTORi)	■	■					■
TCF-JT-S	Serous IIIC G3	High cMET Exp#	Crizotinib (ALK/MET TKI)	■						
TCF-ZF-S	Serous IIIC G3	PTCH1 truncation	Vismodegib (HHi) + Bevacizumab	■		■		■		
TCF-RY-S	Serous IV G3	FGFR1 Amp (12 copies)	JNJ-42756493 (FGFR TKI)	■	■	■	■	■	■	
TCF-AO-S	Serous IIIC G3	High HER2 Exp/Amp##	Trastuzumab (ErbB2 Ab)	NA						

#, 85% 2+ (IHC) ##12-fold Amp (FISH) and 85% 3+ (IHC); Pathway alterations in patient specimen indicated by blue squares, dk blue: BRCA2 and CCNE1 AMP



Bars:Teal, maintenance Rx; Purple, Therapeutic Rx. PR, partial response; SD, stable disease; PD, progressive disease; DOD, dead of disease. Response measurement: *, CA125; **, CA125 + imaging scan

SUMMARY AND CONCLUSIONS

1. Genomic analysis of mostly recurrent specimens from advanced stage ovarian cancer patients revealed that non-HGS and HGS cancers are molecularly distinct but contain genetic alterations in similar cancer pathways.
2. A median of 3 (range 0-7) "actionable" alterations were detected/tumor. >30% harbored alterations in key genes involved in HR DNA repair (HRR), RAS-MAPK, and the cell cycle that are targetable by drugs in clinical development (Fig 2 and 4).
3. Co-existing alterations in >1 pathway were found in 58% of the specimens and may impact responses to agents targeting only one pathway (Fig 4).
4. Low PTEN, p16, and RB protein levels are found in 9-17% of specimens and are not only associated with genomic aberrations (Fig 3).
5. 88 of the 99 specimens contained genetic alterations amenable to targeted therapies (Fig 4). Ten patients received such treatment (Fig 5).
6. Best responses were observed in the patients with endometrioid and clear cell ovarian cancers bearing mutations in KRAS and/or PIK3CA and WT TP53. Long term remission was observed with PARP inhibitor treatment in a HGS patient with a somatic BRCA2 mutation (Fig 5).
7. More clinical trials of targeted agents are needed to establish the value of profile-informed drug selection in the larger population.