Differences in genomic alterations revealed by sequencing of 182 genes in recurrent ovarian cancer specimens compared to TCGA analysis: rearrangements in PTCH1 and FLT3; high frequency of RAS pathway alteration

Deborah A. Zajchowski, PhD[1], Doron Lipson, PhD, Gary Palmer, MD[2] and Laura K. Shawver PhD[1]

[1]The Clearity Foundation, San Diego, CA; [2]Foundation Medicine, Cambridge, MA

INTRODUCTION

Recent comprehensive genomic analyses of advanced stage, high grade serous ovarian carcinomas carried out by TCGA (1) have revealed their heterogeneous nature and provide an explanation for the failure of molecularly targeted drugs evaluated without consideration of the tumor molecular characteristics of the patients treated. Copy number (CN) alterations or mutations in RB, RAS-MAPK, and PI3K-AKT pathway genes occur in ~35%, 20%, and 35% of the tumors, respectively. Activation of these pathways may confer sensitivity to drugs that target them (e.g., PI3K or AKT inhibitors, MEK inhibitors, CDK inhibitors) as well as resistance to drugs (e.g., HER2 or EGFR inhibitors) that have not demonstrated significant activity in ovarian cancer clinical trials. A comprehensive evaluation of the genetic aberrations in targetable pathways may therefore inform the selection of appropriate drugs and combinations for each patient in the context of clinical trials.

APPROACH

As part of molecular profiling analyses performed for recurrent ovarian cancer patients to prioritize selection of their next treatment, the presence of mutations or alterations (e.g., CN changes) in 182 genes (Figure 1) that encode proteins that are key mediators in oncogenic and tumor suppressive pathways were determined using a validated exon-capture sequencing platform provided in a CLIA-certified laboratory setting.

Figure 1. 182 cancer-related genes and rearrangements interrogated in this study

ABL1	ABL2	AKT1	AKT2	AKT3	ALK	APC	AR	
ARAF	ARFRP1	ARID1A	ATM	ATR	AURKA	AURKB	BAP1	
BCL2	BCL2A1	BCL2L1	BCL2L2	BCL6	BRAF	BRCA1	BRCA2	
CARD11	CBL	CCND1	CCND2	CCND3	CCNE1	CD79A	CD79B	
CDH1	CDH2	CDH20	CDH5	CDK4	CDK6	CDK8	CDKN2A	
CDKN2B	CDKN2C	CEBPA	CHEK1	CHEK2	CRKL	CRLF2	CTNNB1	
DDR2	DNMT3A	DUSP1L	EGFR	EPHA3	EPHA5	EPHA6	EPHA7	
EPHB1	EPHB4	EPHB6	ERBB2	ERBB3	ERBB4	ERCC2	ERG	
ESR1	EZH2	FANCA	FBXW7	FGFR1	FGFR2	FGFR3	FGFR4	
FLT1	FLT3	FLT4	FOXP4	GATA1	GNA11	GNAS	GPR124	
GNAQ	GUCY1A2	HOXA3	HRAS	HSP90AA1	IDH1	IDH2	IGF1R	
IGF2R	IKBKE	IKZF1	INHBA	INSR	IRS2	JAK1	JAK2	
JAK3	JUN	KDM6A	KDR	KIT	KRAS	LRP1B	LRP6	
LTK	MAP2K1	MAP2K2	MAP2K4	MCL1	MDM2	MDM4	MEN1	
MET	MITF	MLH1	MLL	MPL	MRE11A	MSH2	MSH6	
MTOR	MUTYH	MYC	MYCL1	MYCN	NF1	NF2	NKX2-1	
NOTCH1	NPM1	NRAS	NTRK1	NTRK2	NTRK3	PAK3	PAX5	
PDGFRA	PDGFRB	PHLPP2	PIK3CA	PIK3CG	PIK3R1	PKHD1	PLCG1	
PRKDC	PTCH1	PTCH2	PTEN	PTPN11	PTPRD	RAF1	RARA	
RB1	RET	RICTOR	RPTOR	RUNX1	SMAD2	SMAD3	SMAD4	
SMARCA4	SMARCB1	SMO	SOX10	SOX2	SRC	STAT3	STK11	
SUFU	TBX22	TET2	TGFBR2	TNFAIP3	TNKS	TNKS2	TOP1	
TP53	TSC1	TSC2	USP9X	VHL	WT1			
Select Rearrangements								
ALK	BCR	BRAF	EGFR	ETV1	ETV4	ETV5	ETV6	
EWSR1	MLL	RAF1	RARA	RET	TMPRSS2			

MATERIALS and METHODS

Patients and tumor specimens. The patients are an unselected population who sought molecular profiling assistance from The Clearity Foundation between November, 2011 and March, 2013. Formalin-fixed, paraffin-embedded (FFPE) tissue specimens, pathology reports, and patient treatment histories were obtained under written informed consent.

DNA extraction and library construction. DNA was extracted from 40 µm of tissue using the Promega Maxwell 16 FFPE Plus LEV DNA Purification kit and molecular barcode-indexed ligation-based sequencing libraries were constructed (2) from at least 50-200 ng sheared DNA (~100-400 bp). Solution-based hybrid capture (2) with a custom Agilent SureSelect biotinylated RNA baitset was used to enrich all 3,320 exons of 182 cancer-related genes and 37 introns from 14 genes recurrently rearranged in cancer representing approximately 1.1 Mb of the human genome.

Illumina Sequencing and data analysis. The selected libraries were sequenced on an Illumina HiSeq 2000 platform using 49x49 paired-end reads. Median exon coverage was 1278. Sequence data were mapped to the reference human genome (hg19) using the Burrows-Wheeler Aligner (3) and were processed using the publicly available SAMtools, Picard, and Genome Analysis Toolkit (4). Genomic base substitutions and indels were detected using custom tools optimized for mutation calling in heterogeneous tumor samples based on statistical modeling of sequence quality scores and local sequence assembly. Variations were filtered using dbSNP and a custom artifact database and then were annotated for known and likely somatic mutations using the Catalogue of Somatic Mutations in Cancer (5). Copy number alterations were detected by comparing targeted genomic DNA sequence coverage with a process-matched normal control sample. Genomic rearrangements were detected by clustering chimeric reads mapped to targeted introns.

REFERENCES

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Sequencing of 182 genes: average of 2.8 "actionable" alterations in each serous ovarian tumor (median 3; range 0-7).
Copy number alterations predominate (21 of 37 altered genes; Figures 2 and 3A).
Mutations in PIK3CA and KRAS only in clear cell and endometrioid histologies (Figure 2).

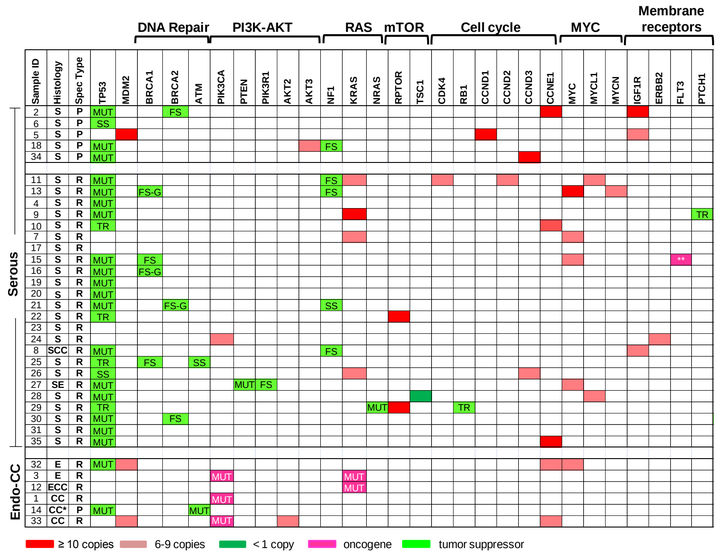
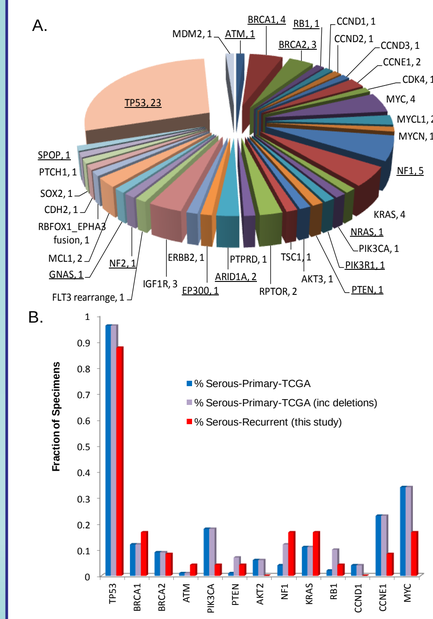


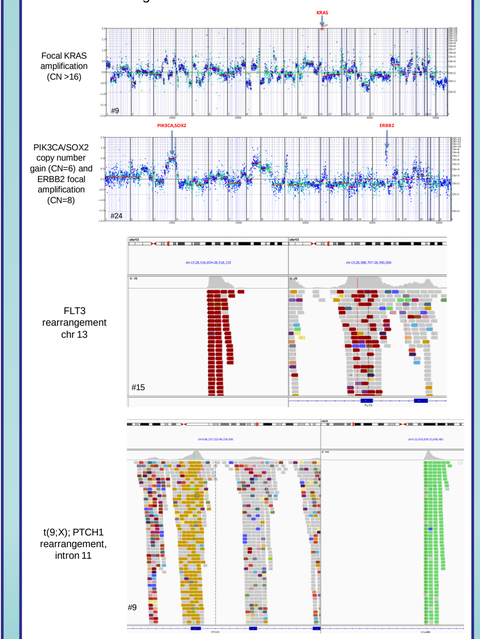
Figure 2. Sequencing data for S, serous; E, endometrioid, CC, clear cell histology specimens from P, primary or R, recurrent tumors. Alterations: MUT, mutation; SS, splice site disruption; TR, truncation; FS, frameshift; G, germline; **, predicted exon duplication

Figure 3. A. Genetic alterations in 29 high grade serous tumors



A. Underlined, mutated genes; regular font, copy number alterations.
B. Comparison of reported frequencies for each gene in recurrent serous specimens in this study (n=24) with data reported by TCGA for 316 primary high grade serous tumors.

Figure 4. Genomic coverage of sequencing reads for specimens with KRAS, PIK3CA/SOX2, and ERBB2 amplifications or PTCH1 and FLT3 rearrangements.



Tumors from 75% of recurrent high grade serous ovarian cancer patients (18/24) contain "actionable" or clinically relevant genetic alterations (Figure 5).

RAS-MAPK pathway alteration was observed in 33% of the tumors (Figure 5A), often in conjunction with RB-cyclin pathway or MYC family member alterations.

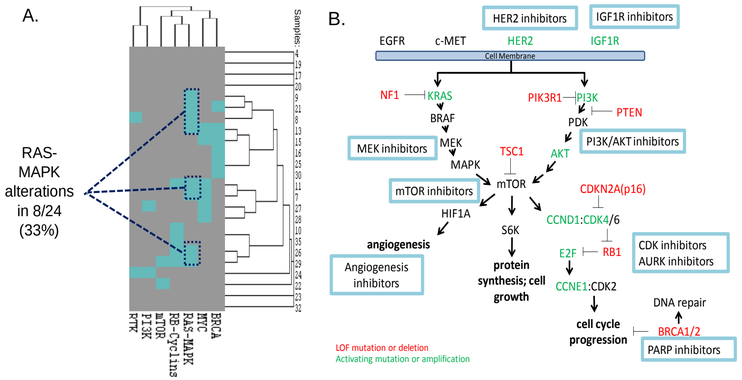


Figure 5. A, Clustering of serous tumor specimens based on pathway alteration status Tumors with at least one alteration in a "pathway" indicated by teal color. BRCA= BRCA1, BRCA2; MYC= MYC, MYCL1, NMYC; RAS-MAPK= KRAS, NF1, NRAS; RB-Cyclins= RB1, CDK4, CCND1-3, CCNE1; PI3K= PIK3R1, PIK3CA, AKT1-3; mTOR= RPTOR, TSC1; RTK= IGF1R, ERBB2, FLT3 B, Observed gene alterations and corresponding cell signaling pathways with reference to drug families in clinical development

SUMMARY and CONCLUSIONS

1. Sequencing of exons from 182 genes associated with cancer etiology and progression identified a median of 3 alterations (range: 0-7) in high grade serous histology ovarian cancers (n=29).
2. 75% of the 24 recurrent serous ovarian tumors contained genetic alteration(s) in at least one pathway that is targeted by approved or clinically developed drugs.
3. The RAS-MAPK pathway was altered in 33% of the recurrent serous ovarian tumors– primarily due to deleterious NF1 gene alterations and KRAS amplification. In such tumors, RB-cyclin pathway or MYC family alterations frequently occurred, suggesting combination therapy strategies with drugs targeting these pathways. Notably, the PI3K-AKT pathway was less frequently altered (i.e., 8%).
4. Rearrangement of the PTCH1 and FLT3 genes were observed in two specimens suggesting the potential for Hedgehog pathway and FLT3 inhibitor therapy in these patients.
5. Increased NF1 alterations and decreased frequency of PIK3CA, CCNE1, and MYC copy number changes were observed relative to the published TCGA analysis of primary tumors. As these studies used different sequencing and analytical methods, additional primary tumor specimens will be analyzed.
6. Additional studies with a larger sample size are required to confirm these observations suggesting that patient selection for clinical trials should be informed by comprehensive molecular characterization of recurrent tumor specimens.