MOLECULAR PROFILING IN RECURRENT OVARIAN CANCER PATIENTS: CONSIDERATIONS FOR THE DESIGN OF CLINICAL STUDIES TO VALIDATE PROFILING FOR THERAPY SELECTION

Deborah A. Zajchowski, PhD[1], Cory Bentley, PhD[1], Jenny Gross[2], Beth Y. Karlan, MD[2], Laura K. Shawver, PhD[1]

[1]The Clearity Foundation, La Jolla, CA; [2]Women's Cancer Research Institute, Division of Gynecological Oncology, Cedar Sinai Medical Center, Los Angeles, CA

BACKGROUND and RATIONALE

Ovarian cancer is the most lethal gynecologic malignancy. In 2010, 21,880 women were diagnosed with ovarian cancer and 13,850 died of this disease (est. American Cancer Society. *Cancer Facts & Figures 2010. Atlanta: American Cancer Society; 2010*). Most patients diagnosed with advanced stage ovarian cancer respond to first-line, standard-of-care platinum-based therapy, but >75% of these patients recur. Therapies for recurrent patients are often empirically selected and usually include taxanes, gemcitabine, anthracyclines, topotecan or other topoisomerase 1 inhibitors, and occasionally fluoropyrimidines and anti-folates (see Table 1). However, limited response or short duration of response are unfortunately observed with all of these agents. Thus, there is a critical need for rational approaches that identify which drugs have the greatest chance to be effective in each individual patient .

Our goal is to improve patient outcomes by enabling selection of chemotherapies based upon individual tumor molecular profiles. Achievement of this goal will require randomized clinical trials designed from hypothesis-generating data sets that characterize expression of candidate molecular markers. In order to identify the markers to include in such profiling, we performed a literature search for evidence supporting the association between biomarkers and clinical responses to drugs currently employed in ovarian cancer treatment and initiated expression studies of those markers in ovarian tumors. By determining the expression characteristics of these markers in a large cohort of ovarian tumors, expression cut-points for future retrospective or prospective studies can be derived. In addition, evaluation of marker expression in specimens obtained from primary diagnosis as well as recurrent tumors would clarify the need for recurrent tumor specimens.

MATERIALS and METHODS

Biomarker reference library generation. A survey of the PubMed database was undertaken to identify all reports that correlated biomarkers with clinical responses and outcomes (i.e., time to progression, progression-free survival, overall survival) following specific chemotherapy. The search terms used were "[drug]", "predictive" and "marker", where each drug is an approved agent (i.e., carboplatin, cisplatin, paclitaxel, doxorubicin, topotecan) or off-label agent (i.e., docetaxel, capecitabine, etoposide, irinotecan) for ovarian cancer treatment. For selected biomarkers, additional queries were carried out with "[marker]" and "ovarian cancer", where the marker was identified as having potential correlation with any of the drugs of interest. Initial searches recovered ~5000 references, from which 585 were selected following review of the abstracts for relevancy. Using a reference manager program, End Note, each abstract was annotated to reflect cancer type, specific drug combinations used in the study, predictive ability, and marker type (protein, RNA, DNA). No specific correlative markers were found in searches for ifosfamide, cyclophosphamide, and altretamine.

Marker selection criteria. Candidate markers were identified from reports of clinical (not pre-clinical) research showing evidence for association with response/outcome and where protein, mRNA, or DNA amplification or function-altering mutations were measured. With the exception of BRCA1/2, germline DNA alterations or polymorphisms were not considered. Markers were considered predictive if ≥ 50% of the studies for that marker showed consistent and statistically significant (p value <0.05) associations with response/outcome. At least 5 references were required for any marker that had only 50% of the studies with consistent results. In other cases, a minimum of two concordant studies was required.

Patients and tumor specimens. The patients are an unselected population who sought molecular profiling assistance from The Clearity Foundation between October, 2008 and March, 2011. Formalin-fixed, paraffin-embedded (FFPE) tissue specimens and patient treatment histories were obtained under written informed consent. Specimens procured during primary surgical procedures were from ovary and fallopian tube (O) or the peritoneal cavity (P; biopsies from omentum, diaphragm, peritoneum, colon, appendix, cul de sac, side wall). Recurrent cancer specimens were from the peritoneal cavity (M), lymph nodes (LN), or distant organs (MD; lung, liver).

Immunohistochemistry. CLIA-certified laboratories have performed these analyses to ensure that the protocols and reagents used have been fully validated and high reproducibility of the test results over the 17 month timeframe of this study. Immunohistochemistry (IHC) analysis of most markers was performed by Caris Life Sciences, Inc using the Ventana or the DAKO automated staining systems. Ki67 and EGFR IHC analyses were performed at Clarient, Inc. Following heat-induced epitope retrieval, antibody incubation was for 20-40 minutes (antibody-specific), visualization was by the Ultraview or Vision Biosystem Novolink Poly-HRP (Ventana) or Biocare envision plus horseradish peroxidase Polymer Detection System (DAKO). All slides were scored manually by a board certified pathologist and results reported as % of tumor cells that stained positive and intensity of staining (0, 1+, 2+, 3+).

Summary of published retrospective clinical research studies for biomarkers correlated with chemotherapy response.

Figure 1. Biomarker reference library generation

Search PubMed Database for "drug" "predictive" "marker" (> 20 million references)

↓

Select relevant references from ~5,000 retrieved by query

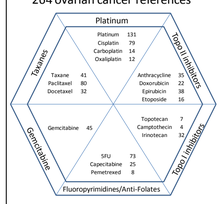
↓

Review 1015 abstracts, select, and annotate

↓

Annotated library

585 total references
264 ovarian cancer references



Platinum – 234
Taxanes – 155
Topo II inh –111
Topo I inh – 43
Gemcitabine –45
5FU – 106

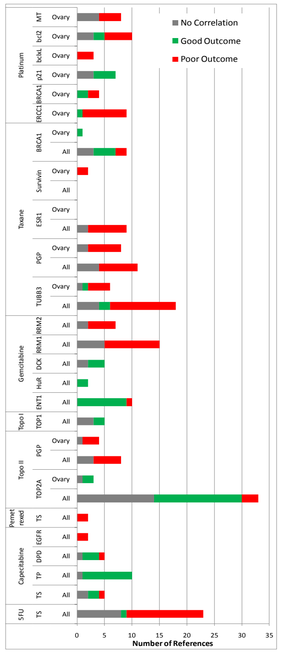


Figure 2. Graphical representation of the number of biomarker references for indicated drug classes in ovarian cancer (Ovary) or all cancers (All). Bars, number of studies that reported high marker expression correlated with good (green) or poor (red) outcomes (ie., drug response, PFS, or OS) or no correlation (gray). * correlation in p53 WT patients or in combination with cyclophosphamide; ** germline loss of function mutation; ***high TP:DPD ratio
For corresponding references and additional information, see www.clearityfoundation.org

Table 1. Chemotherapies and Associated Response Biomarkers*

Drug Class	Drug	Sensitivity	Resistance	Cancer
Platinum	Carboplatin Cisplatin	BRCA1 mutation p21	ERCC1 BRCA1 Bcl-xL Bcl-2 MT	Ovarian
Taxanes	Paclitaxel Docetaxel	BRCA1	TUBB3 PGP Survivin ESR1	Ovarian Breast
	Nab-paclitaxel	SPARC		Head & Neck
Nucleoside Analogs	Gemcitabine	ENT1 DCK HuR	RRM1 RRM2	Pancreatic Ovarian Lung
Topo I Inhibitors	Topotecan Irinotecan	TOP1		Ovarian Colon
Topo II Inhibitors	Doxorubicin Etoposide	TOP2A	PGP	Ovarian
Fluoropyrimidines	5-FU		TS	Colon, Gastric
	Capecitabine	TP	DPD EGFR	Colon, Lung, Breast
Anti-folates	Pemetrexed		TS	Lung, Mesothelioma

*Response prediction markers for each drug class were identified by searching for relevant clinical research reports in the PubMed database. See M&M. Correlation of high marker expression with sensitivity or resistance is indicated. Evidence for cancer type(s) other than ovarian was used to support the indicated biomarkers when no or limited clinical evidence for ovarian cancer exists. Squares, markers used in IHC analyses.

California Ovarian Cancer Awareness Program

Most of the patients profiled were diagnosed with stage IIIC serous ovarian carcinoma and were sensitive to platinum-taxane therapy (**Table 2**). Specimens from primary (ovary and peritoneal) and recurrent lesions were approximately equally represented.

Table 2. Clinical Characteristics of Ovarian Serous or Adenocarcinoma Patients and Specimens Used in this Study

Stage*	n	Stage*	n
II	0	III	4
IIA	0	IIIA	0
IIB	2	IIIB	4
IIC	1	IIIC	29
Total	3	Total	37

Specimen Source		Platinum Response**			
		Sens	Res	Ref	Total
Primary	Ovary	8	4	2	14
	Peritoneal	6	4		10
Recurrent	Peritoneal	18	2		20
	Distant	2			2
Total		34	10	2	46

*Stage, FIGO. Table includes 3 adenocarcinoma specimens (2 primary; 1 recurrent) from platinum-sensitive stage IIIC patients. Unknown stage for 4 patients. **Ref, refractory; no response or progression; Res, resistant: recurrence < 6mos; sens, sensitive: recurrence > 6 mos after cessation of initial platinum-taxane therapy.

HUGO	NAME
TOP1	Topoisomerase 1
TOP2A	Topoisomerase 2A
TS	Thymidylate synthase
RRM1	Ribonucleotide reductase regulatory subunit M1
ERCC1	Excision repair complementation group 1
PGP	P-glycoprotein
SPARC	Secreted protein, acidic, cysteine-rich
ESR1	Estrogen receptor
EGFR	Epidermal growth factor receptor
BCRP	Breast cancer resistance protein
MRP1	Multidrug resistant protein 1
MGMT	O-6-methyl guanine DNA methyltransferase
Ki67	Proliferation antigen

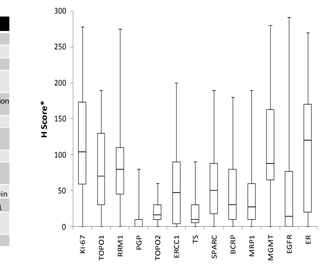


Figure 3. Chemo-response markers and boxplot representation of the range of expression of each protein.
Hscore, histoscore=%positive cells x intensity (see M&M)
Box: bottom 25th percentile; top 75th percentile; band, median. Whiskers: maximum and minimum values

Expression of chemotherapy response markers is variable in ovarian serous carcinomas **(Figure 3).** The overall marker profiles in primary and recurrent ovarian tumors are remarkably similar. However, expression of individual markers such as PGP, Topo1, and RRM1 were significantly different in primary and recurrent lesions **(Table 3 and Figure 4).**

Table 3. Biomarker Expression in Primary and Recurrent Ovarian Serous Carcinoma and Adenocarcinoma Specimens

	Primary	Recurrence	T-test
TOP1	95.8 ± 52.6	62.4 ± 60.9	0.050*
TOP2A	19.7 ± 13.0	20.8 ± 17.9	0.807
TS	25.7 ± 25.0	17.0 ± 23.1	0.232
RRM1	111.0 ± 65.6	74.3 ± 49.0	0.036*
ERCC1	48.3 ± 58.0	66.1 ± 59.4	0.308
PGP	3.9 ± 12.3	18.7 ± 24.9	0.013*
SPARC	52.3 ± 45.2	62.0 ± 54.1	0.509
ESR1	115.4 ± 68.8	114.3 ± 99.7	0.965
EGFR	35.9 ± 13.7	62.8 ± 30.1	0.101
BCRP	41.9 ± 38.2	54.6 ± 57.1	0.375
MRP1	39.3 ± 36.7	45.2 ± 47.7	0.639
MGMT	104.1 ± 62.1	128.9 ± 74.2	0.224
Ki67	127.8 ± 59.5	158.6 ± 98.6	0.208

Mean (±SD) histoscores for 24 primary and 22 recurrent specimens are tabulated.
* Statistically significant correlations with p value <0.05 calculated by two-tailed student's t test.

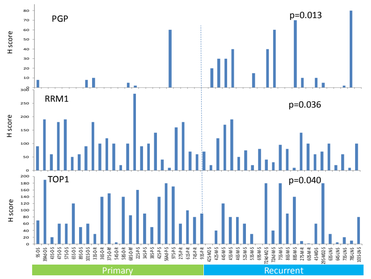


Figure 4. Expression of PGP, RRM1, and TOP1 in primary and recurrent tumor specimens. Levels for the indicated proteins in primary ovary (O) and peritoneal (P) specimens or recurrent (M or MD); see Table 3.

Expression cut-points for the chemo-response biomarkers identified in our survey of published clinical research can be proposed based on this analysis of 46 tumor specimens **(Figure 5A).** Use of these cut-points permits the stratification of 73% of the patients tested **(Figure 5B).**

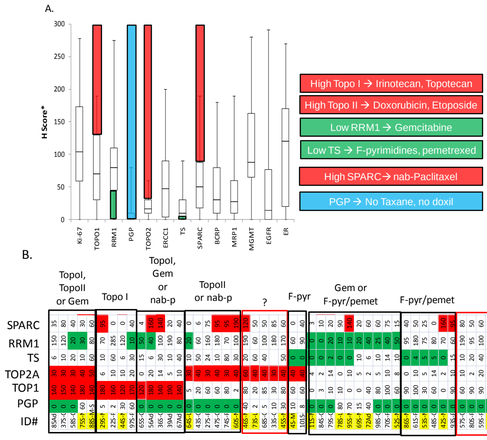


Figure 5. Patient stratification by biomarker expression and predicted responses. A, Population statistics-derived expression cut-points for indicated markers. Predicted drug responses associated with low (<25th percentile, green bars) or high (>75th percentile, red bars) expression values are as indicated. Blue bar, expression of detectable PGP contra-indicates taxanes/anthracyclines. B, Classification of 52 tumor profiles based upon expression of chemo-response markers PGP, TOP1, TOP2A, TS, RRM1, and SPARC. Patients with predicted sensitivity to indicated drugs are identified based on criteria in 6A (exception is PGP ,where no expression determines sensitivity and corresponding sample data are shown in green). ?, specific drugs cannot be assigned based on these biomarkers and expression cut-points.

SUMMARY and CONCLUSIONS

1. Biomarkers that have reproducibly significant associations with response to the chemotherapy agents commonly employed in ovarian cancer treatment were identified from a comprehensive literature review.
2. Expression levels of three key biomarkers (i.e., PGP, RRM1, and TOP1) analyzed in 46 primary and recurrent serous ovarian carcinoma specimens demonstrated significant differences between the two sample cohorts. The expression levels of ten other biomarkers revealed no significant differences between the two groups of samples. Clinical trials that prospectively evaluate the predictive ability of these markers should be performed to further validate these results.
3. Using a percentile-ranking strategy to score tumors for expression of a subset of these chemo-response prediction markers, 73% of the patients could be assigned to chemotherapy, although additional clinical validation of the results are still required. These results demonstrate, however, the feasibility of using similar expression cut-points for patient stratification for prospective clinical trials.
4. Future studies should incorporate the additional markers identified by the meta-analysis (see Table 1) into the multi-marker testing panel and increase the size of the patient cohort in the analysis.

ACKNOWLEDGEMENTS

We thank the patients and physicians who have participated in this study and are also appreciative of the efforts of Brenda Durand for patient support services, Michelle Bernabe and Kathleen Zajchowski for documentation coordination and database entry, and Michael Petka for enhancement of our database capabilities. The literature survey and meta-analysis were supported in part by a grant from the California Ovarian Cancer Awareness Program (COCAP).