SELECTING PATIENTS for OVARIAN CANCER CLINICAL TRIALS by PROFILING TUMORS against a BROAD PANEL of MOLECULAR MARKERS

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BACKGROUND and RATIONALE

Ovarian cancer is a molecularly complex and heterogeneous disease. Over-expression or alteration of any one drug target is uncommon in ovarian cancer and thus targeted therapeutic approaches have had only modest success to date. To improve the identification of patients for clinical trial enrollment and the likelihood of meaningful response, we propose to evaluate patients using a broad marker panel of potential therapeutic targets. We envision a future ovarian cancer clinical trial that is similar to the Bisgrove trial (Von Hoff *et al.* J. ClinOncol 24: No 18s, 138s, 2006), which selected therapeutic agents based on the tumor expression levels of drug targets and efficacy biomarkers. The Bisgrove trial accepted patients with all cancer types and demonstrated significantly increased progression-free survival in 27% of the 66 patients treated (including those with breast, colorectal, and ovarian cancers).

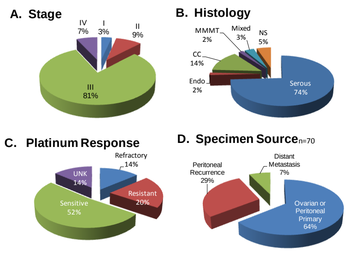
In a pilot study for this approach in ovarian cancer, we characterized the tumors of 58 epithelial ovarian cancer patients for expression of proteins that are targets for drugs currently in clinical development for ovarian cancer treatment. In addition, we measured the expression of chemotherapy response markers in an attempt to improve on the empiric selection of cytotoxic agents for women with recurrent ovarian cancer. Identification of molecular markers which can inform that selection has the potential to improve tumor response and quality of life.

METHODS

Patients and tumor specimens.
The current cohort is an unselected population of ovarian cancer patients who sought molecular profiling assistance from The Clearity Foundation between October, 2008 and March, 2010. Patient tumor specimens and treatment histories were obtained under written informed consent. Formalin-fixed, paraffin-embedded (FFPE) tissue specimens were obtained from the hospital laboratories for these studies and were stored for a median of 1.07 years (range: 2 days-9.4 yrs). 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 obtained from the peritoneal cavity (M) or distant organs (MD; lung, liver).

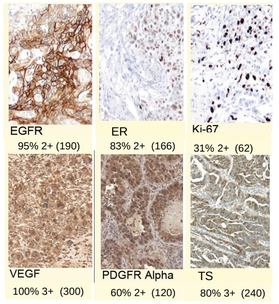
Immunohistochemistry.
CLIA-certified laboratories have performed these analyses to ensure that the protocols and reagents used have been fully validated and that test results over the 17-month timeframe of this study were highly reproducible. Immunohistochemistry was performed by Clarient, Inc. (i.e., ER, AR, c-Kit, EGFR, VEGF, PDGFRa/b, Ki67, TS, COX-2), Caris Lifesciences (i.e., AR, ER, PR, HER2, PGP, BCRP, MRP1, Topo1, TopoIIa, TS, ERCC1, RRM1, MGMT, c-Kit, PDGFRa, SPARC), or Targeted Molecular Diagnostics/ Quintiles (i.e., c-Met, IGF1Rb, HIF1a). All laboratories utilized either the Ventana or the DAKO automated staining systems. Following heat-induced epitope retrieval (except EGFR and VEGF: protease K) , antibody incubation was for 20-40 minutes (dependent on the antibody), 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+). The H score is the product of % and intensity.

Figure 1. Disease Characteristics of the 58 Patients Profiled in this Study



A. Percentage of patients diagnosed with stage I-IV disease.
B. Endometrioid (Endo), clear cell (CC), malignant mixed mullerian tumor (MMMT), not specified (NS).
C. Refractory, no response or progression; resistant, recurrence < 6mos; sensitive, recurrence > 6 mos after cessation of initial platinum-taxane therapy.
D. Source of specimens (see Methods).

Figure 2. Selected IHC Results and H Score Determination



Expression of each protein was scored for % of positive cells and intensity of staining i.e., 0, 1+, 2+,3+.
Histoscore (H score) = product of % and intensity

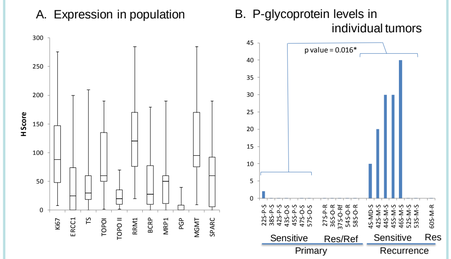
Most of the patients profiled were diagnosed with stage III serous carcinoma and were sensitive to platinum-taxane therapy (**Figure 1A-C**). Two-thirds of the specimens were from the primary surgical procedure, including tumors in the ovary/fallopian tube as well as in the peritoneal cavity (**Figure 1D**).
IHC analyses were performed on FFPE tumor blocks and examples of positively staining tumor sections for several markers are shown in **Figure 2**.

Table 1. Chemotherapy Response Markers

Marker	Name	Drug (s)	High Expression Association*
ERCC1	Excision-repair cross complementation group 1	platinum analogs	Resistant [Kuramoto,K. CancerSci 98:1336-43(2007)]
TS	Thymidylate synthetase	fluoropyrimidines	Resistant [Hu, Y. Clin.CancRes 9:4165-71 (2003)]
RRM1	Ribonucleotide reductase subunit M1	gemcitabine	Resistant [Rosell, R. Clin Cancer Res 10: 4215s-4219s (2004)]
MGMT	O-6-methylguanine-DNA methyltransferase	temozolamide	Resistant [Kovacs, K. Acta Neuropathol 115(2): 261-2 (2008)]
Topo I	Topoisomerase I	Topo I inhibitors (e.g., topotecan, irinotecan)	Sensitive* * [Naimeu, J. Int/J.GynecolCancer 17:76-82 (2007)]
Topo II	Topoisomerase II alpha	Topo II inhibitors (e.g., doxorubicin, epirubicin)	Sensitive (Derbecq,V. Mol CancerTher 3: 1207-14 (2004)]
BCRP	Breast cancer resistance protein (ABCG2)	platinum analogs, some topo I inhibitors, mitoxanthrone	Resistant (Yoh, K. Clin Cancer Res 10): 1691-7(2004)]
PGP (MDR1)	P-glycoprotein (multi-drug resistance; ABCB1)	many drugs, e.g., anthracyclines, paclitaxel, vinblastine	Resistant** [Raspollini, HR Int/J/GynecolCancer 15:255-60 (2005)]
MRP1	Multidrug-Resistance like Protein 1 (ABCC1)	anthracyclines, vinca alkaloids, mitoxantrone	Resistant (Filipits, M. / Clin Oncol 23: 1161-8 (2005)]
SPARC	secreted protein, acidic, cysteine-rich (osteonectin)	nab-paclitaxel	Sensitive [Desai, N. Transl Oncol. 2:59-64(2009)]

* Clinical drug response that is associated with high marker expression is indicated with a representative reference **study in ovarian cancer patients

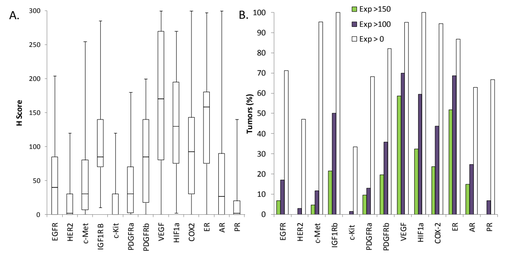
Figure 3. Ovarian Tumor Expression of Drug Response Markers



A. Box plots depict the range of expression (first-third quartiles) and median; whiskers, maximum and minimum values for each marker. n=30 for all but Ki67 and TS, where n=60.
B. PGP levels for tumors from patients sensitive (S), resistant (R), or refractory (Rf) to platinum-taxane therapy. Primary ovary (O) and peritoneal (P) specimens or recurrent (M or MD) were analyzed. *p values calculated by two-tailed students t test.

Expression of the proteins listed in **Table 1** has been correlated with response to chemotherapeutic agents in clinical studies of multiple tumor types. The levels of these markers were variable in ovarian tumors (**Figure 3A**). High levels of the BCRP platinum transporter and ERCC1, the DNA repair protein reported to play a role in platinum resistance, were expressed in a fraction of these tumors, but were not significantly associated with platinum responsiveness in the small cohort that was analyzed (n=12; data not shown). Interestingly, levels of PGP were significantly higher in recurrences from patients that were initially sensitive to platinum therapy (i.e., 6 of 8 samples; **Figure 3B**).

Figure 4. Heterogeneous Expression of Drug Targets in Ovarian Cancer

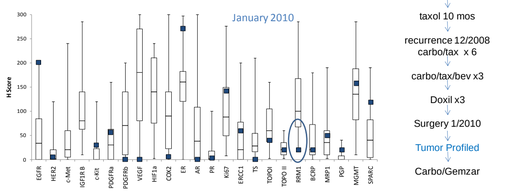


A. Boxplots for expression of each drug target in >50 tumors for all but c-Met, IGF1R(β, and HIF1α, where n=30.
B. Percentage of tumors that express detectable (open bars) or high levels of each marker protein corresponding to the indicated Hscore cut-off levels (solid bars).

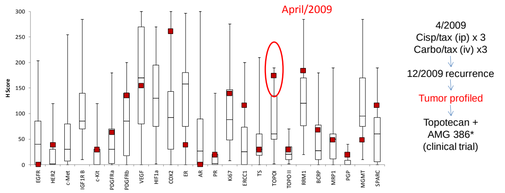
High level expression of HER2, c-Met, and EGFR (i.e., H score >150, which corresponds to >50% positive cells with 3+ staining intensity or 75% with 2+) was detected in <7% of the tumors analyzed (**Figure 4**). In contrast, VEGF and ER were found to be highly expressed in > 50% of the tumors.

Figure 5. Tumor Molecular Profile Informs Therapeutic Decisions

A. Profile for patient diagnosed in 3/2005 with stage IIIB papillary serous carcinoma



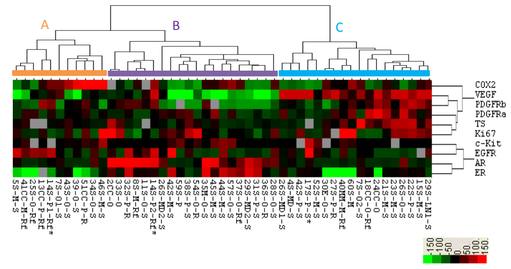
B. Profile for patient diagnosed in 4/2009 with stage IIIC clear cell carcinoma



A. Profile for omentum specimen procured during Jan, 2010 surgery. Patient treatments schematically shown at right. Blue circle, result suggesting use of gemzar
B. Profile of adnexal mass procured during April, 2009 surgery. Red circle, result suggesting use of topotecan. *angiopoietin1/2-neutralizing peptibody

The combined results from the drug response biomarker panel and the drug target expression data provide the molecular profile for an individual patient's tumor (red and blue squares in the plots in **Figure 5**). By comparing the results for the individual patient with the median expression results derived from data for the population, the expression level for the patient sample is interpreted in a relevant context and therapies selected accordingly.

Figure 6. Ovarian Cancer Classification by Marker Expression



H Score data for each marker was median-centered across the samples, average linkage clustering performed using CLUSTER software (Eisen et al. (1998) PNAS 95:14863), and visualization using Java Treeview. Colored bars underneath dendrogram indicate the classes. Naming convention for samples X-Y-Z, where X is ID# and histotype; Y, specimen type; Z, platinum response: sensitive (S), resistant (R), or refractory (Rf).

Hierarchical clustering analysis enables visualization of the data for the entire patient cohort based upon expression of each marker relative to the median for the population. The dendrogram in **Figure 6** reveals that these tumors can be grouped into two major branches, with the first split further into two additional classes. Class A tumors have up-regulated expression of EGFR or COX2; class B tumors are distinguished by higher relative expression of hormone receptors (ER or AR); most class C tumors have higher expression of VEGF, PDGFRα and β, TS, and Ki67.

SUMMARY and CONCLUSIONS

1. The molecular profiling results for 58 ovarian tumors using a panel of 24 drug targets and drug response markers have confirmed that the complexity and heterogeneity observed at the genomic level are maintained at the protein level.

2. With the exception of VEGF and the estrogen receptor, no other drug target is highly expressed in a large fraction of tumors. Profiling a large panel of targets for each patient tumor is feasible and may be key to selecting appropriate therapies or clinical trials.

3. The results from the small patient cohort employed for preliminary correlative analyses of marker expression with platinum response confirmed published reports of PGP over-expression in recurrent tumors (e.g., Ozalp SS *et al* Eur J Gynaecol Oncol. 23:337-40, 2002) and also suggest hypotheses for future study.

4. Individual patient profiles are currently interpreted by comparison with other ovarian tumor profiles stored in The Clearity Foundation database. As correlative data for marker expression and therapy response are generated, more informed and reliable interpretation of individual tumor results should be possible.

5. Ovarian tumors can be classified into at least three groups based on their molecular profiles. Future investigations will determine any correlation of these classes with patient prognosis or treatment outcomes.