

## An Integrated Genomic-Based Approach to Individualized Treatment of Patients With Advanced-Stage Ovarian Cancer

Holly K. Dressman, Andrew Berchuck, Gina Chan, Jun Zhai, Andrea Bild, Robyn Sayer, Janiel Cragun, Jennifer Clarke, Regina S. Whitaker, LiHua Li, Jonathan Gray, Jeffrey Marks, Geoffrey S. Ginsburg, Anil Potti, Mike West, Joseph R. Nevins, and Johnathan M. Lancaster

### A B S T R A C T

#### Purpose

The purpose of this study was to develop an integrated genomic-based approach to personalized treatment of patients with advanced-stage ovarian cancer. We have used gene expression profiles to identify patients likely to be resistant to primary platinum-based chemotherapy and also to identify alternate targeted therapeutic options for patients with de novo platinum-resistant disease.

#### Patients and Methods

A gene expression model that predicts response to platinum-based therapy was developed using a training set of 83 advanced-stage serous ovarian cancers and tested on a 36-sample external validation set. In parallel, expression signatures that define the status of oncogenic signaling pathways were evaluated in 119 primary ovarian cancers and 12 ovarian cancer cell lines. In an effort to increase chemotherapy sensitivity, pathways shown to be activated in platinum-resistant cancers were subject to targeted therapy in ovarian cancer cell lines.

#### Results

Gene expression profiles identified patients with ovarian cancer likely to be resistant to primary platinum-based chemotherapy with greater than 80% accuracy. In patients with platinum-resistant disease, we identified expression signatures consistent with activation of *Src* and *Rb/E2F* pathways, components of which were successfully targeted to increase response in ovarian cancer cell lines.

#### Conclusion

We have defined a strategy for treatment of patients with advanced-stage ovarian cancer that uses therapeutic stratification based on predictions of response to chemotherapy, coupled with prediction of oncogenic pathway deregulation, as a method to direct the use of targeted agents.

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### INTRODUCTION

Ovarian cancer is a leading cause of cancer death among women in the United States and Western Europe and has the highest mortality rate of all gynecologic cancers. Currently, platinum drugs are the most active agents in epithelial ovarian cancer therapy.<sup>1-3</sup> Consequently, the standard treatment protocol used in the initial management of advanced-stage ovarian cancer is cytoreductive surgery, followed by primary chemotherapy with a platinum-based regimen that usually includes a taxane.<sup>4</sup> Approximately 70% of patients will have a complete clinical response to this initial therapy, with absence of clinical or radiographic detectable residual disease and normalization of serum CA-125 levels.<sup>5,6</sup> The remaining 30% of patients will demonstrate residual or progressive platinum-

resistant disease. The inability to predict response to specific therapies is a major impediment to improving outcome for women with ovarian cancer. Empiric-based treatment strategies are used and result in many patients with chemotherapy-resistant disease receiving multiple cycles of often toxic therapy without success before the lack of efficacy is identified. In the course of these empiric treatments, patients may experience significant toxicities, compromise to bone marrow reserves, detriment to quality of life, and delay in the initiation of therapy with active agents. Moreover, the lack of active therapeutic agents for patients with platinum-resistant disease limits treatment options. As such, many patients receive chemotherapy with little or no benefit.

The clinical heterogeneity of ovarian cancer, resulting from the acquisition of multiple genetic

From the Divisions of Gynecologic Surgical Oncology and Cancer Prevention and Control, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; Institute for Genome Sciences and Policy, Department of Molecular Genetics and Microbiology, Department of Obstetrics and Gynecology/Division of Gynecologic Oncology, and Departments of Surgery and Medicine, Duke University Medical Center; Institute of Statistics and Decision Sciences, Duke University, Durham, NC; and Institute of Medical Genetics, University Hospital of Wales, Cardiff, United Kingdom.

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Address reprint requests to Johnathan M. Lancaster, MD, PhD, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Dr, Tampa, FL 33612; e-mail: lancasm@moffitt.usf.edu.

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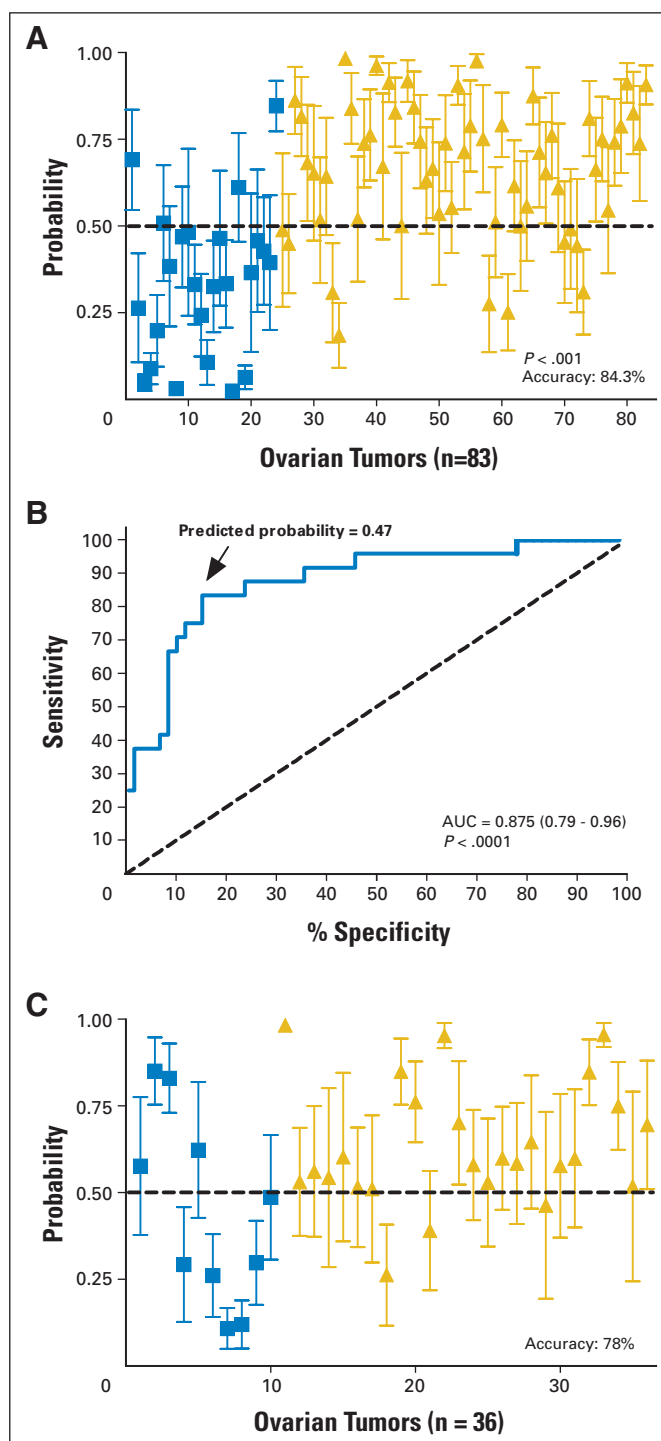
alterations that contribute to the development of the tumor, underlies the heterogeneity of response to chemotherapy.<sup>7</sup> Although a variety of gene alterations have been identified, no single gene marker can reliably predict response to therapy and outcome.<sup>8-12</sup> Recent advances in the use of DNA microarrays, which allow global assessment of gene expression in a single sample, have shown that expression profiles can provide molecular phenotyping that identifies distinct classifications not evident by traditional histopathologic methods.<sup>13-20</sup> Our group and others have applied this approach to describe gene expression profiles associated with ovarian cancer development, surgical debulking, response to therapy, and survival.<sup>21-27</sup> We have now applied genomic methodologies to identify gene expression patterns within primary tumors that predict response to primary platinum-based chemotherapy. We have coupled this analysis with gene expression signatures that reflect the deregulation of various oncogenic signaling pathways to identify unique characteristics of the platinum-resistant cancers that can guide the use of these drugs in patients with platinum-resistant disease. We propose integrating gene expression profiles that predict platinum response and oncogenic pathway status as a strategy for developing personalized treatment plans for individual patients.

## PATIENTS AND METHODS

### Patients and Tissue Samples

Clinicopathologic characteristics of the 119 patients who contributed the ovarian cancer samples included in this study are listed in Table 1. All ovarian cancer samples were obtained at initial cytoreductive surgery from patients treated at Duke University Medical Center and H. Lee Moffitt Cancer Center and Research Institute, who then received platinum-based primary chemotherapy. The samples were divided (70:30 ratio) into training and validation sets. As a result, 83 (70%) of 119 samples were randomly selected for the training set, and 36 (30%) of 119 samples were selected for the validation set. In the training set, a total of 59 (71%) of 83 patients demonstrated a complete response (CR) and 24 (29%) of 83 patients demonstrated an incomplete

Table 1. Clinicopathologic Characteristics of Ovarian Cancer Patients		
Characteristic	Clinical Complete Responders (n = 85)	Clinical Incomplete Responders (n = 34)
Mean age, years	63	65
Stage, No. of patients		
III	72	27
IV	13	7
Grade, No. of patients		
1	2	1
2	42	15
3	41	18
Surgical debulking, No. of patients		
Optimal, < 1 cm	51	12
Suboptimal, ≥ 1 cm	34	22
Chemotherapy, No. of patients		
Platinum/cyclophosphamide	23	11
Platinum/paclitaxel	60	22
Single-agent platinum	2	1
Mean serum CA-125, m/mL		
Before platinum	2,601	4,635
After platinum	16	529
Mean survival time, months	45	31



**Fig 1.** Gene expression pattern associated with platinum response. (A) Leave-one-out cross validation of training set (blue = incomplete responders, yellow = responders). (B) Receiver operating characteristic (ROC) curve of the training set. (C) Validation of the platinum response prediction based on a cutoff of 0.47 predicted probability of response as determined by ROC curve. AUC, area under the curve.

response (IR) to primary platinum-based therapy after surgery. In the validation set, a total of 26 (72%) of 36 patients demonstrated a CR and 10 (28%) of 36 patients demonstrated an IR to primary platinum-based therapy. The distribution of CR and IR in both the training and validation sets was selected

**Table 2.** Highest Weighted Genes in the Platinum Prediction Response Models Using 83-Sample Training Set and Validated in 36-Sample Validation Set

Gene Title	Gene Symbol	Representative Public ID
Sialidase 1 (lysosomal sialidase)	<i>NEU1</i>	U84246
Translocated promoter region (to activated <i>MET</i> oncogene)	<i>TPR</i>	NM_003292
Periplakin	<i>PPL</i>	NM_002705
H3 histone, family 3B (H3.3B)	<i>H3F3B</i>	BC001124
Zinc finger protein 264	<i>ZNF264</i>	NM_003417
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 4	<i>PSMD4</i>	AB033605
Heterogeneous nuclear ribonucleoprotein U	<i>HNRPU</i>	BC003621
Peptidylglycine alpha-amidating mono-oxygenase	<i>PAM</i>	NM_000919
Glyceronephosphate O-acyltransferase	<i>GNPAT</i>	NM_014236
Splicing factor 3a, subunit 3, 60 kd	<i>SF3A3</i>	NM_006802
Glycine cleavage system protein H (aminomethyl carrier)	<i>GCSH</i>	AW237404
Reticulocalbin 1, EF-hand calcium binding domain	<i>RCN1</i>	NM_002901
Hypothetical protein FLJ10404	<i>FLJ10404</i>	NM_019057
Trophinin-associated protein (tastin)	<i>TROAP</i>	NM_005480
Tissue inhibitor of metalloproteinase 2	<i>TIMP2</i>	NM_003255
Ribosomal protein S20	<i>RPS20</i>	BF184532
PTK7 protein tyrosine kinase 7	<i>PTK7</i>	NM_002821
Suppressor of cytokine signaling 5	<i>SOCS5</i>	AW664421
NADH dehydrogenase (ubiquinone) flavoprotein 1, 51 kd	<i>NDUFB1</i>	AF092131
Protein phosphatase 4, regulatory subunit 1	<i>PPP4R1</i>	NM_005134
Cysteine-rich, angiogenic inducer, 61	<i>CYR61</i>	NM_001554
MCM4 minichromosome maintenance deficient 4	<i>MCM4</i>	AA604621
Thyroid hormone receptor-associated protein 1	<i>THRAP1</i>	AB011165
Calcyclin binding protein//calcyclin binding protein	<i>CACYBP</i>	BC005975
Hydroxysteroid (17-beta) dehydrogenase 12	<i>HSD17B12</i>	NM_016142
DnaJ (Hsp40) homolog, subfamily C, member 9	<i>DNAJC9</i>	BE551340
Translocated promoter region (to activated <i>MET</i> oncogene)	<i>TPR</i>	BF110993
PERP, TP53 apoptosis effector	<i>PERP</i>	NM_022121
Importin 13	<i>IPO13</i>	NM_014652
Pleckstrin homology domain interacting protein	<i>PHIP</i>	BF224151
Cyclin B2	<i>CCNB2</i>	NM_004701
CDC5 cell division cycle 5-like ( <i>Schizosaccharomyces pombe</i> )	<i>CDC5L</i>	NM_001253
Zinc finger protein 592	<i>ZNF592</i>	NM_014630
Kazrin	<i>KIAA1026</i>	AB028949
Nuclear receptor coactivator 2	<i>NCOA2</i>	AI040324
DKFZP564G2022 protein	<i>DKFZP564G2022</i>	BG493972
GK001 protein	<i>GK001</i>	NM_020198
IQ motif containing GTPase activating protein 1	<i>IQGAP1</i>	AI679073
Lysosomal associated protein transmembrane 4 beta	<i>LAPTM4B</i>	NM_018407
Protein kinase, interferon-inducible double stranded RNA-dependent inhibitor, repressor of (P58 repressor)		

(continued in next column)

**Table 2.** Highest Weighted Genes in the Platinum Prediction Response Models Using 83-Sample Training Set and Validated in 36-Sample Validation Set (continued)

Gene Title	Gene Symbol	Representative Public ID
Ash2 (absent, small, or homeotic)-like ( <i>Drosophila</i> )	<i>ASH2L</i>	AB020982
Kallikrein 5	<i>KLK5</i>	AF243527
Low-density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor)		
Membrane-associated ring finger (C3HC4) 5	<i>C3HC4</i>	NM_017824
Ring-box 1	<i>RBX1</i>	NM_014248
SET domain, bifurcated 1	<i>SETDB1</i>	NM_012432
Epiplakin 1//epiplakin 1	<i>EPPK1</i>	NM_031308
HIV-1 Tat interacting protein, 60 kd	<i>HTATIP</i>	BC000166
CGI-128 protein	<i>CGI-128</i>	NM_016062
Reticulon 3	<i>RTN3</i>	NM_006054
CGI-62 protein	<i>CGI-62</i>	NM_016010
7-Dehydrocholesterol reductase	<i>DHCR7</i>	AW150953
Chromosome 9 open reading frame 10	<i>C9orf10</i>	BE963765
Replication factor C (activator 1) 1	<i>RFC1</i>	NM_002913
Nuclear transcription factor Y, beta	<i>NFYB</i>	AI804118
Chromosome 8 open reading frame 33	<i>C8orf33</i>	NM_023080
Tumor rejection antigen (gp96) 1	<i>TRA1</i>	NM_003299
Transportin 1	<i>TNPO1</i>	NM_002270
Protein phosphatase 3 (formerly 2B), catalytic subunit	<i>PPP3CB</i>	NM_021132
High-mobility group 20B	<i>HMG20B</i>	BC002552
Lamin A/C	<i>LMNA</i>	AA063189
Phosphoglycerate kinase 1	<i>PGK1</i>	NM_000291
RNA (guanine-7-) methyltransferase	<i>RNMT</i>	NM_003799
HSPC038 protein	<i>LOC51123</i>	NM_016096
Myosin VI	<i>MYO6</i>	AA877789
Lipase A, lysosomal acid, cholesterol esterase	<i>LIPA</i>	NM_000235
DiGeorge syndrome critical region gene 6//DiGeorge syndrome critical region gene 6-like		
Protein kinase C, zeta	<i>PRKCZ</i>	NM_002744
Tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase 2		
Nedd4 binding protein 1	<i>N4BP1</i>	BF436315
Tetraspanin 6	<i>TSPAN6</i>	AF053453
Mitochondrial ribosomal protein L9//mitochondrial ribosomal protein L9		
Chromosome 20 open reading frame 47	<i>C20orf47</i>	AF091085
Macrophage stimulating 1 (hepatocyte growth factor-like)	<i>MST1</i>	NM_020998
Mlx interactor	<i>MONDOA</i>	NM_014938
RAB31, member <i>RAS</i> oncogene family	<i>RAB31</i>	NM_006868
Prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy)		
Solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier)		
Small nuclear ribonucleoprotein polypeptide A	<i>SNRPA</i>	NM_004596
KIAA0247	<i>KIAA0247</i>	NM_014734
Cyclin M3	<i>CNNM3</i>	NM_017623
Zinc finger protein 443	<i>ZNF443</i>	NM_005815
Matrix-remodelling associated 5	<i>MXRA5</i>	AF245505

(continued on next page)

**Table 2.** Highest Weighted Genes in the Platinum Prediction Response Models Using 83-Sample Training Set and Validated in 36-Sample Validation Set (continued)

Gene Title	Gene Symbol	Representative Public ID
RAE1 RNA export 1 homolog ( <i>S pombe</i> )	<i>RAE1</i>	NM_003610
ATP synthase, H <sup>+</sup> transporting, mitochondrial F0 complex, subunit d		
Coenzyme A synthase	<i>COASY</i>	NM_025233
mutS homolog 6 ( <i>Escherichia coli</i> )	<i>MSH6</i>	NM_000179
Ubiquitin-specific protease 25	<i>USP25</i>	NM_013396
Quiescin Q6	<i>QSCN6</i>	NM_002826
Adenylate kinase 2	<i>AK2</i>	W02312
GNAS complex locus	<i>GNAS</i>	AI591100
Nucleolar protein family A, member 3 (H/ACA small nucleolar RNPs)		
Phosphatidylinositol-4-phosphate 5-kinase, type I, gamma	<i>PIP5K1C</i>	AB011161
Microtubule-associated protein 4	<i>MAP4</i>	W28892
Torsin family 3, member A	<i>TOR3A</i>	NM_022371
Ankyrin repeat domain 10	<i>ANKRD10</i>	NM_017664
Muscleblind-like ( <i>Drosophila</i> )	<i>MBNL1</i>	NM_021038
Shank-interacting protein-like 1/// shank-interacting protein-like 1		
Natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)		
Geranylgeranyl diphosphate synthase 1	<i>GGPS1</i>	NM_004837

to reflect clinical CR rates of approximately 70%. The distribution of debulking status within the training and validation sets was equally balanced. All tissues were collected under the auspices of respective institutional review board–approved protocols with written informed consent.

### Measurement of Clinical Response

Response to therapy in ovarian cancer patients was evaluated from the medical record using standard WHO criteria for patients with measurable disease.<sup>28</sup> CA-125 was used to classify responses only in the absence of a measurable lesion; CA-125 response criteria were based on established guidelines.<sup>29,30</sup> A CR was defined as a complete disappearance of all measurable and assessable disease or, in the absence of measurable lesions, a normalization of the CA-125 level after adjuvant therapy. Patients were considered to have an IR if they demonstrated only a partial response, had stable disease, or demonstrated progressive disease during primary therapy. A partial response was considered a 50% or greater reduction in the product obtained from measurement of each bidimensional lesion for at least 4 weeks or a decrease in the CA-125 level by at least 50% for at least 4 weeks. Disease progression was defined as a 50% or greater increase in the product from any lesion documented within 8 weeks of initiation of therapy, the appearance of any new lesion within 8 weeks of initiation of therapy, or any increase in the CA-125 from baseline at initiation of therapy. Stable disease was defined as disease not meeting any of the above criteria.

### RNA and Microarray Analysis

Frozen tissue samples were embedded in optimal cutting temperature medium, and sections were cut and slide mounted. Slides were stained with hematoxylin and eosin to assure that samples included greater than 70% tumor content. Approximately 30 mg of tissue was used for RNA isolation. See Appendix (online only) for further details. Affymetrix DNA microarray analysis was prepared according to the manufacturer's instructions, and targets were hybridized to the Human U133A GeneChip (Affymetrix, Santa Clara, CA).

### Statistical Analysis

The expression intensities for all genes across the samples were normalized using the robust multiarray average (RMA),<sup>31</sup> including probe-level quantile normalization and background correction, as implemented in the Bioconductor software suite.<sup>32</sup> RMA data were prescreened to remove genes/probes with trivial variation across the sample and low median expression levels; thus, 6,088 genes/probes were used in the analysis. The remaining RMA data were further processed by applying sparse regression model methods<sup>33</sup> to correct for assay artifacts; the resulting expression files are available at <http://data.cgt.duke.edu/platinum.php>. A binary logistic regression model analysis and a stochastic regression model search, called shotgun stochastic search (SSS), was used to determine platinum response prediction models in the training set of 83 samples. The details for SSS methods can be found in the Appendix (online only).

### Cell Lines and RNA Extraction

The ovarian cancer cell lines OV90, TOV21G, and TOV112D were grown as recommended by the supplier (American Type Culture Collection, Rockville, MD). FUV01, a human ovarian carcinoma, was grown according to the supplier (DSMZ, Braunschweig, Germany). Eight additional cell lines (C13, OV2008, A2780CP, A2780S, IGROV1, T8, OVCAR5, and IMCC3) were provided by Dr Patricia Kruk (Department of Pathology, College of Medicine, University of South Florida, Tampa, FL). These eight cell lines were grown in RPMI-1640 supplemented with 10% fetal bovine serum, 1% sodium pyruvate, and 1% nonessential amino acids. All tissue culture reagents were obtained from Sigma Aldrich (St Louis, MO). Total RNA was extracted from each cell line and assayed on the Human 133 plus 2.0 arrays (Affymetrix).

### Cell Proliferation Assays

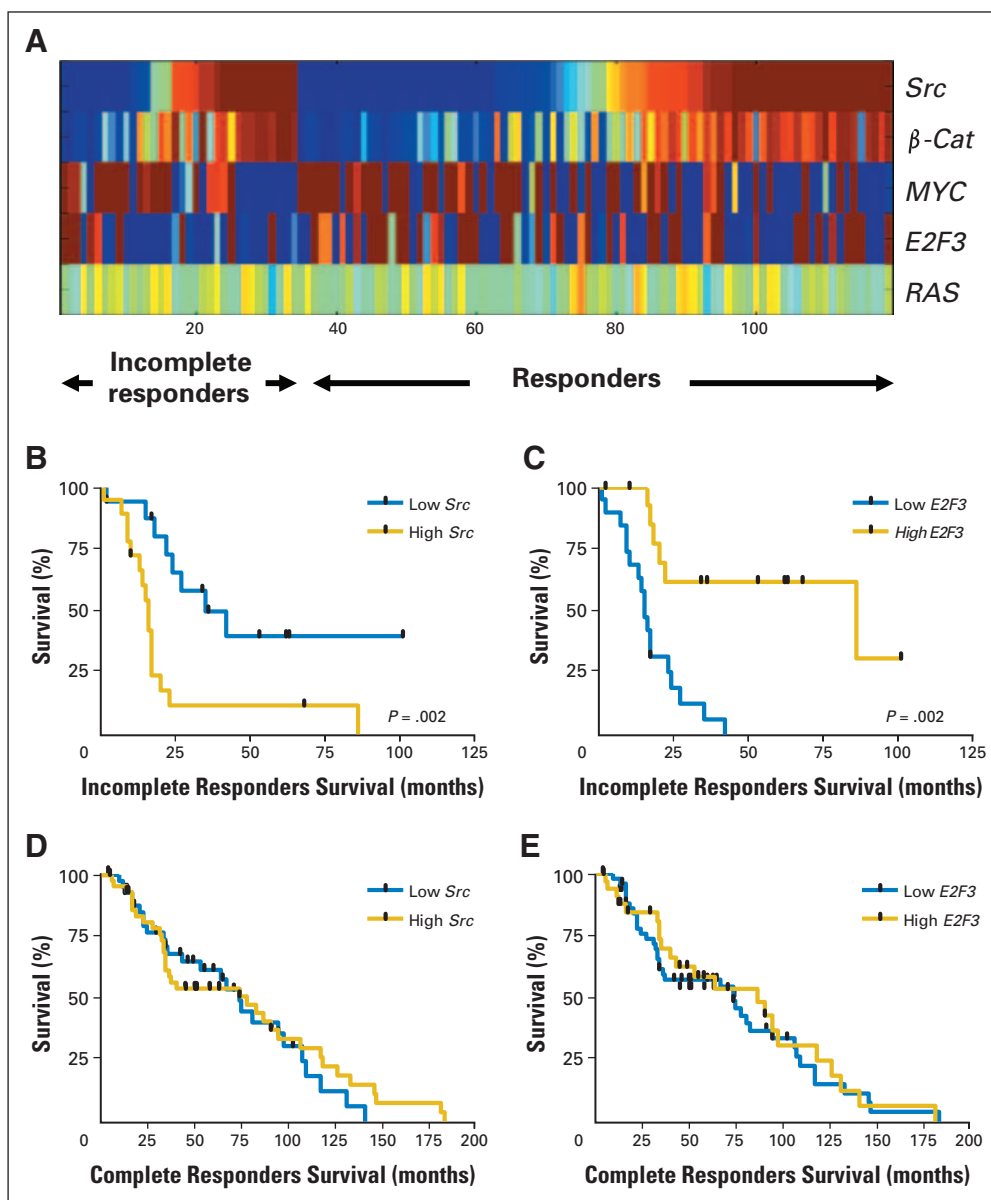
Assays measuring cell proliferation and the effects of targeted agents have been described previously.<sup>34</sup> Details can be found in the Appendix (online only).

## RESULTS

### Gene Expression Profiles That Predict Platinum Response

With the ultimate objective of developing a strategy for determining the most appropriate therapy for an individual patient with ovarian cancer, we developed a predictive tool that identifies patients with platinum-resistant disease at the time of initial diagnosis. The 83-sample training set was used to identify a gene expression pattern that could predict clinical outcome. Using a cutoff of 0.47 predicted probability of response, as determined by receiver operating characteristic curve analysis (Fig 1A), platinum response in patients was predicted accurately in 70 of 83 samples, achieving an overall accuracy of 84.3% (specificity 85%; sensitivity 83%; Fig 1B). Applying a Mann-Whitney *U* test for statistical significance ( $P < .001$ ) demonstrates the capacity of the predictor to distinguish nonresponder patients from responder patients.

A validation of the predictive performance of the gene expression model was performed on a randomly generated set of 36 samples to evaluate the ability of the model to predict platinum response. Both the training and validation sets were balanced with respect to platinum response rates seen in the clinic (ie, approximately 70% complete responders). On the basis of the cutoff of 0.47 as defined in the training set, it is evident that the predicted platinum response in the training set performs well to predict the response within the separate validation set (78% accuracy; Fig 1C). When other clinical variables, such as debulking status and CA-125, were included in the SSS to determine platinum response predictions, there was no effect on the predicted accuracy or gene content of the models, suggesting that the signature of platinum response is independent of other clinical variables.



**Fig 2.** Prediction of oncogenic pathway deregulation and drug sensitivity in ovarian cancer cell lines. (A) Predicted probability of pathway activation (red = high probability, blue = low probability) in ovarian tumors (n = 119). (B) Kaplan-Meier survival analysis demonstrating relationship of *Src* and (C) *E2F3* pathway activation and survival of patients who demonstrated an incomplete response to primary platinum therapy. (D) Kaplan-Meier survival analysis demonstrating relationship of *Src* and (E) *E2F3* pathway activation and survival of patients who demonstrated a complete response to primary platinum therapy.

Given these results, we conclude that it is possible to develop gene expression profiles that have the capacity to predict response to platinum-based chemotherapy and thus serve as a mechanism to stratify patients with respect to treatment. Although the ability to identify responsive patients is not likely a primary goal, a capacity to identify the patients resistant to platinum therapy would be a significant benefit in guiding more effective treatment for these patients. In this context, an emphasis on the specificity of predicting resistance might be the most appropriate goal.

A total of 1,727 genes were included in the averaged predictive model, and the 100 genes most weighted in achieving the prediction are listed in Table 2. Analysis of gene ontology categories represented by these genes is depicted in Appendix Table A1 (online only). The analysis reveals an enrichment for genes reflecting cell proliferation and cell growth, certainly consistent with a mechanism of action of cytotoxic chemotherapeutic agents such as cisplatin and paclitaxel that generally are directed at the proliferative capacity of the cancer cell.

### Identifying Therapeutic Options for Patients With De Novo Platinum-Resistant Ovarian Cancer

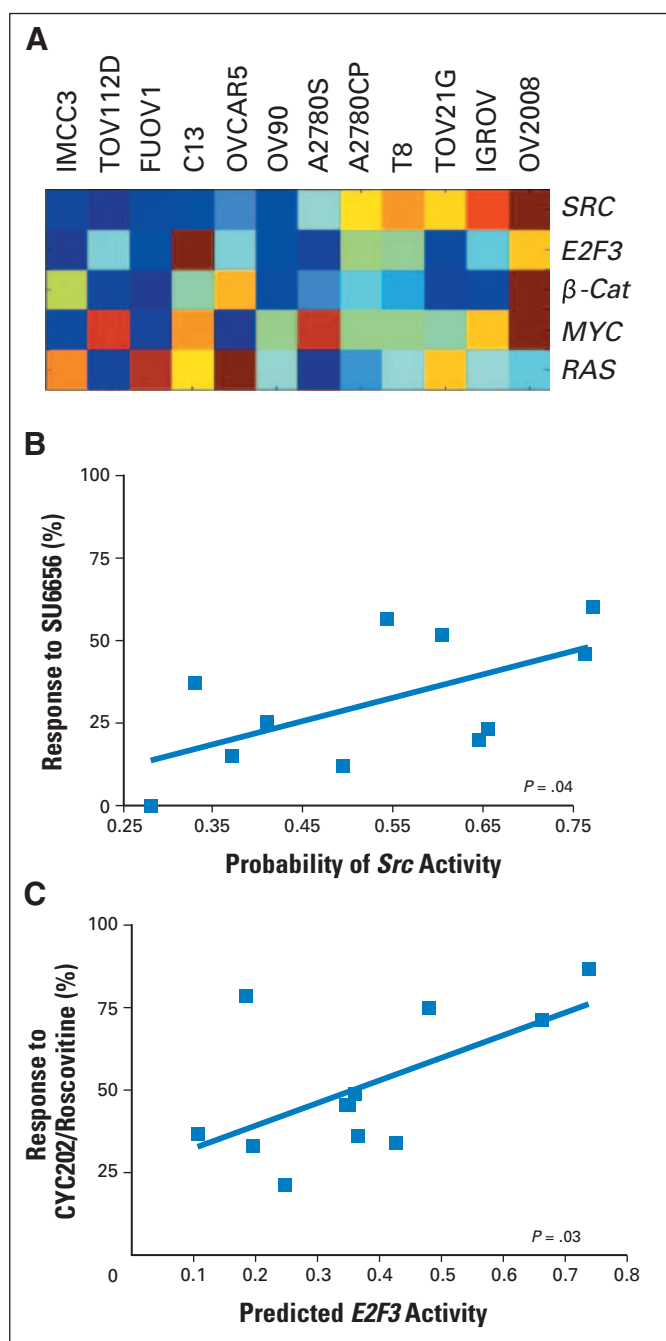
The development of a predictor that can identify patients likely to be resistant to primary platinum therapy provides an opportunity to effectively identify the population most likely to benefit from additional therapeutic intervention. The challenge is determining what other therapies might benefit these patients. Although in principle it might be possible to use the gene expression data to deduce the critical biologic distinction(s) that predicts platinum response, in practice this is difficult because of our limited knowledge of the integration of biologic pathways and systems. We believe an alternative strategy is one that makes use of an ability to profile the status of various oncogenic signaling pathways within the tumor. We have recently described the development of gene expression signatures that reflect the activation status of several oncogenic pathways and have shown that these signatures can evaluate the status of the pathways in a series of tumor samples,

providing a prediction of relative probability of pathway deregulation of each tumor.<sup>34</sup>

To explore the potential for using this as an approach to identify new therapeutic options, we made use of the previously developed signatures to predict the status of these pathways in the tumors. In each case, the probability of pathway activation in a given tumor is predicted from the signature developed by expression of the activating oncogene in quiescent epithelial cell cultures. Evidence for high probability of pathway activation is indicated by red, and evidence for low probability is indicated by blue (Fig 2A). Initial analyses revealed that a substantial number of the tumors exhibit *Src* pathway deregulation. In Figure 2A, the tumor samples are sorted based on the predicted level of *Src* activity. The Kaplan-Meier survival analysis in Figure 2B illustrates further that those patients with deregulated *Src* pathway also exhibit the worst prognosis. However, in complete responders, there was no evident relationship between *Src* and *E2F3* pathway deregulation and survival (Fig 2D and 2E). An examination of other pathways in the context of the *Src* pathway deregulation revealed *Myc* and *E2F3* to be frequently deregulated in the tumors lacking *Src* activity. Although *Myc* pathway deregulation does not link with available therapeutics, *E2F3* deregulation does suggest an opportunity for use of a cyclin-dependent kinase (*CDK*) inhibitor. We further explored the potential of these two pathway signatures (*Src* and *E2F3*) to direct the use of inhibitors that target these pathways.

In parallel with the determination of pathway status in the tumors, we characterized the status of the pathways in a series of ovarian cancer cell lines (Fig 3A). This analysis provides a baseline measure of the status of these pathways that can be compared with the sensitivity of the cells to therapeutic drugs known to target specific activities within given oncogenic pathways. The goal is to determine whether a cell line is sensitive to a drug based on the knowledge of the pathway deregulation within that cell. For the *Src* pathway, we made use of an *Src*-specific inhibitor (SU6656), and for the *E2F3* pathway, we made use of a *CDK* inhibitor (roscovitine). The ability of these agents to inhibit growth of the ovarian cancer cell lines was assessed using assays of cell proliferation. In Figures 3B and 3C, a clear and statistically significant relationship can be seen between prediction of either *Src* or *E2F3* pathway deregulation and sensitivity to the respective therapeutic of that pathway. As such, it is evident from these results that predicted pathway deregulation predicts sensitivity to the pathway-specific therapeutic agent.

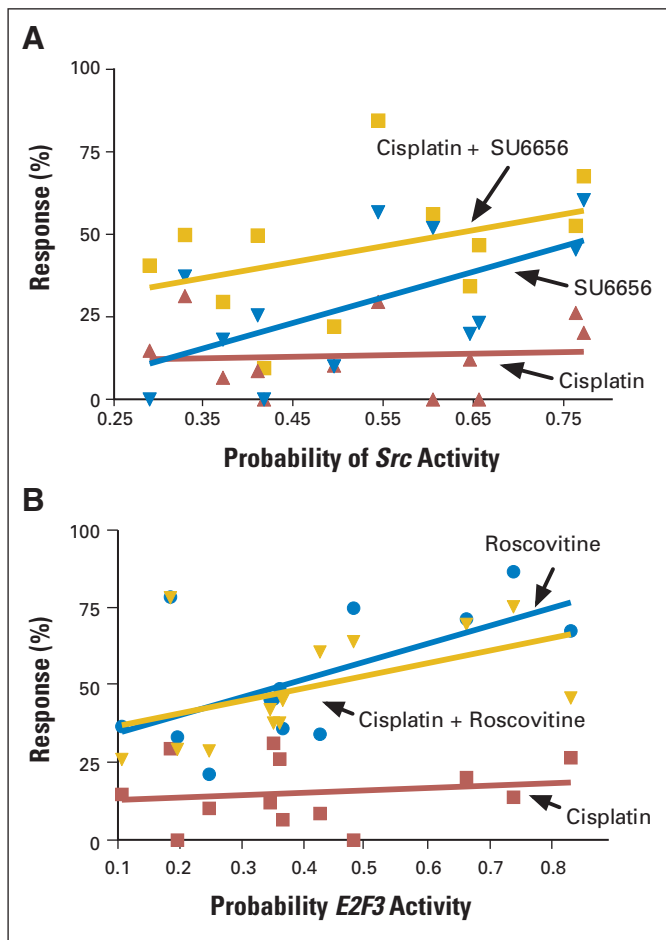
Although the goal of the use of pathway predictions is to identify options for patients with platinum-resistant ovarian cancer, it is nevertheless true that most of the patients with platinum-resistant disease will show some evidence of response to platinum therapy. The utilization of targeted therapeutics, such as the *Src* or *CDK* inhibitor, likely would be in conjunction with standard cytotoxic chemotherapies such as carboplatin and paclitaxel. We have further investigated the extent to which there may be an additive effect of combined therapies. A collection of ovarian cancer cell lines was assayed for sensitivity to cisplatin either with or without SU6656 or roscovitine. In Figure 4, the response was plotted as a function of pathway prediction (either *Src* or *E2F3*), and as seen previously, there is a relationship between pathway deregulation and SU6656 or roscovitine drug sensitivity. In contrast, there was no evident relationship between pathway deregulation and cisplatin sensitivity. Nevertheless, there was evidence for a greater sensitivity to the combination of cisplatin and SU6656 compared with



**Fig 3.** Prediction of *Src* and *E2F3* pathway deregulation predicts sensitivity to pathway-specific drugs. (A) Pathway predictions (red = high probability, blue = low probability) in ovarian cancer cell lines. (B) Sensitivity of cell lines to *Src* inhibitor (SU6656) and (C) cyclin-dependent kinase inhibitor (roscovitine). Growth inhibition assays are plotted as percent inhibition of proliferation versus probability of pathway activation (*Src* and *E2F3*).

either agent alone, whereas there was no evident added benefit of cisplatin combined with roscovitine versus roscovitine alone.

Taken together, these results demonstrate a capacity of a pathway signature to not only predict deregulation of the pathway but also to predict sensitivity to therapeutic agents that target the corresponding pathways. We suggest that this is a viable approach for directing the use of various therapeutic agents.



**Fig 4.** Sensitivity of ovarian cancer cell lines to combinations of pathway-specific and cytotoxic drugs as a function of pathway deregulation. (A) Proliferation inhibition of cisplatin, SU6656, and a combination of SU6656 and cisplatin plotted as a function of probability of *Src* pathway activation. (B) Proliferation inhibition of roscovitine (blue), cisplatin, and a combination of roscovitine and cisplatin plotted as a function of probability of *E2F3* pathway activation.

## DISCUSSION

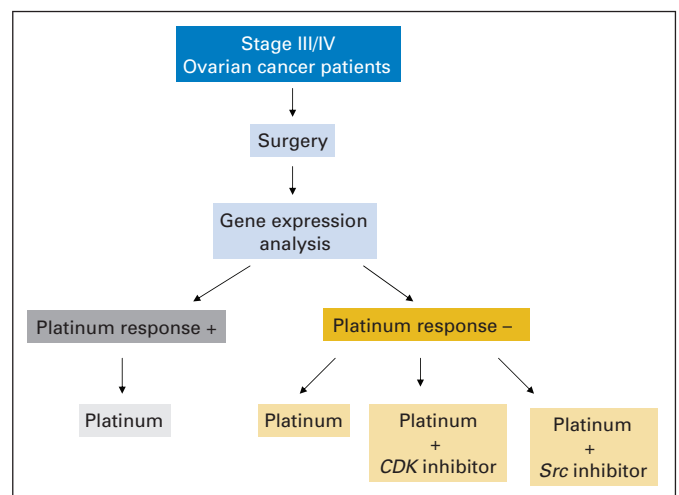
Treatment of patients with advanced-stage ovarian cancer is empiric, and almost all patients receive a platinum drug, usually with a taxane. Although many patients achieve a clinical CR to platinum-based primary therapy, a significant fraction of patients either have an IR or develop progression of disease during primary therapy. Recently, several groups have used genomic approaches to delineate genes that may impact ovarian cancer platinum responsiveness.<sup>24-27</sup> Although we can identify some commonality of gene family/function (ie, zinc finger proteins, ubiquitin-specific proteases, protein phosphatases, and DNA mismatch repair genes) between our platinum predictor and those of others,<sup>24-27</sup> common genes do not seem to be represented, which could be a result of the use of cDNA-based microarrays by other groups.

Strategies for the treatment of patients determined to be resistant to platinum-based chemotherapy involve the use of various empiric-based salvage chemotherapy agents that often have only marginal benefit. Although it is possible that, based on knowledge that the patient is unlikely to benefit from platinum therapy, initiation of

salvage agents as first-line therapy would achieve a greater benefit, we believe a more effective strategy may be the use of agents that target components of pathways that are seen to be deregulated in individual cancers. Thus, the therapeutic strategy is tailored to the individual patient based on knowledge of the unique molecular alterations in their tumor.

Individualizing treatments by identifying those patients unlikely to respond fully to the primary platinum-based therapy coupled with an ability to identify characteristics unique to this group of patients can direct the use of novel therapeutic strategies. This truly represents a move toward the goal of personalized treatment. An outline of the approach afforded by these developments is summarized in Figure 5. The capacity to predict likely response to platinum chemotherapy based on gene expression data obtained from the primary tumor can identify those patients most appropriate for additional therapies. The purpose of this assessment is not to direct the use of primary platinum-based chemotherapy but rather to identify that subset of patients who most likely will benefit from additional therapies. The use of pathway predictions provides a basis for utilization of drugs specific to the deregulated pathway in patients predicted to have platinum-resistant disease. In Figure 5, this might involve a choice of either an *Src* inhibitor or a *CDK* inhibitor based on the observation that these two pathways dominate ovarian cancers and the results that demonstrate a capacity of these pathway predictors to also predict sensitivity to these agents. Given the fact that most patients demonstrate some (if not complete) response to platinum therapy, we would expect that, for now, all patients would still receive standard platinum therapy, but patients predicted to have an IR to platinum would also receive a targeted therapeutic.

We believe that the approach described here, using gene expression profiles that predict primary chemotherapy response coupled with expression data that identify oncogenic pathway deregulation to stratify patients to the most appropriate treatment regimen, represents an important step toward the goal of personalized cancer treatment. We further suggest that a major benefit of this approach (and, in particular, the use of pathway information to guide the use of targeted therapeutics) is the capacity to ultimately direct the formulation of



**Fig 5.** Potential application of platinum response and pathway prediction in the treatment of patients with ovarian cancer.

combinations of therapies (multiple drugs that target multiple pathways) based on information that details the state of activity of the pathways.

#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

#### AUTHOR CONTRIBUTIONS

**Conception and design:** Holly K. Dressman, Andrew Berchuck, Andrea Bild, Jonathan Gray, Joseph R. Nevins, Johnathan M. Lancaster

**Financial support:** Joseph R. Nevins, Johnathan M. Lancaster

**Administrative support:** Joseph R. Nevins, Johnathan M. Lancaster

**Provision of study materials or patients:** Andrew Berchuck, Robyn Sayer, Janiel Cragun, Johnathan M. Lancaster

**Collection and assembly of data:** Holly K. Dressman, Andrew Berchuck, Gina Chan, Andrea Bild, Robyn Sayer, Janiel Cragun, Jennifer Clarke, Regina S. Whitaker, LiHua Li, Joseph R. Nevins, Johnathan M. Lancaster

**Data analysis and interpretation:** Holly K. Dressman, Andrew Berchuck, Gina Chan, Jun Zhai, Andrea Bild, Jennifer Clarke, LiHua Li, Jeffrey Marks, Geoffrey S. Ginsburg, Anil Potti, Mike West, Joseph R. Nevins, Johnathan M. Lancaster

**Manuscript writing:** Holly K. Dressman, Andrew Berchuck, Jun Zhai, Andrea Bild, Jennifer Clarke, LiHua Li, Jonathan Gray, Geoffrey S. Ginsburg, Anil Potti, Mike West, Joseph R. Nevins, Johnathan M. Lancaster

**Final approval of manuscript:** Holly K. Dressman, Andrew Berchuck, Gina Chan, Jun Zhai, Andrea Bild, Robyn Sayer, Janiel Cragun, Jennifer Clarke, Regina S. Whitaker, LiHua Li, Jonathan Gray, Jeffrey Marks, Geoffrey S. Ginsburg, Anil Potti, Mike West, Joseph R. Nevins, Johnathan M. Lancaster

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***Appendix***

The Appendix is included in the full-text version of this article, available online at [www.jco.org](http://www.jco.org). It is not included in the PDF version (via Adobe® Reader®).