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Platinum resistance in gynecologic malignancies: Response, disease free and overall survival are predicted by biochemical signature: A metabolomic analysis

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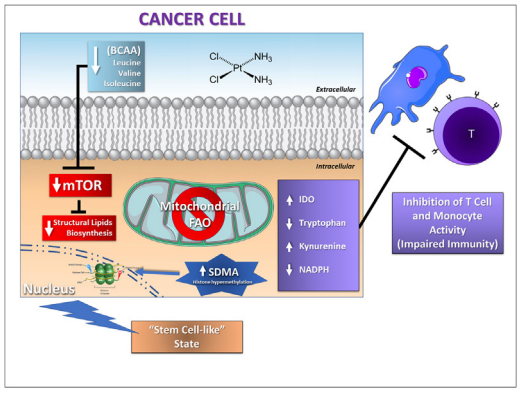
HIGHLIGHTS

- Platinum resistance in gynecologic cancer correlates with metabolic signatures measured by quantitative mass spectrometry.
- Metabolic signatures predict clinical outcome following carboplatin plus Paclitaxel chemotherapy.
- Altered amino acid and lipid profiles characterize a state of tumor cellular quiescence associated with immune dysfunction.

GRAPHICAL ABSTRACT



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ABSTRACT

Objective. Platinum resistance, defined as the lack of response or relapse within six months of platinum-based chemotherapy, is an important determinant of survival in gynecologic cancer. We used quantitative Mass Spectrometry to identify metabolic signatures that predict platinum resistance in patients receiving chemotherapy for gynecologic cancers.

Methods. In this study 47 patients with adenocarcinoma of the ovary or uterus who were candidates for carboplatin plus paclitaxel submitted blood for quantitation of metabolites and surgical specimens for the

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isolation 3-dimensional organoids used to measure individual patient platinum resistance, ex vivo. Results were correlated with response, time to progression and survival.

Results. Of 47 patients, 27 (64.3%) achieved complete remission with a mean time to progression of 1.9 years (± 1.5), disease-free survival of 1.7 years (± 1.4) and overall survival of 2.6 years (± 1.6) and a mean cisplatin lethal concentration 50% (LC50) = 1.15 μg/ml (range 0.4–3.1). Cisplatin LC50's correlated with a non-significant decrease in complete remission (RR [95% CI] =0.76 [0.46–1.27]), diminished disease-free survival (median: 1.15 vs. 2.99 years, $p = 0.038$) and with biochemical signatures of 186 metabolites. Receiver operating curves (ROC) of lipid ratios, branched chain amino acids and the tryptophan to kynurenine ratio identified patients at the highest risk of relapse and death (AUC = 0.933) with a sensitivity of 92.0% and specificity of 86.0% ($p < 0.001$).

Conclusions. Metabolic signatures in gynecologic cancer identify patients at the highest risk of relapse and death offering new diagnostic and prognostic tools for management of the advanced gynecologic tumors.

1. Introduction

Of the over 117,000 new gynecologic cancers diagnosed in the US annually, adenocarcinomas of the ovary and uterus are the most common. With 21,750 new diagnoses and 13,900 deaths, ovarian cancer is the leading cause of death from female reproductive system cancer followed closely by endometrial cancer with 65,620 new cases and 12,520 deaths [1].

Total abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy with optimal de-bulking is the most important determinant of long-term survival in ovarian [2] and uterine cancer [3]. Patients with advanced ovarian and endometrial cancers of adequate performance status receive postoperative platinum-based chemotherapy. After the introduction of cisplatin (CDDP) plus paclitaxel [4], carboplatin plus paclitaxel became the gold standard of treatment for ovarian and endometrial cancer [5].

Over 80% of ovarian and 60% of endometrial cancer patients initially respond to platinum-based chemotherapy, but the majority relapse. The term "platinum-resistant" [6] refers to patients with ovarian cancer who progress within six months of platinum-based therapy and identifies those at the highest risk for disease related mortality. "Platinum resistance" applied to endometrial cancer has also been found significantly associated with progression free and overall survival ($P < 0.0001$) [7].

The critical role of platinum in the management of gynecologic malignancies has led to intensive research into the cellular mechanisms of platinum resistance including drug uptake, neutralization by intracellular thiols and DNA repair [8].

Since the 1980s platinum adduct repair by nucleotide excision ERCC1 (excision repair cross-complementation group) and mismatch repair have been a major focus of research [9]. More recently small interfering RNA HOTAIR (HOX transcript antisense intergenic RNA discovered by transcript profiling) has been examined [10,11].

The growing recognition that malignant transformation is associated with alterations in cellular bioenergetics has led to a renewed interest in the study of cancer metabolism. To examine the metabolic basis of platinum resistance, we conducted a prospective study of patients with advanced ovarian and endometrial cancer who received post-operative carboplatin plus paclitaxel following cytoreductive surgery and applied targeted mass spectrometry to assess the correlation between biochemical signatures, platinum resistance measured ex vivo and patient response and survival.

2. Materials and methods

2.1. Patients and study procedures

Patients referred to the Gynecology Oncology Service of the University of California, Irvine Long Beach Memorial Medical Center with advanced intraabdominal/pelvic malignancy between August 2013 and July 2018 were screened for eligibility. Of 51 patients who underwent surgical exploration during this period, 39 had advanced stage ovarian, 7 had uterine and 1 had both ovarian and uterine cancer. Four patients were found to have unrelated malignancies and were excluded from this study: appendiceal pseudo myxoma peritonei ($n = 1$), colon carcinoma ($n = 1$), and squamous cancer of the cervix ($n = 2$). The remaining 47 patients were evaluable for ex vivo platinum sensitivity and metabolomic analysis. A total of 31 healthy post-menopausal women ages 54–78 years (mean = 66) with no history of ovarian cancer with normal physical exam, ultrasound, mammogram, and biochemical findings served as controls.

Prior to surgery, eligible patients signed informed consent for the collection of tumor tissue and peripheral venous blood samples for metabolomic analysis at the time of surgery and clinical follow up. Tumor specimens were collected from the Department of Pathology and transferred to the laboratory within 30 min following surgery, in RPMI and immediately processed. Blood samples were collected in ethylendiaminetetraacetic acid (EDTA) tubes, and immediately centrifuged (5 min at 4000 rpm). Plasma samples were aliquoted, frozen, and stored at −80 °C for analysis. All 47 patients had both tissue culture and blood metabolic analyses conducted in parallel.

After recovery from surgery, all patients with adequate white count, hemoglobin, and platelet count and Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1 received carboplatin plus paclitaxel with an area under the curve (AUC) of 5 or 6 and a paclitaxel dose of 135 mg/m^2 or 175 mg/m^2, depending on performance status and tolerance [12]. All patients were followed through the course of treatment and monitored every three months by CA-125, CT scan, MRI, or PET/CT, depending upon the clinical determination of the treating physicians.

2.2. Demographic and clinical data

Patient demographics and baseline health characteristics were extracted from medical records. These included age at time of surgery, clinical stage (I-IV) at baseline, and receipt of neoadjuvant and adjuvant chemotherapy. For patients with measurable disease, clinical response was assessed by RECIST criteria [13]. Patients optimally debulked and rendered free of measurable disease at the time of surgery were followed for evidence of clinical recurrence.

Overall survival was measured as the number of months from the date of surgery until death or last contact. Progression-free survival was measured as the number of months from date of surgery until clinical progression, death, or end of follow-up.

The study was approved by Memorial Care Health System Institutional Review Board (project # 292-13) under the Department of Obstetrics and Gynecology, University of California, Irvine, and the Institutional Review Board of São Paulo Hospital (CEP/UNIFESP) from the Federal University of São Paulo, São Paulo, Brazil (approval # CAAE 40652915.2.0000.5505).

2.3. Ex vivo analysis of drug response

To develop a metric of individual patient platinum resistance, tissue culture studies were conducted by ex vivo analysis of programmed cell

death (EVA-PCD), a short-term suspension culture method that examines the morphologic and metabolic features of drug-induced programmed cell death in primary culture. The EVA-PCD laboratory method has previously been described [14,15].

Briefly, surgical specimens were mechanically and enzymatically dis-aggregated in 0.2% (w/v) DNAse and 0.4% (w/v) Collagenase IV. Tumor clusters of desired size were isolated by density centrifugation. Cell counts were adjusted by dilution and distributed into 96-well plates. Serial dilutions of Cisplatin were added by micropipette. Tumor cell/drug mixtures were incubated for 72 h at 37 °C in 5% CO2 in a humidified incubator.

After drug exposure, air dried slides were counterstained with H & E with percent viability measured against saline-exposed controls (normalized to 100%). Five-point dose response curves were interpolated to calculate platinum sensitivity as the lethal concentration of CDDP required to kill 50% of tumor cells (LC50 CDDP). Due to the highly right-skewed distribution, LC50 CDDP was log-transformed or dichotomized above vs. below the median (0.9 μg/mL) as ex vivo platinum resistant vs. sensitive in analyses.

2.4. Clinical outcome analyses

We conducted time-to-event analyses to explore whether ex vivo platinum sensitivity was associated with time to disease progression, disease-free-survival, and overall survival using Kaplan-Meier tables and multivariable-adjusted Cox proportional hazards regression model, adjusted for age, clinical stage, and neoadjuvant chemotherapy. All analyses on clinical outcomes were conducted both in the full sample of $n = 47$ participants and replicated among patients with high grade serous cancers only ($n = 30$) as a sensitivity analysis. A second time to event analysis explored whether the metabolomic signature define as

{[(Val/Phe)/(C18 : 2/lysoPC a C16 : 0)]/Tryptophan} was associated with time to progression, disease free and overall survival.

2.5. Targeted quantitative MS/MS analysis

Absolute quantification (μmol/L) of blood metabolites was achieved by targeted quantitative profiling of 186 annotated metabolites by electrospray ionization (ESI) tandem mass spectrometry (MS/MS) in each patient's biological samples, blinded to any clinical information, on a centralized, independent, fee-for-service basis at the quantitative metabolomics platform from BIOCRATES Life Sciences AG, Innsbruck, Austria. In brief, the measurement technique consisted of a targeted profiling scheme used to quantitatively screen for fully annotated metabolites using multiple reaction monitoring, neutral loss, and precursor ion scans. Quantification of metabolite concentrations and quality control assessment was performed with the MetIQ software package (BIOCRATES Life Sciences AG, Innsbruck, Austria), yielding sample identification and plasma concentrations (μmol/L) of each metabolite.

2.6. Metabolite panel

The metabolite panel comprised 40 Acylcarnitines (ACs), 21 Amino Acids (AAs), 19 Biogenic Amines (BA), sum of Hexoses (Hex), 76 Phosphatidylcholines (PCs), 14 Lysophosphatidylcholines (LPCs) and 15 Sphingomyelins (SMs), Glycerophospholipids were further differentiated with respect to the presence of Ester (a) and Ether (e) bonds in the Glycerol moiety, where two letters denote that two Glycerol positions are bound to a fatty acid residue (aa = diacyl, ae = acyl-alkyl), while a single letter indicates the presence of a single fatty acid residue (a = acyl or e = alkyl). In addition, the metabolite panel included the following energy metabolism components (TCA intermediates): Glucose, Ribose, 2-Hydroxyglutaric Acid, Alpha-Ketoglutaric Acid, Fumaric Acid, Malic Acid, Succinic Acid, 2-Hydroxybutyric Acid, 3-Hydroxybutyric Acid, Lactic Acid, Pyruvic Acid, Citric Acid, and Isocitric Acid.

2.7. Metabolomic analysis and validation tests

For metabolomic data analysis, log-transformation was applied to all quantified metabolites to normalize the concentration distributions and uploaded into the web-based analytical pipelines MetaboAnalyst 3.0 for the generation of uni- and multivariate Receiver Operating Characteristic (ROC) curves obtained through Support Vector Machine (SVM), Partial Least Squares-Discriminant Analysis (PLS-DA) and Random Forests as well as Logistic Regression Models to calculate Odds Ratios of specific metabolites [16,17].

ROC curves were generated by Monte-Carlo Cross Validation (MCCV) using balanced sub-sampling where two-thirds of the samples were used to evaluate the feature importance. Significant features were then used to build classification models, which were validated on the remaining third of the samples. The same procedure was repeated 10–100 times to calculate the performance and confidence interval of each model.

To further validate the statistical significance of each model, ROC calculations included bootstrap 95% confidence intervals for the desired model specificity and accuracy after 1000 permutations and false discovery rates (FDR) calculation [16,17].

The AbsoluteIDQ p180 kit includes quality control (QC) samples that are pipetted onto each measurement plate in order to allow harmonization of large datasets and comparability of results between batches. These QC samples are plasma-based reference samples with the exact same composition in every aliquot that are used to normalize all data, allowing a greater degree of precision across larger studies and minimizing batch effects. QC-based normalization was conducted for every measurement plate using Biocrates' software Met IDQ.

3. Results

Forty-seven patients with ovarian and/or uterine cancers met eligibility criteria for this analysis, 30 (64%) of whom were classified as having high-grade serous cancers (Table 1). Participant age ranged from 32 to 83 years with mean (± standard error [SE]) age 62.3 ± 12 years. The majority had ovarian cancer and were clinical stage III or IV at the time of surgery. Twenty-seven (64.3%) achieved complete remission after initial treatment but there was wide variability in time to progression, and disease-free and overall survival (Table 1); mean time to progression was 1.9 years (±1.5), disease-free survival was 1.7 years (±1.4), and overall survival was 2.6 years (±1.6). Based on EVA-PCD testing, mean LC50 CDDP was 1.15 (range 0.4–3.1).

Kaplan-Meier plots for time to progression, disease-free survival, and overall survival by ex vivo platinum resistance vs. sensitivity (LC50 CDDP dichotomized above vs. below the median 0.9 μg/mL) and metabolomic signature are shown in Fig. 1 A-F. Results indicate that those with ex vivo platinum resistance (high LC50 CDDP) have shorter median time to progression (1.30 vs. 1.86 years, $p = 0.071$), disease-free survival (1.15 vs. 2.99, $p = 0.038$) and overall survival (median not observed for ex vivo platinum resistant group, $p = 0.097$); however, only the differences in disease-free survival were statistically significant. Kaplan-Meier plots for time to progression, disease-free survival, and overall survival by metabolic signature resistant vs. sensitive (dichotomize above vs. below the median of 60 units, indicate that those with a resistant metabolic signature greater than 60 have shorter median time to progression (1.3 vs. 2.99 years, $p = 0.03$), disease-free survival ($p = 0.024$) and overall survival. Findings did not notably change when analyses were restricted to the high-grade serous cancers only (Supplemental Fig. 1A-F). Results from Cox proportional hazards models, adjusting for age, neoadjuvant chemotherapy, and clinical stage (where possible), showed similar patterns for each outcome by

Table 1
Study group characteristics.

Variable	Category	**Overall ($n = 47$)	Ovarian high grade serous only ($n = 30$)	Healthy controls ($n = 31$)	Healthy controls ($n = 76$)
		n (%) or mean ± SD (range)	n (%) or mean ± SD (range)	n (%) or mean ± SD (range)	n (%) or mean ± SD (range)
Total		47 (100)	30 (100)	31 (100)	76 (100)
Age years		65.3 ± 12 (35–87)		66 ± 12 (54–78)	50.7 ± 12 (20–89)
Diagnosis	Ovary	39 (83)	30 (100)	Healthy Menopausal Women	Healthy Female Volunteers
	Uterine	7 (14.9)	0 (0)		
	*Ovary with Uterine	1 (2.1)	0 (0)		
FIGO stage	I	7 (15.2)	1 (3.3)	n/a	n/a
	II	8 (17.4)	4 (13.3)		
	III	21 (45.7)	16 (53.3)		
	IV	10 (21.7)	9 (30.0)		
Histological types	Ovarian Serous Adenocarcinoma	30 (63)			
	Ovarian Mucinous Adenocarcinoma	2 (4.2)			
	Ovarian Endometrioid Adenocarcinoma	1 (2.1)			
	Ovarian Borderline Tumor	4 (8.5)			
	Ovarian Clear Cell Carcinoma	1 (2.1)			
	Ovarian Granulosa Cell Tumor	1 (2.1)			
	Endometrial Adenocarcinoma	4 (8.5)			
	Endometrial Serous Carcinoma	2 (4.2)			
	Uterine Leiomyosarcoma	2 (4.2)			
Neoadjuvant chemo (yes/no)		13 (27.7)	13 (43.3)	n/a	n/a
Adjuvant chemo (yes/no)		36 (76.6)	25 (83.3)		
Treatment response	Complete remission	27 (64.3)	17 (65.4)	n/a	n/a
	Partial remission	1 (2.4)	1 (3.8)		
	Progressed disease	14 (33.3)	8 (30.8)		
Platinum sensitive (LC50 CDDP high vs. low)		20 (60.6)	16 (66.7)	n/a	n/a
Clinical Platinum Resistance (DFS)	DFS < 180 days (resistant)	8 (17)	6 (21)		
	DFS > 180 days (sensitive)	38 (83)	23 (79)		
Time to progression (years)		1.8 ± 1.3 (0.1–4.5)	1.7 ± 1.2 (0.1–4.5)		
Disease-free survival (years)		1.6 ± 1.2 (0.1–4.5)	1.5 ± 1.2 (0.1–4.5)		
Overall survival (years)		2.3 ± 1.3 (0.1–6.7)	2.3 ± 1.3 (0.1–5.1)		
Recurrent Disease (yes/no)		28 (62.2)	21 (75.0)		

Footnote - FIGO = staging according to classification by International Federation of Gynecology and Obstetrics; n/a = not applicable;*Ovary with Uterine = subject with concurrent ovary and uterine cancers; **Overall = Total cohort consists of 39 ovarian cancer, 6 endometrial and 2 uterine sarcomas (30 ovarian serous; 2 ovarian mucinous; 1 ovarian endometrioid; 4 ovarian borderlines; 1 ovarian clear cell; 1 ovarian granulosa; 4 endometrial adenocarcinomas; 2 endometrial serous and 2 uterine leiomyosarcoma).

platinum resistance and metabolic signature but none of the results were statistically significant (Table 2).

Fig. 2 shows a heatmap of the results of the unsupervised quantitative multivariate analysis applied to a training set to identify the 60 most discriminating biochemical parameters (indicated by black arrows). As cryopreserved samples for metabolic study were conducted by batch, the first 13 specimens served as the "training set" followed by the full 47 samples used in the "validation set". Results were confirmed in a validation set (power = 0.8). Findings include elevations of short and long chain acylcarnitines and a decrease in structural lipids, specifically phosphatidyl cholines and sphingomyelins compared with controls (Fig. 2).

We examined the relationship between clinical platinum resistance, defined as relapse within 6 months of platinum-based chemotherapy, and laboratory CDDP resistance defined as LC50 falling above the median value 0.9 μg/ml. Results indicate a strong trend with a 2 fold higher likelihood of relapse within 6 months of platinum based chemotherapy for patients' with a CDDP falling above the median value LC50 > 0.9 μg/ml that, due to limited sample size, did not achieve significance (χ^2 test, $P = 0.259$).

Fig. 3 provides Pearson Moment correlation coefficients (R-values) as *Z*-scores distributed around the mean that compare CDDP resistance (LC50) with the described metabolites and with disease-free-survival (DFS).A broad array of lipid species, specifically lysophosphatidyl cholines associated with inflammation, track with CDDP resistance (LC50) while DFS tracks in the opposite direction along with the amino acids Phe and Met, linoleic acid (C18:2) and Sphingomyelin (SM C20:2) which have been correlated with altered metabolism [18].

A second analysis using a combination of liver function related metabolites, valine, and phenylalanine (Val/Phe), combined with the previously described acylcarnitine/lyso-phosphatidyl choline C18:2/LysoPC ratio together with the immune related tryptophan ratio provides a highly discriminating ROC that identifies CDDP resistance in the blood with an AUC = 0.933 ($P = 1.24$ e-6), a sensitivity of 0.92, and specificity of 0.86 (Fig. 4A–B). The Valine/Phenylalanine ratio reflects Fisher's quotient [19] an established measure of liver health and function, that we previously applied in a metabolomic analysis of breast cancer [20] while the lipid and Tryptophan ratios reflect altered bio-energetic and immune signatures. The analyses that examined specific lipid species and ratios with a focus upon acyl-carnitines, phosphatidylcholines and sphingomyelins that were used to define the shift in mitochondrial function from structural lipids to inflammation and energy production are provided in Supplementary Figs. 1 (A–G; I, J); 2 (A–F); and 3 (D). The described alterations in amino acid concentrations that are reflective of liver dysfunction are provided in Supplementary Fig. 3 (A–C, E, J, K) while immune dysfunction is reflected in levels of tryptophan metabolites Supplementary Fig. 3 (A, F).

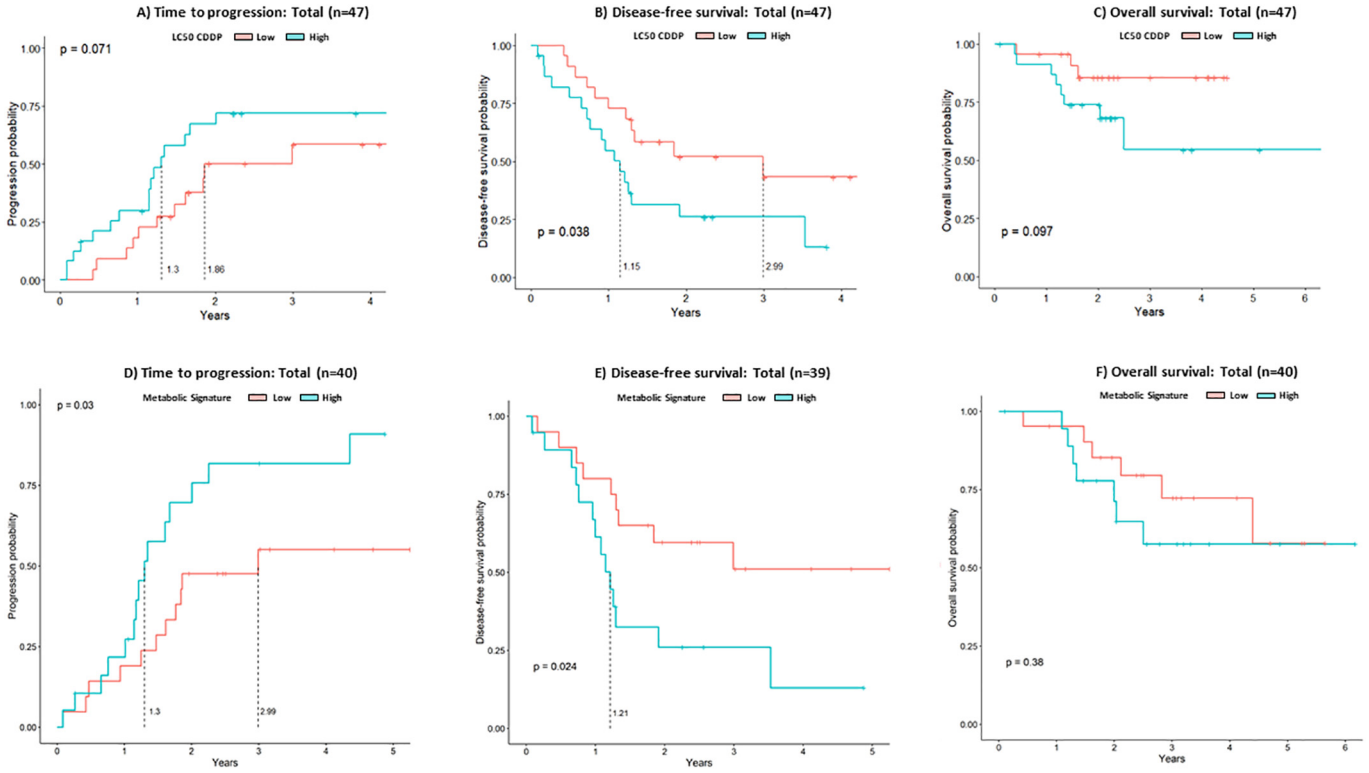


Fig. 1. (A–F). Kaplan-Meier curves of time to progression, disease-free survival, and overall survival by LC50 CDDP (A–C) and metabolic signature (D–F).

4. Discussion

As platinum resistance in advanced gynecologic malignancies is a principal determinant of survival [21], there is a pressing need to explore the biological basis of this phenomenon.

The capacity to identify platinum-resistance using laboratory measures at the time of diagnosis could have important implications for the choice and intensity of therapy. Among the approaches that have been applied are tissue culture techniques to measure relative drug responsiveness [22,23]. With the description of apoptosis [24] laboratory methods that measure drug-induced cell death have been applied for the prediction of response to chemotherapy in a variety of cancers [25,26].

The recognition that cancer arises in a state of altered metabolism has led to a renewed interest in the biochemical basis of malignant transformation and the capacity of cancer cells to resist therapeutic intervention. [27].

The current study correlated individual patient cisplatin resistance measured ex vivo with clinical outcomes and metabolic signatures in patients with advanced gynecologic carcinomas who received carboplatin plus paclitaxel as post-operative treatment.

We chose cisplatin based on its critical role in gynecologic malignancies. Prior studies that compared single agent carboplatin or cisplatin with multi-drug combinations established platinum's primacy as a determinant of outcome [28–32].

Table 2
Cox proportional hazards model for time to progression. Overall (n = 47). The Cox model was used for analysis of survival data with and without censoring, for identifying differences in survival due to treatment and prognostic factors. The Cox regression model for survival data provides an estimate of the hazard ratio and its confidence interval.

Variable		Hazard ratio	p
High LC50 CDDP (>0.9 μg/mL)		1.55 (0.68, 3.57)	0.299
Age		1.02 (0.98, 1.06)	0.432
Clinical Stage (I-IV)	1	Reference	
	2	0.69 (0.10, 4.71)	0.707
	3	3.33 (0.71, 15.62)	0.127
	4	3.97 (0.78, 20.10)	0.096
Neoadjuvant chemotherapy		0.60 (0.24, 1.54)	0.291

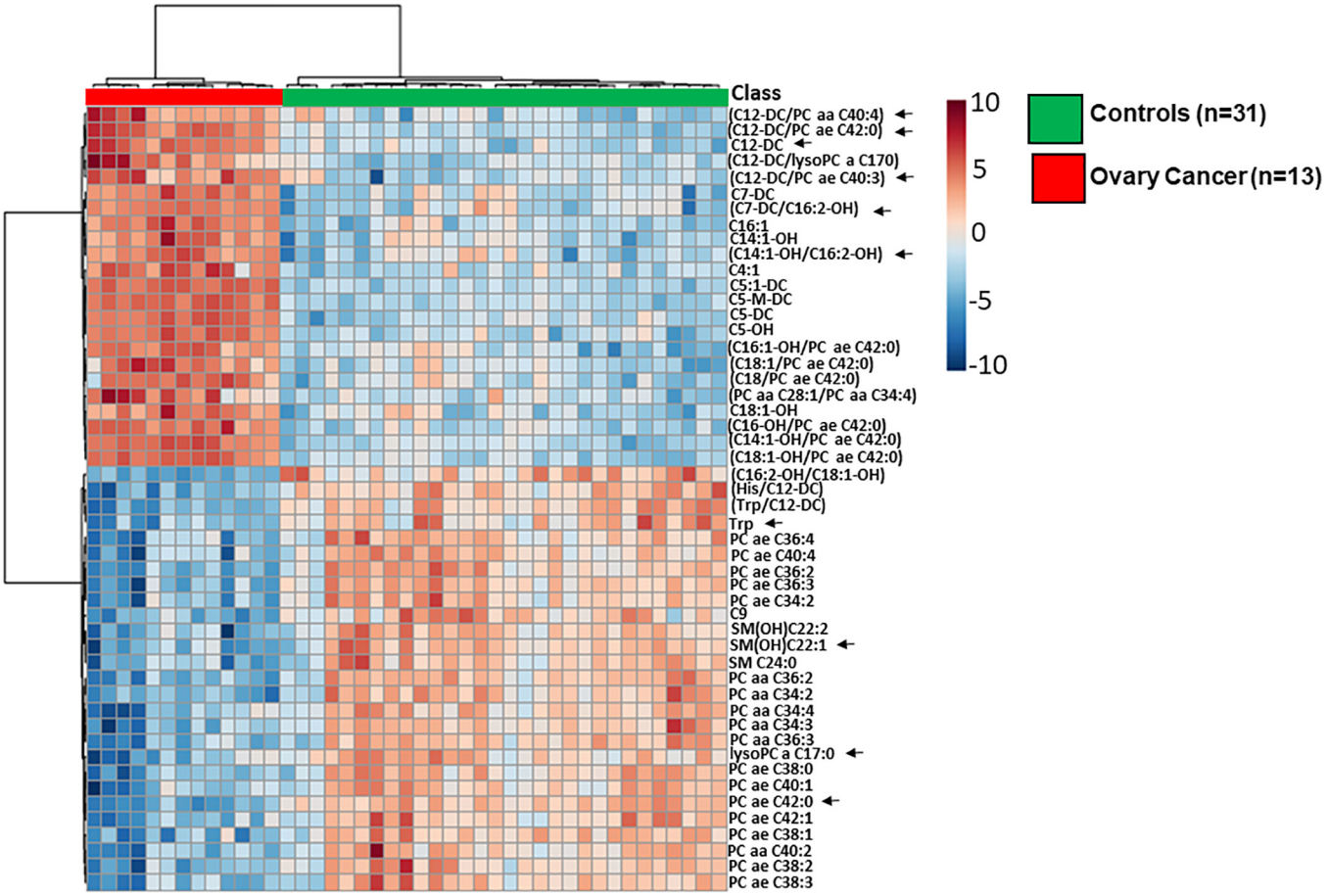
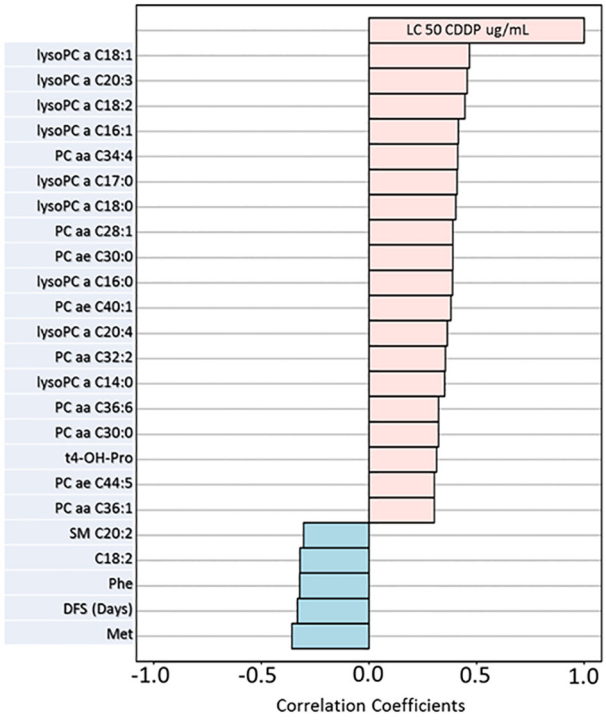


Fig. 2. Heatmap with unsupervised quantitative multivariate analysis applied to training set reveals that ovary cancer patients exhibit systemic biochemical changes suggestive of disorders in mitochondrial function. Controls and ovary cancer groups were discriminated with the 60 most discriminating biochemical parameters described.



Name	Correlation	p-value	FDR
lysoPC a C18:1	0.47	2.09E-03	1.65E-01
lysoPC a C20:3	0.46	2.71E-03	1.65E-01
lysoPC a C18:2	0.45	3.51E-03	1.65E-01
lysoPC a C16:1	0.42	6.92E-03	2.07E-01
PC aa C34:4	0.41	7.41E-03	2.07E-01
lysoPC a C17:0	0.41	7.82E-03	2.07E-01
lysoPC a C18:0	0.40	8.90E-03	2.07E-01
PC aa C28:1	0.39	1.18E-02	2.07E-01
PC ae C30:0	0.39	1.20E-02	2.07E-01
lysoPC a C16:0	0.39	1.21E-02	2.07E-01
PC ae C40:1	0.38	1.42E-02	2.23E-01
lysoPC a C20:4	0.36	1.92E-02	2.78E-01
PC aa C32:2	0.35	2.29E-02	2.86E-01
lysoPC a C14:0	0.35	2.44E-02	2.87E-01
PC aa C36:6	0.32	3.95E-02	3.69E-01
PC aa C30:0	0.32	3.97E-02	3.69E-01
t4-OH-Pro	0.31	4.54E-02	3.88E-01
PC ae C44:5	0.30	5.32E-02	4.08E-01
PC aa C36:1	0.30	5.43E-02	4.08E-01
SM C20:2	-0.30	5.34E-02	4.08E-01
C18:2	-0.32	4.13E-02	3.69E-01
Phe	-0.32	4.01E-02	3.69E-01
DFS (Days)	-0.36	2.11E-02	2.31E-01
Met	-0.36	2.18E-02	2.86E-01

Fig. 3. Correlation coefficients comparing CDDP resistance (LC50) measured ex vivo with the described metabolites measured in plasma and disease-free-survival (DFS).

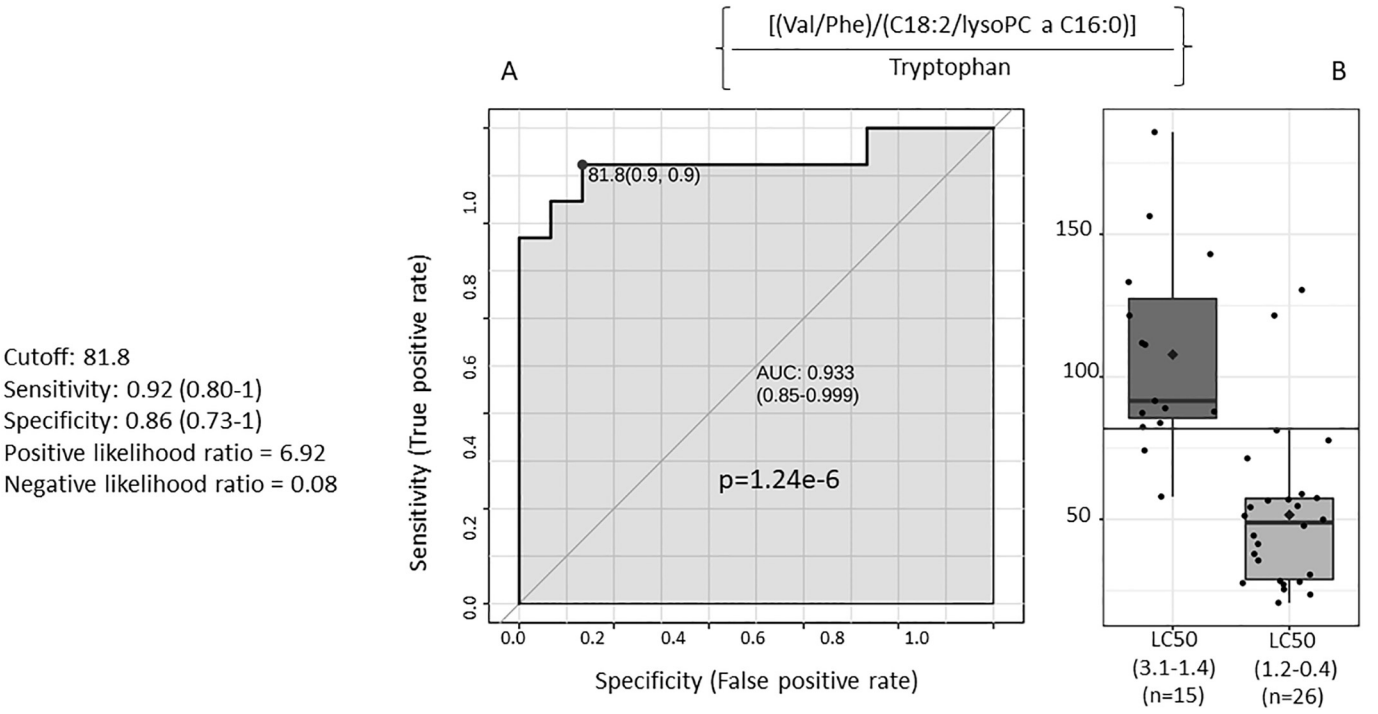


Fig. 4. (A, B). Multivariate ROC curve analysis depicting the performance of a biochemical equation in detecting platinum resistant patients. The ratio takes into consideration metabolites related to liver function (Val/Phe), lipid metabolism (C18:0/lyso PC C16:0) and Immunity (Tryptophan) and compares the results with the CDDP LC50 values, generated ex vivo.

To interrogate the biochemical basis of platinum resistance in gynecologic tumors we examined metabolic signatures in plasma obtained from patients prior to surgery and chemotherapy. This provided the opportunity to correlate response, disease free and overall survival, and platinum resistance with 186 metabolites quantified using targeted mass spectrometry (MS/MS). The findings suggest that chemotherapy response and survival in advanced gynecologic malignancies may reflect individual patient metabolic abnormalities associated with liver dysfunction (Val/Phe), altered lipid metabolism (18:2/LysoPC) and immune dysfunction (TRP/Kyn). We show that these metabolic aberrancies can be identified and quantified in patients prior to therapy via blood testing.

Among the findings are disturbances in branched chain amino acids (BCAA) reminiscent of Hartnup's disease, an inborn error of metabolism associated with BCAA malabsorption that can extend to Citrulline, a non-proteinogenic amino acid synthesized in the small intestine. Additional evidence of intestinal malabsorption is reflected by lower levels of essential fatty acids like linoleic as measured by lysophatidylcholine (C18:2).

Lower citrulline levels could also reflect urea cycle dysregulation, particularly, ornithine transcarbamylase deficiency [33]. The increased ornithine to citrulline (Orn/Cit) ratio further supports urea cycle dysregulation as described previously in other cancers [34].

The non-proteinogenic amino acid kynurenine, identified in patient's plasma, is the byproduct of tryptophan catabolism by indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase 2 (TDO2). The ratio of kynurenine to tryptophan (Kyn/Trp) reflects activation of these pathways that are known to diminish T-cell function and are found up-regulated in many human malignancies [35].

The elevated ratio (C18:2/lysoPC a C18:0) suggests that cancer patients shift their metabolism of free fatty acids toward the synthesis of acylcarnitines (C18:2) at the expense of structural lipids (lysoPC a C18:0). As we show, higher values of this ratio are associated with the highest levels of resistance to cisplatin. This ratio also segregates cancer cases from controls in both the training and validation sets. The specific lipidomic results that characterize platinum resistance (as LC50 values) are provided in Supplementary Figs. 1 (A–G, I, J) and 2 (A–F). Similar results in some of the controls suggest that platinum sensitivity could be an inborn biochemical characteristic.

Prior metabolomic analyses in ovarian cancer have correlated alterations in amino acids, phospholipids, acylcarnitines and other metabolic intermediates with clinical outcomes [36–38]. Cell line and patient derived xenografts as well as knock out mouse models have been explored for actionable metabolic vulnerabilities [39]. More recently fatty acid synthesis has been suggested as a novel target for therapy against ovarian peritoneal metastases [40].

The limitations of the study include the relatively small sample size and possible selection bias based upon single institution accrual. Patients were accrued by clinical research coordinators based upon clinical presentation and protocol criteria, but unintended biases may have arisen. There could be concern that the metabolic results reflect algorithms or ratios that were selectively defined to achieve desired results. To address this concern, we used training sets followed by confirmatory analyses that examined established biochemical parameters, previously described in the literature, to statistically support our findings.

We recognize that laboratory platforms can only approximate the complexity of human biology. In this study, plasma metabolites were used to provide insights into each individual's metabolic status as conditions of metabolic stress can promote malignant transformation.

Recognizing that plasma measurements are a surrogate for cellular changes, we have begun the study of tumor cell metabolism by extracting peri-tumoral culture media for MS/MS analysis and will plan to report those findings as that data matures.

Our findings indicate clinically significant increases in disease-free survival in patients found to be platinum sensitive. We and others have shown that platinum resistance can be addressed with gemcitabine-based doublets in some patients [31,32], suggesting that the pre-treatment measurement of platinum responsiveness could offer prognostic and therapeutics insights.

The results identify metabolic changes in patients with gynecologic malignancies that appear to promote carcinogenesis. Altered lipid and amino acid metabolism creates an environment for malignant transformation. Cells confronting nutritional deficiencies re-program their

metabolism in a manner that promotes malignantly transformed cell survival at the expense of the host. This survival advantage functions not only establish the malignant clone but to protect it from exogenous stresses like those associated with cytotoxic chemotherapy and heightened immune surveillance. Our findings re-define individual patient response and survival not as a function of "drug resistance" but instead as a function of inherent (biochemical) "drug insensitivity".

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Declaration of Competing Interest

Krishnansu S. Tewari, Robert E. Bristow, Fabio Cappuccini, Marcia B. Salzgeber, Anton M. Palma, Dirce M. L. Marchioni, Antonio A.F. Carioca, Kristine R. Penner, Jill Alldredge and Teresa Longoria have no conflicts of interest to declare. Paulo D'Amora (research grants from funding agencies: São Paulo Research Foundation (FAPESP); financial support for attending symposia and for educational programs: Nagourney Cancer Institute, American Association for Cancer Research, Rivkin Center; consultation: Metabolomycs, Inc.; intellectual property rights: Metabolomycs, Inc.; stockholder: Metabolomycs, Inc.). Steven S. Evans (employment: Nagourney Cancer Institute; stockholder: Metabolomycs, Inc.). Paula J. Addis-Bernard (employment: Nagourney Cancer Institute). Ismael Dale C.G. Silva (board director: Metabolomycs, Inc.; stockholder: Metabolomycs, Inc.). Robert A. Nagourney (board director: Nagourney Cancer Institute and Metabolomycs, Inc.; stockholder: Metabolomycs, Inc.). We certify that the submission is original work and is not under review at any other publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2021.08.001.

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