



Original research

Clinical relevance of cancer stem cell chemotherapeutic assay for recurrent ovarian cancer[☆]

Candace M. Howard^a, Nadim Bou Zgheib^b, Stephen Bush II^c, Timothy DeEulis^d, Antonio Cortese^e, Antonio Mollo^f, Seth T. Lirette^g, Krista Denning^h, Jagan Valluriⁱ, Pier Paolo Claudio^{j,*}

^a Department of Radiology, University of Mississippi Medical Center, Jackson, MS, USA

^b Gynecologic Oncology, Edwards Comprehensive Cancer Center, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV, USA

^c Gynecologic Oncology, Charleston Area Medical Center Hospital, Charleston, WV, USA

^d Gynecologic Oncology, Women's Oncologic Palliative Medicine, St. Mary's Hospital, Huntington, WV, USA

^e Department of Medicine and Surgery, University of Salerno, Italy

^f Obstetric and Gynecologic Unit, Department of Medicine and Surgery, University of Salerno, Salerno, Italy

^g Department of Data Science, University of Mississippi Medical Center, Jackson, MS, USA

^h Department of Anatomy and Pathology, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV, USA

ⁱ Department of Biological Sciences, Marshall University, Huntington, WV, USA

^j Department of BioMolecular Sciences, National Center for Natural Products Research, and Department of Radiation Oncology, University of Mississippi Medical Center Cancer Institute, Jackson, MS, USA

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ABSTRACT

Introduction: Disease recurrence and progression of ovarian cancer is common with the development of platinum-resistant or refractory disease. This is due in large part to the presence of chemo-resistant cancer stem cells (CSCs) that contribute to tumor propagation, maintenance, and treatment resistance. We developed a CSCs drug cytotoxicity assay (ChemoID) to identify the most effective chemotherapy treatment from a panel of FDA approved chemotherapies.

Methods: Ascites and pleural fluid samples were collected under physician order from 45 consecutive patients affected by 3rd-5th relapsed ovarian cancer. Test results from the assay were used to treat patients with the highest cell kill drugs, taking into consideration their health status and using dose reductions, as needed. A retrospective chart review of CT and PET scans was used to determine patients' outcomes for tumor response, time to recurrence, progression-free survival (PFS), and overall survival (OS).

Results: We observed that recurrent ovarian cancer patients treated with high-cell kill chemotherapy agents guided by the CSCs drug response assay had an improvement in the median PFS corresponding to 5.4 months (3rd relapse), 3.6 months (4th relapse), and 3.9 months (5th relapse) when compared to historical data. Additionally, we observed that ovarian cancer patients identified as non-responders by the CSC drug response assay had 30 times the hazard of death compared to those women that were identified as responders with respective median survivals of 6 months vs. 13 months. We also found that ChemoID treated patients on average had an incremental cost-effectiveness ratio (ICER) between -\$18,421 and \$7,241 per life-year saved (LYS).

Conclusions: This study demonstrated improved PFS and OS for recurrent ovarian cancer patients treated with assay-guided chemotherapies while decreasing the cost of treatment.

Introduction

Cytoreductive surgery followed by platinum-based chemotherapy are standard of care for new cases of epithelial ovarian cancer (EOC) [1]. Although this regimen is initially effective in a high percentage of cases,

unfortunately, most patients relapse. This is mostly attributed to the presence of ovarian cancer stem cells (CSCs), which are chemo-resistant and responsible for the recurrence of cancer [2]. CSCs account not only for the primary tumor growth, the peritoneal spread and relapse of ovarian cancer, but also for the development of chemoresistance, thus having profound

Abbreviations: rEOC, recurrent epithelial ovarian cancer; PFS, progression-free survival; OS, overall survival; CSCs, cancer stem cells; RT, radiation therapy.

[☆] Keypoint: Cancer Stem Cell Chemotherapeutics Assay improves the outcome of recurrent ovarian cancer patients by guiding their chemotherapy treatment.

* Corresponding author at: Department of BioMolecular Sciences, National Center for Natural Products Research, Department of Radiation Oncology, University of Mississippi, Jackson, MS 39126, USA.

E-mail address: pclaudio@olemiss.edu. (P.P. Claudio).

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implications for the treatment of this deadly disease [3]. Indeed, CSCs account for a very small subpopulation in the primary tumor that is enriched in recurrent disease, both because of their expansion to fuel the relapse and because of the possible selection of drug-resistant CSCs after the first-line treatment. Malignant ascites is common in advanced EOC, both at initial diagnosis and upon recurrence, and contain CSCs which can survive and proliferate even under non-adherent conditions, leading to self-organized spheroids of ovarian cancer cells that, result in peritoneal seeding [4–7].

Recurrent EOC is associated with significant mortality and a median survival of only 12–24 months [8]. The combination of platinum drugs with a taxane is the standard of care for systemic treatment of EOC after primary cytoreductive surgery [9]. However, this treatment results in response rate (RR) of ~70% in patients with suboptimally debulked disease, and of ~80% in patients with optimal cytoreductive surgery [10,11]. Disease recurrence is common in these patients, and most of them eventually develop platinum-resistant disease (defined as disease recurring within 6 months after the last receipt of platinum-based chemotherapy) [12,13].

RRs and duration of response to second-line chemotherapy for patients with recurrent platinum-resistant disease are significantly lower than those with platinum-sensitive disease. In women with platinum-resistant disease, RRs range from 10%–15%, and duration of response is typically <6 months, with PFS (3–4 months), and OS (~12 months) to chemotherapeutic agents such as pegylated liposomal doxorubicin (PLD), topotecan, taxanes, etoposide, and gemcitabine [14]. In comparison, the RRs are usually >30% and/or duration of response >8 months in women with platinum-sensitive disease [15,16].

While treatment guidelines for the primary occurrence of advanced stage EOC recommend numerous platinum-based combination therapies, an even greater number of treatment regimens are recommended for recurrent disease. Nearly 10 different platinum-based therapies are recommended for treatment of patients experiencing recurrence following >6 months from first-line treatment (platinum-sensitive recurrent disease), and over 20 different therapies (mostly single agents) for treatment of patients experiencing recurrence within 6 months following first-line treatment (platinum-resistant recurrent disease) [17], with little to no guidance on how to select among the treatment options. Thus, in the absence of specific directives beyond the primary setting, treatment choices for recurrent EOC patients are made empirically [18]. Regimens to treat recurrent EOC are normally informed by responses to first-line therapies and vary significantly; therefore, choice of which agent to use is usually based on toxicity profile, the previous toxicities experienced by the patient, and patient preference. Several clinical trials, many supported by the National Cancer Institute's consortium the Gynecologic Oncology Group (GOG), have investigated chemotherapy drugs, regimens, and reductive surgery methods in search of effective strategies to prevent EOC recurrence. Recently, the AURELIA, OCEANS, and GOG-0213 phase-3 randomized trials have assessed combination therapy with a targeted agent such as bevacizumab for recurrent platinum-resistant and -sensitive ovarian cancer [19–23] and found that in patients receiving bevacizumab/chemotherapy, the primary endpoint of PFS was significantly prolonged (6.8 months versus 3.4 months, hazard ratio (HR) 0.48, 95% confidence interval (CI) 0.38–0.60, $p < 0.001$) compared to patients treated with chemotherapy alone. In these studies, the median overall survival was 16.6 months for the bevacizumab/chemotherapy combination versus 13.3 months for chemotherapy alone; however, the difference was not statistically significant (HR 0.85, 95% CI, 0.66–1.08, $p < 0.174$).

Nonetheless, none of these trials have addressed or explored the idea of reducing the burden of cancer stem cells in recurrent EOC to enable a greater and more durable response to therapy. Despite results demonstrating treatment advances, regimens for platinum-resistant recurrent EOC are unfortunately not curative. Thus, there is an urgent need for the development of alternative strategies given the poor response of platinum-resistant recurrent ovarian epithelial malignant disease.

Individual patient responses to standardized treatments vary significantly. Oncologists point to patient race/ethnicity, age, and comorbidities, as well as nuances in how EOC lesions are graded as challenges

to standardization [24]. Toxicity profiles are extensive for most chemotherapy drugs with no guarantee of success at the patient level. As such, there is an urgent need for ways to tailor chemotherapy regimens to patients based on their individual tumor characteristics.

We have developed a cytotoxicity assay (ChemoID), a CLIA (Clinical Laboratory Improvement Amendments) and CAP (College of American Pathologists) certified test, performed by an independent Hospital Pathology laboratory to help physicians' select appropriate chemotherapies for individual patients based on the cytotoxicity profile of CSCs and bulk of tumor cells response to FDA approved chemotherapies.

The drug response assay measures the effect of clinical doses of standard-of-care chemotherapies on CSCs and bulk of tumor cells with a prioritized list of effective and ineffective chemotherapies. The goal of the assay is to find the most efficacious agents that would reduce CSC-burden in ovarian cancer, thereby limiting metastatic and recurrent disease potential to help improve outcomes and to reduce health care costs. Previous economic analyses showed cost savings in cohorts of patients treated with therapies guided by a cytotoxic assay [25,26], thus warranting an economic analysis of the ChemoID assay based on patient clinical outcomes.

We report here for the first time the clinical benefit we observed from the chart review of a cohort of consecutive recurrent ovarian cancer patients who were treated using the ChemoID drug response assay. The current analysis sought also to investigate the relative cost-effectiveness of assay-guided treatment regimen relative to the assay-uninformed, empiric standard of care, assuming the payer's perspective. Additionally, the survival analysis of the patients treated with assay-guided regimens was used to inform the power analysis calculations for randomized multi-institutional clinical trials that are currently being conducted in the USA.

Material and methods

Patients

We have retrospectively reviewed the charts of 45 consecutive female 18 years and older, clinically diagnosed with poor-prognosis recurrent EOC (3rd–5th relapse), who were prospectively treated with the highest cell kill drugs as identified by the ChemoID drug response assay, according to their overall functional status and ability to tolerate the recommended treatment and using dose reductions, as needed. Sample collections were acquired from standard-of-care therapeutic paracentesis, or thoracentesis to manage their symptoms after obtaining patients' written informed consent under the ethical standards of the Helsinki Declaration (1964, amended most recently in 2008) of the World Medical Association. Any information, including radiological imaging, was blinded. Marshall University Institutional Review Board (IRB) has approved this research under protocol #326290. Participants provided their written consent on an IRB approved informed consent form to participate in this study after being educated about the research protocol. Ethics committees at Marshall University approved the consent procedure of the study. Fresh fluid collections were sent to the Pathology lab for confirmation of diagnosis and a portion of the biopsy specimen was sent for ChemoID drug response assay by physician order. Radiological data were collected at baseline before to fluid collection and after chemotherapy with a computed tomography (CT) scan and/or positron emission tomography (PET) with follow-up every 2–3 months. Repeated measures of drug response were not obtained, and therefore each woman's data appears once. Supportive care was allowed at the discretion of the treating physician. Response to treatment was assessed by radiologic examination (CT scan as the primary imaging method) and measurements using the RECIST 1.1 criteria.

ChemoID assay

Details regarding the CSCs cytotoxicity assay (ChemoID) procedure have been described elsewhere [27–33]. In brief, primary cancer cell cultures were initiated from malignant cells present in the ascites or pleural aspirates. CSCs were enriched from the primary cancer cell cultures (bulk of

tumor cells) as described previously [27–33] using a novel cell culture bioreactor with a membrane that allows for gas exchange. Cells were counted and a range of 1×10^6 – 1×10^7 cells were cultured for 7–10 days. The cells were maintained in RPMI 1640 medium in the rotating vessel at 25 rpm with airflow constantly set at 20%, 5% CO₂ at 37 °C. Cells were removed and their viability and cell number were confirmed. The cells were also incubated with fluorescent antibodies for phenotypic characterization.

For the ChemoID assay, the bulk of tumor cells and CSCs were counted using trypan blue exclusion to determine cellular viability and cell number before chemosensitivity testing. Percent of cell survival was assessed using an MTT assay on 1×10^3 cells plated in 5 replicates into 96-well plates. An equal number of the bulk of tumor cells and CSCs were seeded in 96-well dishes and incubated at 37 °C for 24-h. Three concentrations of each chemotherapy treatment were prepared (dose 1: clinically equivalent dose to serum C_{Max} reported after 1 h from an infusion of a drug; dose 2: a double dose of serum C_{Max}; dose 3: 1/2 dose of serum C_{Max}).

Clinical grade chemotherapy drugs were used in the assay provided by the Hospital Pharmacy. The in vitro concentration equivalent to clinical dose was calculated taking into account the molecular weight (MW) of the drug, the average body surface area of female individuals, and the stock molarity concentration of the clinical grade drugs. Each concentration was added to five replicate wells on the microtiter plate. Additionally, three replicate wells (control 1 = no treatment) and three replicate wells (control 2 = equal amount of solvent) were associated with each treatment. Only clinically equivalent dose to serum C_{Max} is reported by the clinical lab as per CLIA laboratory guidelines for testing. The cells were challenged for a 1-h pulse with the panel of anticancer drugs (Table 1). MTT assay was performed 48-h following chemotherapy treatment to assess cell survival as previously described [27,28,30,32–34]. Inhibition of bulk of tumor cells and CSCs survival was measured for each concentration (average counts in five replicates $\pm$ SE) of a given treatment (15–18 different treatments per patient). Survival of tumor cells at each concentration was calculated as compared to control-2 and overall percent of the bulk of tumor cells and CSCs killed were calculated for each treatment as the primary measures of potential therapy efficacy.

Table 1

List of single and combined chemotherapeutic agents used to treat recurrent epithelial ovarian carcinoma.

Regimen#	Single or combination drug(s) clinical dose tested
1	Liposomal Doxorubicin (30 or 60 mg/m ²)
2	Docetaxel (75 mg/m ²)
3	Paclitaxel (80 or 175 mg/m ²)
4	Carboplatin (400 mg/m ²)
5	Cisplatin (60 or 100 mg/m ²)
6	Gemcitabine (800 or 1000 mg/m ²)
7	Topotecan (1.25 mg/m ²)
8	Etoposide (100 mg/m ²)
9	Oxaliplatin (85 mg/m ²)
10	Vinorelbine (30 mg/m ²)
11	Vinblastine (3.75 mg/m ²)
12	Carboplatin (400 mg/m ²) + Gemcitabine (800 or 1000 mg/m ²)
13	Cisplatin (50 mg/m ²) + Gemcitabine (800 or 1000 mg/m ²)
14	Carboplatin (400 mg/m ²) + Liposomal Doxorubicin (30 mg/m ²)
15	Carboplatin (400 mg/m ²) + Paclitaxel (175 mg/m ²)
16	Carboplatin (400 mg/m ²) + Docetaxel (75 mg/m ²)
17	Gemcitabine (1000 mg/m ²) + Paclitaxel (80 or 175 mg/m ²)
18	Gemcitabine (1000 mg/m ²) + Docetaxel (75 mg/m ²)
19	Paclitaxel (175 mg/m ²) + Cisplatin (80 mg/m ²)
20	Etoposide (100 mg/m ²) + Cisplatin (80 mg/m ²)

Transplantation assay in immunodeficient animals

Female *nu/nu* mice (nude; Jackson Labs) were used for in vivo transplantation studies. Prepared cell suspensions were injected intraperitoneally using 1 mL syringes with a 25 G needle into 8-week-old mice ($n = 6$ or 10). Tumors were assessed by necroscopy and sizes were recorded by measuring the length and width of the tumors in two dimensions using a caliper. The tumor volume (TV) was calculated using the formula volume = $1/2 \times \text{length (mm)} \times (\text{width [mm]})^2$. Engraftment was determined according to progressive nodule growth at the injection site. The tumorigenicity of ovarian cancer cells was evaluated by measuring tumor-forming capacity following implantation of 1×10^1 , 1×10^2 , 1×10^3 , and 1×10^4 , 1×10^5 , and 1×10^6 cells in the abdomen the nude mice. Tumor initiating properties of cultured CSCs were verified by injecting them in limited dilution in nude mice and observing after 30 days their tumor formation capacity at necropsy. Fig. 1 illustrates the tumor-forming capacity of 1×10^2 ovarian CSCs enriched with the ChemoID culture method compared to an equal number of CSCs sorted by a column (Milteny Biotech, Auburn, CA) using a specific antibody following intraperitoneal injection in nude mice, compared to 1×10^6 bulk ovarian cancer cells derived from a patient of our cohort. Column sorted and enriched CSCs were also phenotypically characterized by flow cytometry using fluorescent antibodies as previously described [27]. In brief, cells were analyzed by the antigenic criteria using anti-CD44 (BD Bioscience, Sparks, MD), -CD117 (Milteny Biotech, Auburn, CA), and -CD133/2 (prominin1) (Milteny Biotech, Auburn, CA). Briefly, cells were detached using 0.02% EDTA in PBS and pelleted (10 min at 1000 rpm), washed in 0.1% BSA in $1 \times$ PBS at 4C, and incubated in a solution of 1 mg antibody + 9 mL 0.1% BSA in $1 \times$ PBS. Cells were washed in the same solution once and were analyzed using a C6 Accuri flow cytometer (BD Biosciences, San Jose, CA).

Statistical analysis

Two different responder categories were defined: the bulk of tumor responders were those women who received a treatment identified by the drug response assay as 55% or above cell kill for the bulk of tumor and CSC responders were those women who received a drug in which the test identified as 40% or above cell kill of CSCs. These cell kill values were derived from previous research and validated in this sample via Youden indices. Summary statistics were calculated where appropriate and all relevant graphs were constructed. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated according to standard approaches. Kaplan-Meier graphs were constructed and hazard ratios were calculated from Cox models adjusted for the number of relapses and age. Model assumptions were graphically checked and tested via Schoenfeld residuals and were found to be satisfactory. All statistical analyses were completed using Stata v15.1 (StataCorp LP, College Station, TX).

Cost calculation

The major costs associated with the treatment of recurrent EOC are the cost of the drug response assay, cost of surgery, cost of paracentesis/thoracentesis, cost of chemotherapy, cost of adverse events and toxicities, and cost of end of life care. It was assumed that all our patients incurred the same cost per surgery, paracentesis/thoracentesis, and end of life care and treatment of toxicities; therefore, we did not include these costs in our analysis. For our analysis, we considered only the cost of chemotherapies. The main source for the cost data in this analysis reflects current Medicare pricing.

Cost of chemotherapy

All patients in the current model were prospectively treated with a chemotherapy regimen appropriate to their ovarian cancer at the time of their

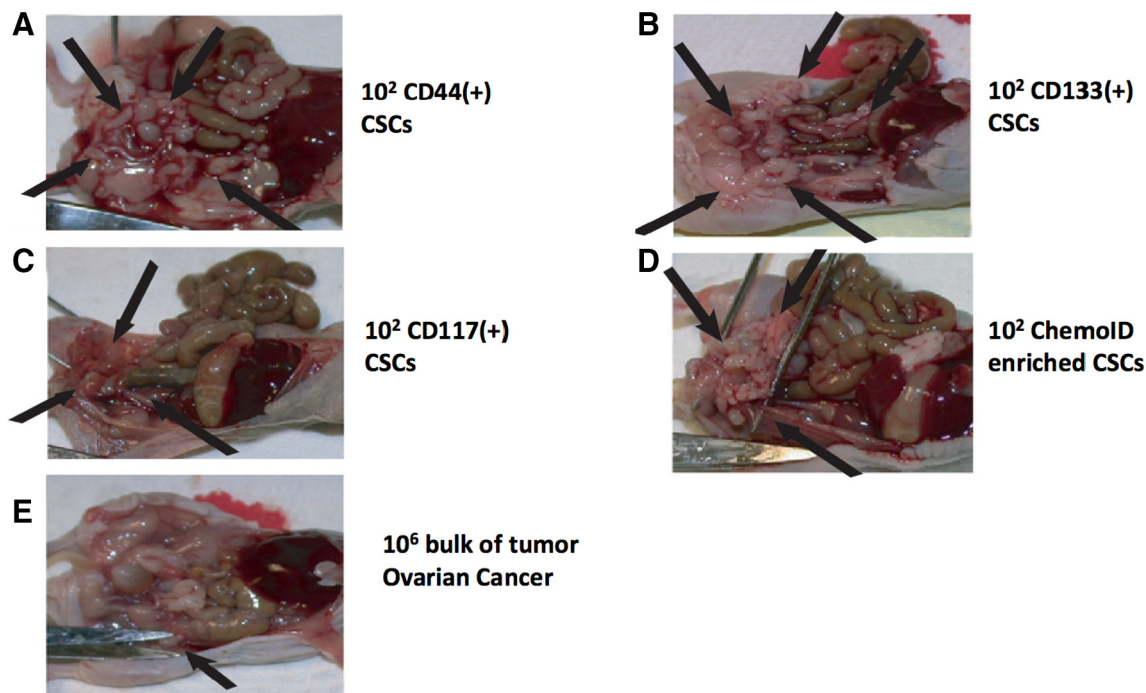


Fig. 1. Limiting dilution transplantation assay in immunodeficient animals. A-C) Necropsic examination of intraperitoneal tumor nodules observed following injections of 1×10^2 CD44, CD133, or CD117 positive ovarian CSCs, respectively. Arrows point at tumor nodules D) Necropsic examination of intraperitoneal tumor nodules observed following injections of 1×10^2 ChemoID enriched ovarian CSCs. Arrows point at the several tumor nodules formed. E) Necropsic examination of intraperitoneal tumor nodules observed following injections of 1×10^6 bulk of tumor ovarian cancer cells. Only one tumor was observed in the control bulk of tumor injected mice.

recurrence. The costs associated with 6 cycles of each chemotherapy regimen, as well as the associated administration costs (in the physician office setting), were estimated using the current Medicare physician fee schedule for administration payments and drug pricing database for chemotherapy agents [35,36]. Doses were calculated assuming a body surface area of 1.8 m^2 , serum creatinine of 0.9 mg/dL , the weight of 170 lb. , the height of 62 in. , and the age of 63 years . To estimate the cost of the second-line chemotherapy, the distribution of administered therapies in our cohort, and the costs calculated for the historical cohort were used (Supplementary Tables 1 and 2). Thus, the average cost of six cycles of salvage chemotherapy in the historical cohort was estimated at \$20,311 in current dollars. The cost of the second-line chemotherapy in the assay treated cohort was estimated by the average cost of all therapies in the highest category of assay sensitivity for each patient; this cost was \$20,528 in current dollars.

Cost-effectiveness and sensitivity analysis

The relative cost-effectiveness of the intervention is expressed by the incremental cost-effectiveness ratio per life-year saved (ICER/LYS), which is the ratio of the difference in the average costs per patient to the difference in the mean overall survivals. The standard threshold for a healthcare intervention to be deemed cost-effective is an expenditure of between \$50,000 and \$100,000 per additional year of life saved [37]. Keeping the costs for surgery, chemoresponse assay, end of life care, and adverse event treatment constant between our cohort and historical data, the model results are affected only by the cost of chemotherapies and by survival outcomes. To account for the uncertainty in the hazard ratio (HR) estimates associated with the assay and its impact on the ICER/LYS, the range in the ICER/LYS was estimated by 1000 bootstrap samples for the assay. Several stratified analyses for the reference model are also reported. To assess the sensitivity of the model due to the cost of chemotherapy, the scenario when the oncologist chooses the least expensive treatment within the highest category of sensitivity for each patient in the assay consistent cohort was also investigated.

Results

In vivo tumor formation by CSCs derived from primary recurrent ovarian cancer

The existence of CSCs and their ability to initiate a tumor was first demonstrated by injecting in immunodeficient animal populations of CSC marker-positive cells using a limiting dilution assay (LDA). Populations of CSC marker-positive cells that give rise to transplantable tumors, which histologically recapitulate the cellular heterogeneity of the parental tumors can be classified as CSCs, whereas populations of CSC marker-negative cells with no or limited tumor-propagating activity can be excluded from the CSC candidates [38–40]. We measured tumor forming capacity of CSCs enriched from patients' derived ovarian cancer primary cell lines using our culture method and compared it to that of CSCs that were column sorted cells using specific antibodies against CD133, CD44, and CD117 using an LDA assay. The tumor-initiating properties of patient-derived primary ovarian cancer cells was evaluated by measuring at necropsy their tumor-forming capacity following implantation of 1×10^1 – 1×10^6 cells in the abdomen the *nude* mice after 30 days from an injection. Fig. 1 illustrates the tumor-forming capacity of 1×10^2 ovarian CSCs enriched with the ChemoID culture method compared to an equal number of CSCs sorted by a column using a specific antibody following intraperitoneal injection in *nude* mice, compared to 1×10^6 bulk ovarian cancer cells derived from a patient of our cohort.

Assay guided clinical outcomes

Fresh tissue samples were prospectively collected from forty-five consecutive recurrent EOC patients (3rd–5th relapse) (Table 2) from a therapeutic peritoneal/pleural aspiration, as per physician order. The sample was sent to the Hospital Pathology lab for confirmation of diagnosis and, at the same time, for the ChemoID CSC cytotoxicity assay. Patients were treated with assay-guided chemotherapy according to their overall functional status and ability to tolerate the recommended treatment.

Table 2
Patient characteristics (*n* = 45).

Characteristics	No patients	%
Age (median 60)		
Range (28–77)		
< 50	6	(13.5)
50–59	14	(31)
60–69	19	(42)
≥ 70	6	(13.5)
Tumor relapse		
3rd	31	(68.8)
4th	6	(13.5)
5th	8	(17.7)
ECOG		
0	22	(49)
1	15	(33)
2	5	(11)
3	3	(7)
Tumor site		
Ovarian	39	(87)
Peritoneal	5	(11)
Fallopian tube	1	(2)
Histology		
Serous	40	(89)
Endometrioid	3	(7)
Clear cell	1	(2)
Mucinous	1	(2)
Unknown	0	(0)
Tumor grade		
1	0	(0)
2	3	(7)
3	42	(93)
Stage		
III	40	(89)
IV	5	(11)

Two years after the last patient was treated, we performed a retrospective chart review of recurrent EOC patients treated using the test results of the ChemoID assay. The mean age of the patients was 59.4 years (SD = 9.99). The majority of the women (31) were on their 3rd relapse (69%), while 6 (13%) were on their 4th, and 8 (18%) were on their 5th relapse. The CSC cytotoxicity assay identified 28 (62%) women as expected responders based upon bulk tumor and 34 (76%) as expected responders based upon CSCs test. Historical data reports that patients affected by 3rd, 4th, and 5th relapse have a median PFS of 5.6, 4.4, and 4.1 months, respectively [8]. The median PFS observed in our recurrent patients treated with sensitive drugs as indicated by the CSC cytotoxicity assay for 3rd, 4th, and 5th relapse was 11, 8, and 8 months, respectively (Table 3). This potentially demonstrates a marked advantage of treating patients with assay-guided therapy.

Fig. 2 illustrates the relationship between the CSC assay results (%-cell kill on the y-axis) and bulk tumor assay results (%-cell kill on the x-axis) characterized by recurrence outcomes at 6 month, with blue solid circles representing treatment responders (non-recurrence at 6 months) and red open circles representing patients with recurrence within 6 months from treatment start. Referent lines are drawn at thresholds of 40% for CSC, and 55% cell kill for the bulk of tumor. In the upper-right quadrant are represented patients treated with high-cell kill chemotherapy for both CSC and bulk tumor assays, who were non-recurrent at 6 months. In the lower-left quadrant are represented patients treated with low-cell kill chemotherapy

Table 3
Comparison of historical median PFS and ChemoID-guided treated poor prognosis ovarian cancer patients.

Relapse	Historical Median PFS (months)	ChemoID Median PFS (months)	Difference Median PFS (months)
3rd	5.6	11.0 (8.5–12.0)	5.4
4th	4.4	8.0 (7.0–NA)	3.6
5th	4.1	8.0 (2.0–NA)	3.9

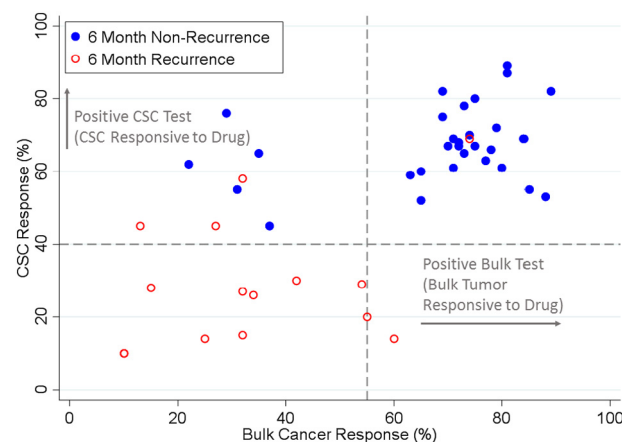


Fig. 2. Quadrant diagram of the relationship between CSC assay results (%-cell kill on the y-axis) and bulk tumor assay results (%-cell kill on the x-axis) characterized by 6-months recurrence outcomes.

for both CSC and bulk tumor assays, who had a recurrence in < 6 months. We chose to analyze the recurrence outcome at 6 months because historical data shows that patients affected by 3rd, 4th, and 5th relapse have a median PFS of < 6 months [8].

The CSC test demonstrated 97% sensitivity, 77% specificity, 91% positive predictive value (PPV), and 90% negative predictive value (NPG). The bulk test demonstrated 84% sensitivity, 92% specificity, 96% PPV, and 71% NPG for the 6-month response.

Figs. 3 and 4 show Kaplan-Meier plots of progression-free and overall survival across the study period stratified by dichotomized test results on bulk of tumor test or CSC test, respectively. In the CSC model for overall survival (Fig. 3B), the ovarian cancer patients that the test identified as non-responders had over 30 times the hazard of death compared to those women that were identified by the test as responders ($p < 0.001$), with respective median survivals of 6 months vs. 13 months ($p < 0.001$). Likewise, in the CSC model for progression-free survival (Fig. 4B), the ovarian cancer patients that the test identified as non-responders had over 60 times the hazard of progression compared to those women that were identified by the test as responders ($p < 0.001$), with respective median survivals of 3 months vs. 9 months ($p < 0.001$). More modest but highly predictive hazards were observed based on the bulk of tumor models. Moreover, we observed that adding the bulk of tumor and CSC test response to a Cox model containing only relapse number and age increased Harrell's C statistics from 0.63 to 0.79 for overall survival and from 0.62 to 0.84 for PFS.

Fig. 5 shows the baseline CT images of an ovarian cancer patient in her third recurrence (Fig. 5A–C), the ChemoID drug response assay results (Fig. 5D), and the subsequent CT images (Fig. 5E–G) following treatment with ChemoID-guided therapy. Ascites was aspirated in November 2016 and a concomitant sample was sent to the pathology lab to confirm the presence of malignant ovarian cancer cells and to the ChemoID lab for functional chemoresponse testing.

Pattern of assay response

Fig. 5 shows in the baseline CT imaging the presence of large ascites (panel A), ovarian mass measuring 9.3×5.4 cm (panel B), and peritoneal carcinomatosis measuring 3.5×5.1 cm (panel C). Fig. 5D shows the ChemoID test results with several high suppression chemotherapy drugs. Based on the patient clinical status (kidney disorder) and prior use of chemotherapy regimens, the patient was treated with a full course of single chemotherapy agent (Doxorubicin) that showed a high percentage of cell kill for both Bulk of Tumor (75.2% cell kill) and CSC (66.4% cell kill) by the ChemoID assay. Other high cell kill drugs indicated by the assay were not administered due their known side effects (kidney toxicity) which were contraindicated by the specific health status of the patient.

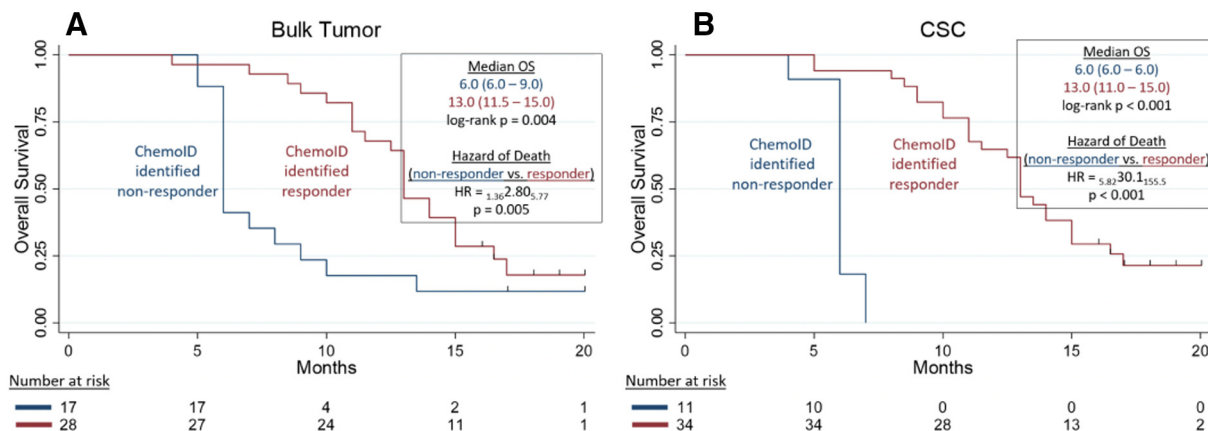


Fig. 3. Kaplan-Meier plots of overall survival across the study period. Overall survival is shown stratified by dichotomized test results for (A) bulk test >55% cell kill - responder or <55% cell kill - non-responder and (B) CSC test >40% cell kill - responder or <40% cell kill - non-responder; Hazard ratios are from Cox proportional hazard models adjusting for number of previous relapses and age.

Images in February of 2017 showed a dramatic reduction of ascites (panel E), smaller ovarian mass measuring 5.8×3 cm (panel F), and reduction in peritoneal carcinomatosis measuring 3.4×3 cm (panel G).

Assay guided cost of treatment

To better understand the health care benefit and the economical impact of the use of the drug response assay, we compared the health benefit observed and the cost of therapies used in our patients' cohort to the historical data of patients treated empirically with chemotherapies, similarly to previously published investigations [26].

The mean cost of therapies administered to the 45 patients was \$20,311 (Table 4). If the therapy with the minimum cost from the top three ChemoID recommended therapies was administered, the average patient would have saved \$7,956/regimen. This difference was highly statistically supported ($p < 0.001$).

We also compared the cost of PARPi (4 cycles) or Avastin therapies (4–22 cycles) (Supplementary Table 3) to 4 cycles of the administered drugs, ChemoID's first three recommendations, and the therapy with the minimum cost of the first three choices (Table 5). Cost savings ranged from a minimum of \$31,689 to \$273,644 (all $p < 0.001$). Interestingly, of the 45 patients, 33 (73%) could have received therapy either as effective or more effective than the administered therapy for a reduction of the cost.

Table 6 illustrates the incremental cost-effectiveness ratio per life-year saved (ICER/LYS), which is the ratio of the difference in the average costs per patient to the difference in the mean overall survival. For the ChemoID

study, the reference model yielded an ICER of \$860–\$32,473, \$1,100–\$48,890, and \$0–\$46,154 per LYS of 3rd, 4th, and 5th relapse patients, respectively (Table 6). We also found that ChemoID treated patients on average had an incremental cost-effectiveness ratio (ICER) between -\$18,421 and \$7,241 per life-year saved (LYS).

Results for the model using the least expensive chemotherapy within the patient's highest category of sensitivity in the assay consistent cohort showed that the range in the ICER/LYS estimated by 1000 bootstrap samples for the assay was -\$5,590 (Supplementary Table 4).

When choosing the least expensive therapy within the test three highest category of sensitivity, the average cost saving of six cycles of chemotherapy becomes \$7,955 in the assay consistent cohort vs. historical control ($p < 0.001$). This change alone in the model makes the chemoresponse assay a positively dominant intervention that greatly changes the health care cost.

Discussion

This study for the first time shows the utility of a new cancer stem cell cytotoxicity assay for the management of poor prognosis recurrent ovarian cancer patients. The current study also served to inform the power calculations for an ongoing larger follow-up randomized clinical trial on the use of the CSCs assay to guide individualized chemotherapy choices and to improve recurrent ovarian cancer patient outcomes. The test is a functional assay that uses patient's live tumor cells and CSCs isolated by tumor biopsies or malignant fluid aspirates (peritoneal and/or pleural fluid) to indicate

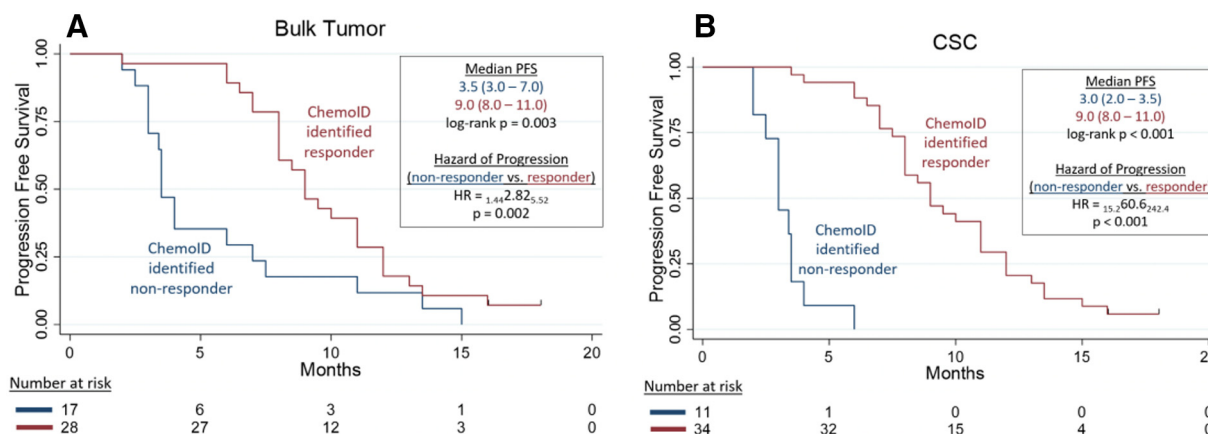


Fig. 4. Kaplan-Meier plots of progression-free survival across the study period. Progression-free survival is shown stratified by dichotomized test results for (A) bulk test >55% cell kill - responder or <55% cell kill - non-responder and (B) CSC test >40% cell kill - responder or <40% cell kill - non-responder; Hazard ratios are from Cox proportional hazard models adjusting for number of previous relapses and age.

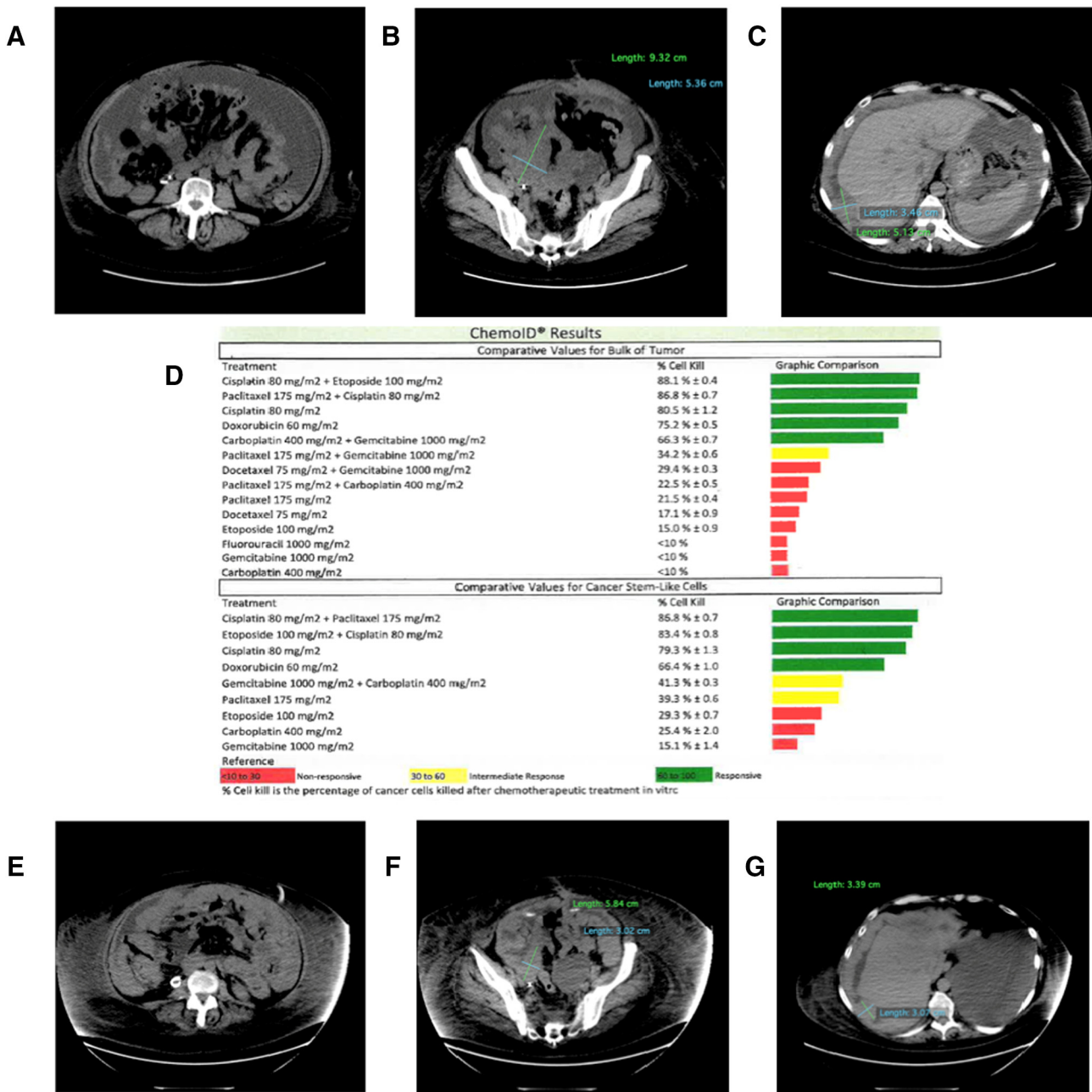


Fig. 5. CT Images and comparative analysis of ChemoID test results on Bulk of Tumor and Cancer Stem Cells of a patient affected by third recurrence of an ovarian cancer. A) Baseline CT images show presence of large amount of ascites in December of 2016, with B) ovarian mass measuring 9.3 × 5.4 cm, and C) peritoneal carcinomatosis measuring 3.5 × 5.1 cm. D) Comparative ChemoID analysis on Bulk of Tumor and Cancer Stem Cells obtained from fresh ascites aspirate. E) Control CT images in November of 2017 following a Doxorubicin regimen showing regression of ascites, F) smaller ovarian mass measuring 5.8 × 3 cm, and G) smaller peritoneal carcinomatosis measuring 3.4 × 3 cm.

which chemotherapy agent (or “combinations”) are more effective. Targeting of CSCs alongside the bulk of other cancer cells is a new paradigm in cancer treatment [41].

The current study evaluated the correlation of CSCs and bulk of tumor cells chemoresponse assay results of recurrent EOC patients to treatment

outcomes independently of other biomarkers. All patients were treated with a chemotherapy regimen that was chosen among those with the highest cell kill guided by the CSCs drug response assay, taking into consideration their health status and using dose reductions, as needed. CT and PET scans were used to prospectively monitor patients for tumor response,

Table 4

Cost comparison of administered drugs vs. ChemoID recommended drugs.

Actual drug cost	Vs. cost of ChemoID 1st choice	Vs. cost of ChemoID 2nd choice	Vs. cost of ChemoID 3rd choice	Vs. min of ChemoID first three choices
\$20,311.11	\$18,356.56 (−\$1,955.56) <i>p</i> = 0.121	\$18,533.33 (−\$1,777.78) <i>p</i> = 0.159	\$17,555.56 (−\$2,755.56) <i>p</i> = 0.029	\$12,355.56 (−\$7955.56) <i>p</i> < 0.001

Table 5

Cost comparison of ChemoID recommended drugs vs. PARP Inhibitor (PARPi) or Bevacizumab (Avastin).

	Vs. PARPi (4 cycles) \$72,000	Vs. Avastin (4 cycles) \$52,000	Vs. Avastin (22 cycles) \$286,000
Actual drug cost	–\$51,689	–\$31,689	–\$265,689
	$p < 0.001$	$p < 0.001$	$p < 0.001$
Vs. cost of ChemoID 1st choice	–\$53,644	–\$33,644	–\$267,644
	$p < 0.001$	$p < 0.001$	$p < 0.001$
Vs. cost of ChemoID 2nd choice	–\$53,467	–\$33,467	–\$267,467
	$p < 0.001$	$p < 0.001$	$p < 0.001$
Vs. cost of ChemoID 3rd choice	–\$54,444	–\$34,444	–\$268,444
	$p < 0.001$	$p < 0.001$	$p < 0.001$
Vs. min of ChemoID First three choices	–\$59,644	–\$39,644	–\$273,644
	$p < 0.001$	$p < 0.001$	$p < 0.001$

time to recurrence, progression-free survival (PFS), and overall survival (OS). This is the first-in-human case study to demonstrate that prospective use of the CSCs drug response assay to select treatment for 45 patients affected by poor prognosis (3rd–5th relapse) recurrent ovarian cancer resulted in significantly better PFS (median PFS of 11 months – 3rd relapse; 8 months – 4th relapse; and 8 months – 5th relapse, respectively), compared to historical data (5.6 months – 3rd relapse; 4.4 months – 4th relapse; and 4.1 months – 5th relapse, respectively) (Table 3). We observed that in the CSC model, the ovarian cancer patients that assay identified as non-responders had over 30 times the hazard of death compared to those women that were identified by the assay as responders ($p < 0.001$) with respective median survivals of 6 months vs. 13 months ($p < 0.001$) (Fig. 3).

Medical management of newly diagnosed epithelial ovarian carcinoma (EOC) is typically a multimodal treatment plan constituted by surgical resection (when possible), followed by platinum-based chemotherapy [11]. Disease recurrence is common in these patients, and most of them eventually develop the platinum-resistant disease (defined as disease recurring within 6 months after the last receipt of platinum-based chemotherapy) [12,13]. Recurrent EOC is associated with significant mortality and a median survival of only 12–24 months that becomes progressively worse with each additional recurrence [8]. Regimens to treat recurrent EOC are normally informed by responses to first-line therapies and vary significantly; therefore, choice of which agent to use is usually based on toxicity profile, the previous toxicities experienced by the patient, and patient preference (3). Additionally, the aggressiveness of recurrent EOC is mostly attributed to the presence of ovarian cancer stem cells (CSCs), which are chemo-resistant and responsible for the recurrence of cancer [42–45]. Individual patient responses to standardized treatments greatly vary and unfortunately, toxicity profiles are extensive for most chemotherapy drugs with no guarantee of success at the patient level. As such, there is an urgent need for ways to tailor chemotherapy regimens based on patients' individual tumor profiles to identify treatments that may lead to improvement in PFS and OS.

Prospective and retrospective investigations conducted in the past years have shown that in this era of personalized medicine, patients with recurrent ovarian cancer deserve better than the 25% response rate that is associated with drugs selected based on clinical information alone [46]. Previous studies, which were conducted using cytotoxicity assays against the bulk of tumor only reported improved clinical outcomes for ovarian cancer patients treated with sensitive chemotherapies as indicated by a chemoresponse assay, compared with those patients treated with non-

sensitive therapies [46–50]. An analysis of survival from patients cohorts in the control arm of phase-III randomized clinical trials indicated that the various treatment regimens had similar efficacy when therapies were randomly assigned [50]. Therefore, in the comparative analysis, even though therapies from the same set of approved and recommended treatment options were administered to participants, patients whose treatment was assay-informed had improved survival when compared to patients whose treatment was randomly assigned.

In our study, the ChemoID cytotoxicity assay identified high-suppression drugs against CSCs and the bulk of tumor cells contributing to a durable clinical response in a statistically significant manner. This study reveals that patients who were treated with a chemotherapy-sensitive regimen against CSCs had an improvement in their time to progression and overall survival compared to patients who could not receive assay sensitive regimens. In the CSC model for overall survival (Fig. 3B), the ovarian cancer patients that assay identified as non-responders had over 30 times the hazard of death compared to those women that were identified by the assay as responders ($p < 0.001$), with respective median survivals of 6 months vs. 13 months ($p < 0.001$). In the CSC model for progression-free survival (Fig. 4B), ovarian cancer patients that the assay identified as non-responders had over 60 times the hazard of progression compared to those women that were identified by the assay as responders ($p < 0.001$), with respective median survivals of 3 months vs. 9 months ($p < 0.001$). More modest but highly predictive hazards were observed based on the bulk of tumor models, demonstrating the superiority of prediction based on the CSCs model. Moreover, we observed that adding the bulk tumor and CSC assay response to a Cox model containing only relapse number and women age increased Harrell's C statistics from 0.63 to 0.79 for overall survival and from 0.62 to 0.84 for PFS. Overall, this data demonstrates the importance of determining the response of CSCs to chemotherapy and their role in patients' tumor response following chemotherapy. This method of determining the response of CSCs to available FDA approved chemotherapies for the treatment of ovarian cancer, as well as other cancers, may provide critical information about an individual patient's likelihood to achieve a durable tumor response before implementing the patient's treatment plan.

Although this study was conducted on a limited cohort of consecutive patients affected by poor prognosis EOC, results indicate that a drug cytotoxicity assay that targets CSCs may be a useful tool for optimizing treatment selection when first-line therapy fails, and when there are multiple clinically-acceptable and -equivalent treatments available. These results

Table 6

ICER/LYS Comparison of ChemoID recommended drugs for 3rd–5th relapse of recurrent EOC.

	Historical median PFS	Assay-guided cohort	ICER/LYS (range)
3rd relapse- average cost per patient	~\$20,000–\$35,000	\$20,387	\$860–(–\$32,473)
Median OS	5.6 months	11 months	
4th relapse - average cost per patient	\$20,000–\$35,000	\$20,333	\$1110–(–\$48,890)
Median OS	4.4 months	8 months	
5th relapse - average cost per patient	~\$20,000–\$35,000	\$20,000	\$0–(–\$46,154)
Median OS	4.1 months	8 months	

further suggest that the CSCs drug response assays may provide more treatment options for improved outcomes than currently achieved by empiric population-based treatment in recurrent ovarian cancer. This concept is especially valuable and important with emerging value-based healthcare models where outcomes-based contracts linked to payment for the indication of specific anticancer-drug prices raise concerns about the accessibility and affordability for treatment of recurrent EOC patients. The power of precision medicine lies in its ability to guide health care decisions toward the most effective treatment for a given patient, and thus, improve care quality while reducing the use of ineffective therapies. In a recent retrospective study involving metastatic cancer patients, researchers at the University of Utah compared data from patients who received precision medicine targeted therapy with data from patients who received standard chemotherapy or best supportive care [51]. It was observed that patients receiving targeted treatment after precision oncology had doubled median overall survival compared to patients receiving chemotherapy or supportive care. Moreover, it was found that the average costs per week over the study period were almost half for patients receiving targeted therapy compared to those receiving chemotherapy or supportive care indicating that precision health and directed therapies have the potential to lower health care costs.

The current study compares the cost of treatment decisions that adhere to chemo response assay results at the time of second-line therapy in patients with recurrent EOC with those that are made empirically, in the absence of chemoresponse testing, such as the historical control.

The cost-effective benefit of a healthcare intervention such as a drug response assay is realized if the comprehensive cost of its use is less than \$100,000 per additional life-year saved [37]. In the current study, the use of and adherence to chemoresponse assay results yield an ICER of \$5,200 per additional life-year saved, suggesting that the assay intervention is cost-effective even at a conservative \$50,000 threshold. Furthermore, in analyses of patients with an assay-sensitive result for at least one therapy, the ICER dropped to -\$5,590 per LYS in our cohort of platinum-resistant patients, where the need for decision support tools is greater due to poorer prognosis than platinum-sensitive patients. While the current study examines cost-effectiveness in the recurrent EOC setting, future studies are planned which will evaluate the cost-effectiveness of this chemoresponse assay across the entire treatment duration of patients with advanced EOC, accounting for its influence in both the primary and recurrent settings. Additionally, the use of a chemoresponse assay in making treatment decisions may potentially reduce toxicities and their associated costs, as well as improve the patients' overall quality of life, by reducing the number of ineffective treatment rounds. Therefore, healthcare cost-effectiveness of the assay may be further enhanced when accounting for these differences.

More clinical studies with a larger number of patients are needed to determine the clinical and economic implications of the CSCs cytotoxicity assay. Although the current study represents a small sample cohort and lacks randomization, nevertheless our results clearly showed that women affected by poor-prognosis ovarian cancer treated with assay-guided regimens against CSCs had an improvement in their time to progression and overall survival compared to patients treated empirically. Our study provides proof of concept for CSCs drug response assay in personalizing treatment strategies to increase survival time for recurrent EOC patients and was useful in developing a larger multi-center randomized trial on the use of this assay to guide therapy in recurrent ovarian cancer. We are conducting a multi-institutional prospectively treated, randomized, blinded, controlled trial on the use of the CSC cytotoxicity assay to guide therapy vs. physician's choice chemotherapy in patients with platinum-resistant recurrent ovarian cancer (NCT03949283), with the intent to determine the clinical impact of CSC assay-directed therapy for recurrent ovarian cancer.

Conclusions

Our results indicate that the prediction of response to high cell kill therapy against CSC was consistent with expected better patients' response rates. Eliminating more effectively the CSC load from the recurrent ovarian tumor improved OS and PFS of women treated with high cell kill

chemotherapies against CSCs as indicated by the assay. More importantly, the prediction of tumor responses to chemotherapy treatment in vitro was directly associated with the OS and PFS of the treated patients. Interestingly, the data also suggests that the CSCs assay may identify alternate treatments when tested in vitro that may be more effective in the subset of patients whose tumors have relapsed and that have been classified clinically non-responsive to platinum agents. Together, this information suggests that the CSCs drug response assay has the potential to help to guide individualized chemotherapy choices to benefit patients by allowing the physician to consider alternate regimens earlier in the treatment plan for improving outcomes in recurrent ovarian cancer.

Declarations

Ethics approval and consent to participate

Marshall University Institutional Review Board (IRB) has approved this research under the protocol #326290. Assay was performed after obtaining patients' written informed consent in accordance with the ethical standards of the Helsinki Declaration (1964, amended most recently in 2008) of the World Medical Association.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated and/or analyzed during the current study are not publicly available due to individual privacy restriction on medical records, but are available from the corresponding author on reasonable request.

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Authors' contributions

All authors contributed significantly to the present research and reviewed the entire manuscript.

CMH: Participated substantially in the conception and execution of the study and the analysis and interpretation of radiological imaging data; also participated substantially in the drafting and editing of the manuscript.

NBZ: Participated in providing patients' treatment samples and data; also participated substantially in execution of the study and in the drafting and editing of the manuscript.

SB: Participated in providing patients' treatment samples and data; also participated substantially in execution of the study and in the drafting and editing of the manuscript.

TDE: Participated in providing patients' treatment samples and data and in the execution of the study.

AC: Participated in providing patients' treatment samples and data; also participated substantially in execution of the study and in the drafting and editing of the manuscript.

AM: Participated in providing patients' treatment samples and data; also participated substantially in execution of the study and in the drafting and editing of the manuscript.

STL: Participated in the statistical analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

KD: Participated substantially in providing patients' ChemoID test results, analysis, and interpretation of data in the drafting and editing of the manuscript.

JV: Participated substantially in conception and execution of the study; participated in providing patients' ChemoID test results, analysis, and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

PPC: Participated substantially in conception and execution of the study; participated in providing patients' ChemoID test results, analysis, and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tranon.2020.100860>.

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Supplementary Materials

Table S1. Estimated cost of chemotherapy drugs including their administration cost of all therapy regimens used in the clinical study ^a.

Docetaxel	\$16,000
Topotecan	\$19,000
Gemcitabine	\$14,000
Pemetrexed	\$24,000
Cisplatin	\$6,000
Doxorubicin ^b	\$12,000
Paclitaxel	\$16,000
Carboplatin	\$6,000
Etoposide	\$5,000
5-Fluorouracyl	\$1,000
Oxaliplatin	\$7,000
Docetaxel+ Carboplatin	\$22,000
Cisplatin+Topotecan	\$25,000
Cisplatin +Paclitaxel	\$22,000
Topotecan+Paclitaxel	\$35,000
Carbopatin+Paclitaxel	\$22,000
Gemcitabine+Cisplatin	\$20,000
Pemetrexed+ Cisplatin	\$30,000
5-Fluorouracyl +Cisplatin	\$7,000
Pemetrexed+ Doxorubicin ^b	\$36,000
Topotecan+ Doxorubicin ^b	\$31,000
Etoposide+ Doxorubicin ^b	\$17,000
Gemcitabine+Docetaxel	\$30,000
Etoposide+Paclitaxel	\$21,000
Docetaxel+Cisplatin	\$22,000

^a Inclusive of the cost of chemotherapy agent(s) included in the regimen plus the cost of administration in the physician office setting, for a total of 4 cycles.

^b Pegylated liposomal doxorubicin

Table S2. Cost of ChemoID assay recommended therapies used to treat patients' cohort including their administration cost and cost of other drugs tested by the assay but not used to treat patients^a.

Patient number	Cost of drugs used	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested	Other drug tested
1	\$16,000	\$22,000	\$25,000	\$22,000	\$6,000	\$16,000	\$12,000	\$19,000	\$24,000	\$14,000	\$6,000							
2	\$19,000	\$25,000	\$20,000	\$6,000	\$22,000	\$22,000	\$35,000	\$14,000	\$24,000	\$16,000	\$6,000							
3	\$30,000	\$8,000	\$6,000	\$22,000	\$7,000	\$12,000	\$36,000	\$6,000	\$5,000	\$16,000	\$16,000	\$24,000						
4	\$30,000	\$20,000	\$21,000	\$6,000	\$21,000	\$17,000	\$22,000	\$16,000	\$31,000	\$12,000	\$6,000	\$16,000	\$5,000	\$19,000	\$14,000			
5	\$6,000	\$22,000	\$20,000	\$16,000	\$30,000	\$22,000	\$6,000	\$14,000										
6	\$12,000	\$30,000	\$25,000	\$22,000	\$22,000	\$20,000	\$6,000	\$35,000		\$16,000	\$14,000	\$6,000	\$16,000	\$24,000	\$14,000			
7	\$22,000	\$8,000	\$30,000	\$6,000	\$7,000	\$12,000	\$36,000	\$6,000	\$1,000	\$5,000	\$16,000	\$16,000	\$14,000	\$19,000	\$20,000	\$20,000	\$18,000	\$22,000
8	\$20,000	\$22,000	\$20,000	\$21,000	\$16,000	\$12,000	\$14,000	\$16,000	\$6,000	\$22,000								
9	\$22,000	\$11,000	\$22,000	\$6,000	\$20,000	\$30,000	\$16,000	\$5,000	\$14,000	\$6,000								
10	\$12,000	\$6,000	\$6,000	\$16,000	\$24,000													
11	\$20,000	\$22,000	\$20,000	\$6,000	\$5,000	\$21,000	\$12,000	\$16,000	\$22,000	\$6,000	\$14,000							
12	\$22,000	\$11,000	\$6,000	\$20,000	\$12,000	\$16,000	\$30,000	\$30,000	\$5,000	\$16,000	\$14,000							
13	\$12,000	\$11,000	\$22,000	\$6,000	\$20,000	\$30,000	\$30,000	\$22,000	\$16,000	\$16,000	\$5,000	\$1,000	\$14,000	\$6,000				
14	\$20,000	\$11,000	\$6,000	\$22,000	\$12,000	\$30,000	\$22,000	\$16,000	\$22,000	\$6,000	\$30,000	\$5,000	\$16,000	\$14,000	\$1,000			
15	\$22,000	\$18,000	\$22,000	\$20,000	\$20,000	\$19,000	\$16,000	\$16,000	\$5,000	\$6,000								
16	\$20,000	\$22,000	\$11,000	\$6,000	\$12,000	\$30,000	\$16,000	\$30,000	\$22,000	\$22,000	\$5,000	\$6,000	\$16,000	\$14,000				
17	\$20,000	\$22,000	\$6,000	\$19,000	\$6,000	\$12,000	\$20,000	\$18,000	\$5,000									
18	\$30,000	\$28,000	\$22,000	\$8,000	\$30,000	\$6,000	\$12,000	\$20,000	\$18,000	\$6,000	\$16,000	\$14,000	\$24,000	\$16,000	\$19,000			

19	\$22,000	\$22,000	\$20,000	\$6,000	\$7,000	\$11,000	\$21,000	\$16,000	\$12,000	\$22,000	\$6,000	\$14,000	\$5,000					
20	\$20,000	\$28,000	\$22,000	\$30,000	\$30,000	\$7,000	\$6,000	\$12,000	\$6,000	\$16,000	\$1,000	\$14,000	\$24,000	\$16,000	\$19,000			
21	\$16,000	\$22,000	\$21,000	\$12,000	\$14,000	\$16,000	\$6,000											
22	\$22,000	\$22,000	\$25,000	\$22,000	\$18,000	\$16,000	\$6,000	\$16,000	\$12,000	\$19,000	\$24,000	\$14,000						
23	\$22,000	\$30,000	\$6,000	\$8,000	\$12,000	\$36,000	\$6,000	\$7,000	\$5,000	\$16,000	\$16,000							
24	\$14,000	\$16,000	\$12,000	\$22,000	\$6,000	\$5,000	\$16,000											
25	\$20,000	\$11,000	\$6,000	\$22,000	\$20,000	\$12,000	\$16,000		\$6,000	\$14,000	\$16,000	\$5,000						
26	\$22,000	\$20,000	\$21,000	\$11,000	\$6,000	\$16,000	\$5,000	\$14,000	\$6,000	\$16,000								
27	\$22,000	\$11,000	\$6,000	\$12,000	\$20,000	\$22,000	\$30,000	\$16,000	\$22,000	\$30,000	\$5,000	\$16,000	\$14,000	\$6,000				
28	\$22,000	\$25,000	\$22,000	\$20,000	\$6,000	\$30,000	\$12,000	\$35,000	\$16,000	\$16,000	\$19,000	\$6,000	\$14,000	\$24,000				
29	\$18,000	\$28,000	\$22,000	\$30,000	\$30,000	\$20,000	\$19,000	\$14,000	\$24,000	\$16,000	\$16,000							
30	\$12,000	\$25,000	\$22,000	\$20,000	\$6,000	\$30,000	\$35,000	\$16,000	\$22,000	\$16,000	\$19,000	\$6,000	\$14,000	\$24,000				
31	\$22,000	\$22,000	\$22,000	\$11,000	\$20,000	\$6,000	\$30,000	\$22,000	\$5,000	\$30,000	\$16,000	\$16,000	\$6,000	\$12,000	\$14,000			
32	\$22,000	\$18,000	\$22,000	\$20,000	\$12,000	\$16,000	\$16,000	\$5,000	\$6,000	\$6,000	\$19,000							
33	\$19,000	\$22,000	\$18,000	\$22,000	\$5,000	\$16,000	\$6,000	\$6,000	\$14,000									
34	\$22,000	\$11,000	\$22,000	\$20,000	\$12,000	\$30,000	\$21,000	\$22,000	\$6,000	\$14,000								
35	\$30,000	\$16,000	\$14,000	\$16,000														
36	\$22,000	\$22,000	\$16,000	\$25,000	\$6,000	\$16,000	\$12,000	\$19,000	\$24,000	\$14,000								
37	\$20,000	\$20,000	\$22,000	\$22,000	\$18,000	\$12,000	\$19,000	\$14,000	\$16,000	\$16,000	\$5,000							
38	\$22,000	\$6,000	\$11,000	\$12,000	\$22,000	\$20,000	\$30,000	\$5,000	\$6,000	\$16,000	\$1,000	\$16,000	\$30,000	\$14,000				
39	\$20,000	\$22,000	\$22,000	\$12,000	\$30,000	\$11,000	\$16,000	\$22,000	\$6,000	\$14,000								
40	\$22,000	\$18,000	\$22,000	\$19,000	\$5,000	\$6,000	\$16,000	\$30,000	\$14,000	\$24,000								

41	\$22,000	\$12,000	\$36,000	\$6,000	\$6,000	\$8,000	\$30,000	\$5,000	\$16,000	\$20,000	\$16,000	\$14,000		\$19,000				
42	\$12,000	\$8,000	\$22,000	\$6,000	\$16,000													
43	\$22,000	\$30,000	\$6,000	\$12,000	\$16,000	\$16,000	\$32,000	\$24,000	\$6,000									
44	\$20,000	\$11,000	\$6,000	\$22,000	\$12,000	\$30,000	\$22,000	\$16,000	\$22,000	\$5,000	\$16,000	\$14,000						
45	\$30,000	\$12,000	\$22,000	\$6,000	\$14,000	\$6,000	\$19,000	\$5,000										

Table S3. Estimated cost of chemotherapy drugs including their administration cost of therapy regimens that have not been used in the clinical study ^a.

PARP Inhibitors ^b	\$72,000
Avastin ^c	\$52,000 - 286,000

^a Inclusive of the cost of chemotherapy agent(s) included in the regimen plus the cost of administration in the physician office setting.

^b 4 cycles

^c 4-22 cycles

Table S4. Results for the model using the least expensive chemotherapy within the patient's highest category of sensitivity in the assay consistent cohort. The range in the ICER/LYS was estimated by independently sampling 1000 times from each of the two 95% CI of the HRs for the assay.

	Baseline cohort	Assay consistent cohort	ICER/LYS (range)
Average cost per patient	\$ 17,400	\$ 16,571	
Median overall survival	10.0 months	13.0 months	
Mean overall survival	10.2 months	11.9 months	\$ -5,590 (-\$18,421, \$7,241)