Efficacy of ChemoID® guided drug selection for palliative chemotherapy in advanced recurrent high-grade ovarian adenocarcinoma: Case study

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Abstract

Notwithstanding tremendous advances in surgery, primary chemotherapy, and novel treatments for recurrent disease, the diagnosis of advanced epithelial ovarian cancer (EOC) in 2016 remains ultimately fatal in the majority of cases. Advanced therapy refractory EOC patients are often treated in hospice and with chemotherapy palliative care. The main goals of chemotherapy for recurrent/refractory ovarian cancer are the palliation of disease-related symptoms, and improvement of quality and quantity of life. Unfortunately, there is contradicting evidence suggesting that chemotherapy has a role in palliation of symptoms with an apparent improvement in quality of life. Anticancer drugs have a high rate of failure and chemotherapy response assays have been used to identify which drugs are more likely to be effective against those of gynecological origin. ChemoID® is a functional drug response assay which measures the sensitivity of cancer stem cells (CSCs) and bulk of tumor cells to chemotherapy to determine the most effective combination of anticancer drugs for solid tumors. We present a clinical case that demonstrates the utility of ChemoID® guidance to commonly available drugs with identified toxicity profiles and predictable cost profile available at POS, along with rapid response in correcting symptomatic disease features, and minimal treatment burden from toxicities. We observed in the

reported case the survival and symptom management benefits of ChemoID® guided therapy even after plateau of response to cisplatin. Further studies are indicated to increase the clinical adoption of ChemoID® for gynecological malignancies in the palliative setting.

Introduction

Epithelial ovarian cancer (EOC) is the leading cause of death from gynecologic malignancies with more than 23,000 cases annually in the United States, and 14,000 women that are expected to die from the disease.[1] Although ovarian cancer accounts for only 3% of all cancers in women, it has one of the highest death-to- incidence ratios, which has been primarily attributed to the unavailability of effective screening tools, the absence of early symptoms, and its typical presentation at advanced stages when prognosis is poor.[2,3] In particular, patients with high- grade serous carcinoma (which constitute 60-80% of EOC, and which represent the archetypical *ovarian cancer*) most frequently present at advanced clinical stage and have a very poor overall survival.[4,5]

The common initial management for newly diagnosed epithelial ovarian cancer (EOC) includes aggressive cytoreductive surgery accompanied by the administration of platinum and taxane-based chemotherapy before or after surgery. Despite the inherent resistance to chemotherapy in some patients, about 80% of patients achieve an initial clinical complete response.[6] However, the majority of EOC patients will eventually relapse. Some patients relapse within 6 months and have a short survival due to platinum-resistant disease; other patients have late relapses with platinum-sensitive disease, and substantially longer survival.

Currently, clinicians do not have good prognostic tools to estimate which patients are destined to have platinum-resistant or sensitive disease. As a result, the current standard of care is to apply a general paradigm to all women with ovarian cancer; either surgery followed by adjuvant platinum and taxane-based chemotherapy or neo-adjuvant chemotherapy with the same agents preceding surgery.[6] Despite improvements in the management of ovarian cancer patients over the last 30 years, there has been only a minimal improvement in overall survival. In fact, while targeted therapeutic approaches for the treatment of cancer have evolved, major challenges in ovarian cancer research still persist, includ-

ing the identification of predictive biomarkers with clinical relevance, so that empirical drug selection can be avoided. Selection of effective chemotherapy is important not only when therapy is first initiated, but especially for recurrent disease. In fact, administration of ineffective anticancer therapy is associated with unnecessary toxicity and the development of more aggressive cancer cell clones that is resistant to subsequent therapies.[7,8] The ability to choose the most effective chemotherapy may help improving patients' quality of life by avoiding the physical, emotional, and financial burden of ineffective therapy.[9] Anticancer drugs for different reasons have a high rate of failure and cell culture chemotherapy testing has been used during the recent past to identify which drugs are more likely to be effective especially against those of gynecological origin.[10,11] Many attempts have been made over the years to develop *an in-vitro* anti-cancer test that can provide clinically relevant treatment information. However, this approach has been hampered by the chemotherapy testing only being performed on bulk of tumor cells derived from cancer biopsies.[12-21] Ovarian cancers contain a population of self- renewing cancer stem cells (CSCs) that contribute to tumorigenesis, treatment resistance and tumor recurrence.[22-29]

ChemoID® is a functional test that uses patient's live tumor cells to indicate which chemotherapy agent (or *combinations*) will kill not only the bulk tumor cells, but more importantly the cancer stem cells (CSCs) that are known to cause cancer to recur. Targeting of CSCs alongside the bulk of other cancer cells is a new paradigm in cancer treatment. This constitutes an important advantage of ChemoID® approach over

other cell culture testing methods. Our recent clinical studies showed that patient derived CSCs from primary cancer cell cultures can be used in a drug response assay.[9,30-36] We have optimized the enrichment of CSCs from tumor biopsies and aspirates of effusions and have developed the ChemoID® chemotherapy response assay, which measures the sensitivity of CSCs and bulk of tumor cells to chemotherapy to determine the most effective combination of anticancer drugs for solid tumors.[9,30-36]

Materials and Methods

Patient

Patient is a 77-year-old female presenting with venous thromboembolism and evidence of IIIC ovarian cancer, who underwent initial cytoreductive surgery in June 02, 2008. Patient relapsed to first, second, third, and fourth line standard-of-care chemotherapy treatment before being diagnosed with ChemoID® drug response assay. Subject was enrolled in the study only after a discussion of her treatment options, including chemotherapy. ChemoID® assay was performed after obtaining patient's consent. Marshall University Institutional Review Board (IRB) has approved this research under the protocol #695141.

Drug sensitivity assay

Ascites aspirate containing a high cellularity of malignant cells for ChemoID® *in vitro* functional testing was collected in the operating room from the patient. Details regarding the assay procedure have been described elsewhere.[9,30-34,36] In brief, primary cultures were initiated by spinning down the malignant cells present in the ascites aspirate and by culturing the cells to sub confluence in RPMI-1640 medium supplemented with 5% irradiated, heat inactivated, defined fetal bovine serum (Thermofisher/Hyclone), and 50 U of penicillin and 5 µg of streptomycin/mL of medium (Thermofisher/Mediatech). Proliferation of CSCs was obtained using a culture methodology previously described[9,31] in which culture media, oxygenation, rotational speed of the culture vessel, temperature and CO2 were kept consistently constant for seven days. Cells were then removed and counted using trypan blue exclusion to determine cellular viability and cell number. Equal number of bulk of tumor cells and CSCs, were counted and seeded separately in 96-well dishes and incubated at 37°C for 24-hours. Three concentrations of each treatment were prepared by serial dilution.

Each concentration was added to five replicate wells on the microtiter plate. Three replicates wells (control 1=no treatment) and three replicates wells (control 2=equal amount of solvent) were associated with each treatment also. The cells were challenged for a 1-hour pulse with the panel of anticancer drugs. Sensitivity to chemotherapy was assessed using a WST8 viability assay (Dojindo Molecular Technologies, Rockville, MD, USA) on 1×10^{-3} cells plated in 5 replicas into 96-well plates. The WST8 assay was performed 48- hours following chemotherapy treatment to assess cell viability as previously described.[9]

The inhibition of bulk of tumor cells and CSCs survival was measured for each concentration (average counts in five replicates ± SE) of a given treatment. The survival of tumor cells at each concentration was calculated as compared to control-2 and overall percent of bulk and CSC tumor cells killed were calculated for each treatment as the primary measures of potential therapy efficacy.
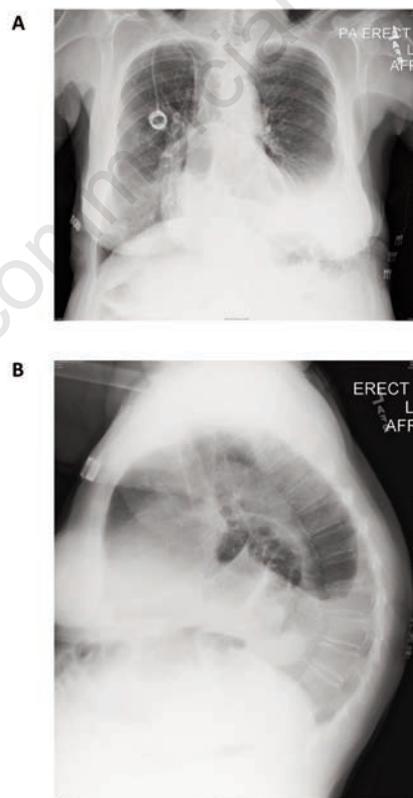

Figure 1. Recurrent malignant effusion at fourth relapse of epithelial ovarian cancer: posterior/anterior (A) and left lateral (B) chest x-ray showing moderate malignant left pleural effusion causing passive atelectasis of the left lower lobe of the lung. Note is also made of a large hiatal hernia.
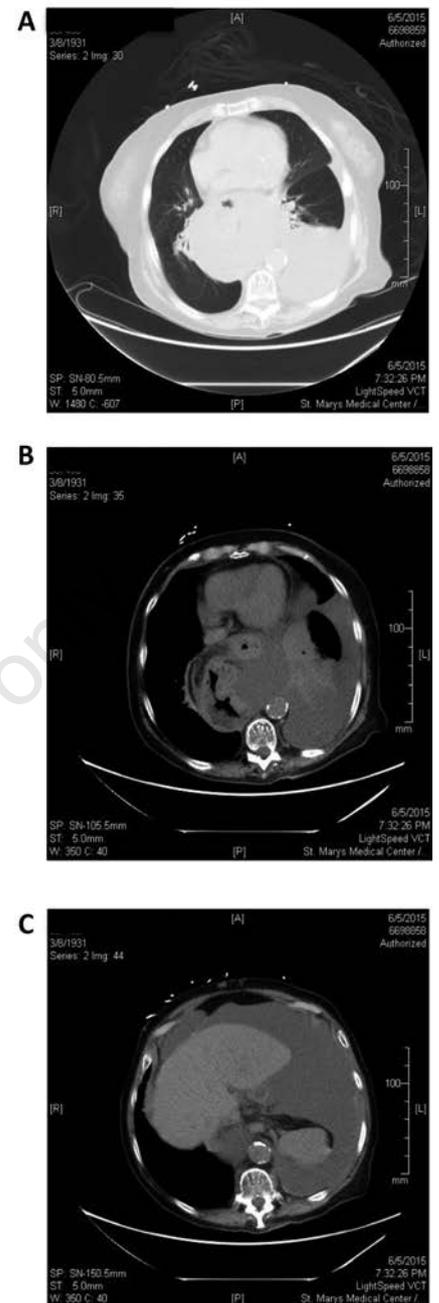

Figure 2. Chest and abdomen computed tomography with contrast enhancement showing detail of the left pleural effusion: A) is a cross sectional image at the lung base in lung windows; B) is a cross sectional image of the upper abdomen at the level of the liver dome with soft tissue windowing; C) is the same image with soft tissue windowing. The images demonstrate increased abdominal pressure from large volume abdominal ascites secondary to peritoneal carcinomatosis causing large hiatal hernia with herniation of the bowel into the retro cardiac portion of the thorax with mass affect on the left atrium of the heart. Moderate malignant left pleural effusion causing passive sub-segmental atelectasis of the left lower lobe of the lung. No morphologic features of cirrhosis in the liver.

Results

A 77-year-old female presented with venous thromboembolism (VTE) and evidence of IIIC ovarian cancer, with a CA125 of 340 and 3,000 cc of ascites at exploration in 2008. She had pulmonary embolism that was treated with an inferior vena cava (IVC) filter. The initial surgery of radical cytoreduction included positive paraaortic lymph nodes to optimal status less than 1 cm. Pathology specimens involved both ovaries, omentum, urinary bladder, bilateral fallopian tubes cervical stroma gall bladder serosa and paraaortic lymph nodes. Pathology report described the presence of a high-grade serous ovarian adenocarcinoma. Within 42-days post surgery, patient was treated with first-line adjuvant chemotherapy (carboplatinum 560 mg and taxol 255 mg IV q4 weeks) beginning July 15, 2008 through July 27, 2009. Immediate post-therapeutic monitoring of CA125 showed a satisfactory decrease (CA 125 = 340 pre chemo, 99.2 = post-op, and 49.9 =

post cycle 1 of carboplatinum/taxol), consistent with the history of a complete platinum response. Unfortunately, following the first two years of no evidence of disease (NED), the EOC relapsed several times in spite of chemotherapy management. Upon a follow-up visit, a relapse of the EOC was observed in September 2011, which was treated with second-line chemotherapy consisting of 11 cycles of carboplatinum 300 mg and gemcitabine 1,000 mg from October 4, 2011 to September 20, 2012. A second relapse in July 2013 was treated with third-line pegylated doxorubicin 51mg administered over 3 of 4 weeks for 9 cycles. A third relapse in March of 2014 with CA 125 = 308 was treated with fourth-line

Figure 4. Chest and abdomen computed tomography with contrast enhancement showing positive therapeutic response of the malignant pleural effusion and abdominal ascites from peritoneal carcinomatosis after one cycle of ChemoID® predicted cisplatinum and taxol chemotherapy. Cross sectional image at the lung bases (A) and cross sectional image of the mid abdomen below the liver and the spleen (B). Both sections are displayed using soft tissue windows. Imaging shows marked reduction in the left pleural post chemotherapy with minimal residual left pleural effusion and improved aeration in the left lung base. Additionally, there is a dramatic decrease in the abdominal ascites. Note the large hiatal hernia has re-expanded following resolution of effusion.

biweekly topotecan $4mg/m^2$ from April 2014 to December 2014 and in January 2015 the CA125 measured 395. In June 2015, a follow-up visit discovered a fourth relapse with CA125 = 655, with pleural effusion and large volume ascites, both cytologically positive for adenocarcinoma, consistent with primary EOC (Figures 1 and 2). An ascites aspirate from the fourth EOC relapse was sent to the clinical laboratory for ChemoID® testing which resulted in four responsive (60-100% cell kill *in vitro*) possible regimens (Table 1). Patient was treated with fifth-line therapy consisting of cisplatinum 40 mg/m^2 plus paclitaxel 60 mg/m^2 with dose reduction due to chronic kidney disease (CKD) and prior grade-2 peripheral neuropathy.

Fifth-line chemotherapy was initiated in July 08, 2015 after supportive care management for pleural effusion and large volume of rapidly recurrent ascites requiring Aspira catheter (over 1,000cc/day daily drainage). Six cycles of cisplatinum and taxol fifth-line chemotherapy, as guided by ChemoID®, were administered without dose modification between July 2015 (Figures 3 and 4) and December 2015 (Figure 5) and resulted in a constant decline of CA125 as follows: 06/06/2015 = 655.5; 07/25/2015 = 201.9 post cycle 1; 08/31/2015 = 68.7 post cycle 2; 09/23/2015 = 53.4 post cycle 3; 10/19/2015 = 43.1 post cycle 4; 11/16/2015 = 59.8 post cycle 5; 12/28/2015 = 59.7 post cycle 6. On 12/28/2015 despite stable CA-
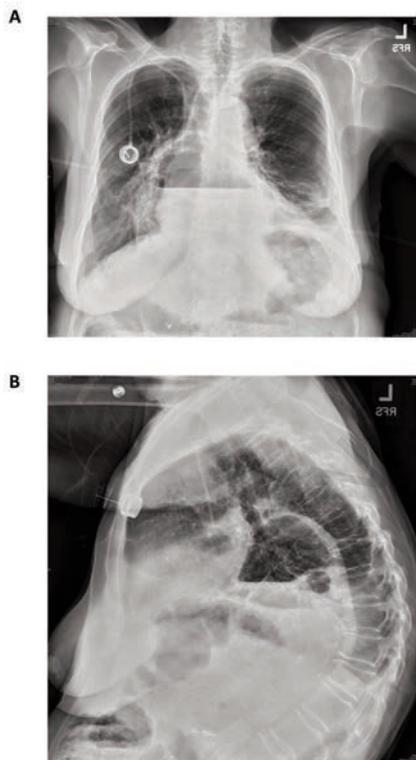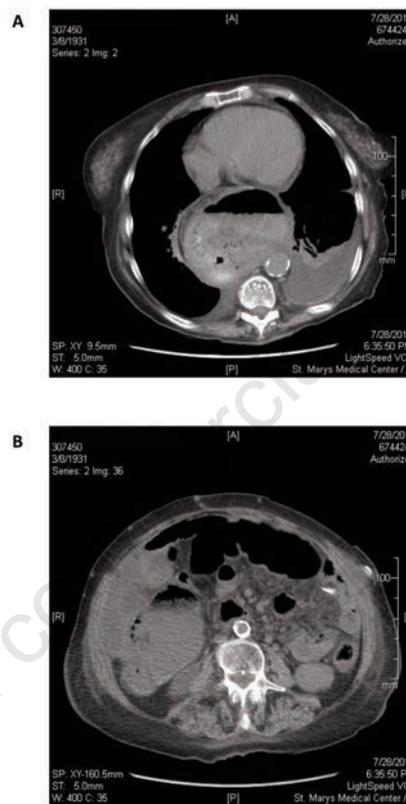

Figure 3. Marked decrease of pleural effusion after one cycle of ChemoID® predicted cisplatinum and taxol chemotherapy: posterior/anterior (A) and left (B) lateral chest x-ray showing marked decrease of prior moderate malignant left pleural effusion and improved aeration of the left lower lobe of the lung. Trace residual left pleural effusion. Note is also made of a large hiatal hernia.

Figure 5. Abdominal computed tomography showing further positive therapeutic response after six cycles of ChemoID® predicted cisplatinum and taxol chemotherapy with resolution of ascites. Cross-sectional image with IV and oral contrast of the mid abdomen below the level of the liver and spleen. Interval complete resolution of abdominal ascites and marked reduction of peritoneal implants and lymphadenopathy.

Table 1. ChemoID® assay results from ascites aspirate from a fourth recurrence of a high-grade serous carcinoma of the ovaries.

Comparative values for bulk of tumor		Comparative values for cancer stem cells	
Responsive 100-60% cell kill			
Cyclophosphamide 600 mg/m^2 +Cisplatin 100 mg/m^2	72.3%±0.9	Pemetrexed 500 mg/m^2 +Cisplatin 100 mg/m^2	70.1%±1.0
Pemetrexed 500 mg/m^2 +Cisplatin 100 mg/m^2	71.3%±0.5	Cisplatin 100 mg/m^2	68.7%±1.7
Cisplatin 100 mg/m^2	63.3%±1.3	Cyclophosphamide 600 mg/m^2 +Cisplatin 100 mg/m^2	66.5%±1.2
Paclitaxel 175 mg/m^2 +Cisplatin 100 mg/m^2	62.5%±1.2	Fluorouracil 1000 mg/m^2 +Cisplatin 100 mg/m^2	60.4%±1.2
Intermediate response 60-30% cell kill			
Fluorouracil 1000 mg/m^2 +Cisplatin 100 mg/m^2	58.9%±1.5	Paclitaxel 175 mg/m^2 +Cisplatin 100 mg/m^2	58.5%±1.4
Doxorubicin 75 mg/m^2	54.2%±2.5	Pemetrexed 500 mg/m^2 +Doxorubicin 75 mg/m^2	54.2%±1.5
Pemetrexed 500 mg/m^2 + Doxorubicin 75mg/m^2	51.9%±0.7	Doxorubicin 75 mg/m^2	51.5%±1.9
-	-	Carboplatin 350 mg/m^2	42.5%±1.0
Not responsive 30-0% cell kill			
Carboplatin 350 mg/m^2	24.5%±4.0	Pemetrexed 500 mg/m^2	14.0%±4.6
Fluorouracil 1000 mg/m^2	12.8%±3.7	Methotrexate 500 mg/m^2	28.8%±3.6
Etoposide 50 mg/m^2	11.5%±1.4	Fluorouracil 1000 mg/m^2	<10%
Cyclophosphamide 600 mg/m^2	<10%	Etoposide 50 mg/m^2	<10%
Paclitaxel 175 mg/m^2	<10%	Cyclophosphamide 600 mg/m^2	<10%
Docetaxel 75 mg/m^2	<10%	Paclitaxel 175 mg/m^2	<10%
Pemetrexed 500 mg/m^2	<10%	Docetaxel 75 mg/m^2	<10%
Methotrexate 500 mg/m^2	<10%	-	

Table 2 ChemoID® assay results from pleural effusion of fifth recurrence from a high-grade serous carcinoma of the ovaries.

Comparative values for bulk of tumor		Comparative values for cancer stem cells	
Responsive 100-60% cell kill			
Gemcitabine 1250 mg/m^2 +Cisplatin 100 mg/m^2	69.3%±1.3	Gemcitabine 1250 mg/m^2+Docetaxel 75 mg/m^2	79.3%±2.4
Ifosfamide 5000 mg/m^2	64.3%±0.4	Docetaxel 75 mg/m^2+Cisplatin 80 mg/m^2	69.8%±1.4
Intermediate response 60-30% cell kill			
Docetaxel 75 mg/m^2 +Cisplatin 80 mg/m^2	59.3%±0.9	Gemcitabine 1250 mg/m^2+Cisplatin 100 mg/m^2	53.1%±1.0
Cisplatin 100 mg/m^2	52.2%±1.9	Docetaxel 75 mg/m^2	52.8%±2.2
Etoposide 100 mg/m^2 + Paclitaxel 175 mg/m^2 + Carboplatin 350 mg/m^2	49.9%±1.8	Ifosfamide 5000 mg/m^2	52.1%±0.6
Gemcitabine 1250 mg/m^2 +Docetaxel 75 mg/m^2	48.5%±2.7	Etoposide 100 mg/m^2+ Doxorubicin 50 mg/m^2+Cisplatin 50 mg/m^2	49.3%±0.4
Etoposide 100 mg/m^2 + Doxorubicin 50 mg/m^2 + Cisplatin 50 mg/m^2	51.9%±0.7	Cisplatin 100 mg/m^2	43.9%±1.2
Oxaliplatin 85 mg/m^2	45.5%±3.8	Etoposide 100 mg/m^2+ Paclitaxel 175 mg/m2+Carboplatin 350 mg/m^2	42.6%±1.6
Paclitaxel 175 mg/m^2 +Carboplatin 350 mg/m^2	41.1%±0.9	Doxorubicin 60 mg/m^2	40.3%±2.8
Docetaxel 75 mg/m^2	32.5%±1.0	Fluorouracil 1000 mg/m^2+Oxaliplatin 85 mg/m^2	39.4%±3.5
Topotecan 1.5 mg/m^2 +Doxorubicin 60 mg/m^2	31.9%±0.3	Topotecan 1.5 mg/m^2+Doxorubicin 60 mg/m^2	37.4%±0.7
Doxorubicin 60 mg/m^2	31.0%±1.8	Paclitaxel 175 mg/m^2 +Carboplatin 350 mg/m	35.9%±2.0
Fluorouracil 1000 mg/m^2 +Oxaliplatin 85 mg/m^2	30.1%±4.5	-	-
Not responsive 30-0% cell kill			
Carboplatin 350 mg/m^2	22.2%±3.2	Oxaliplatin 85 mg/m^2	28.1%±2.2
Paclitaxel 175 mg/m^2	19.0%±2.7	Etoposide 100 mg/m^2	27.3%±2.1
Fluorouracil 1000 mg/m^2	13.7%±1.3	Fluorouracil 1000 mg/m^2	19.4%±1.3
Etoposide 100 mg/m^2	<10%	Gemcitabine 1250 mg/m^2	17.8%±3.5
Topotecan 1.5 mg/m^2	<10%	Topotecan 1.5 mg/m^2	16.8%±3.0
Gemcitabine 1250 mg/m^2	<10%	Carboplatin 350 mg/m^2	16.4%±3.7
-	-	Paclitaxel 175 mg/m^2	<10%

125, no recurrent pleural effusion was found. Supportive management of pleural effusion with thoracic Aspira catheter maintained KPS over 90 and ECOG 0 off therapy for 3 additional months (CA-125 01/11/2016 = 102.7; 02/11/2016 = 296.8; 03/08/2016 = 465.8; 04/08/2016 = 662.2 at baseline). Another fluid aspirate (pleural this time) was sent to the ChemoID lab for testing, which suggested resistance to cis-platinum and paclitaxel, but sensitivity to gemcitabine, ifosfamide and docetaxel (Table 2). Due to CKD the patient was treated with sixth-line Avastin 15mg/m² q3weeks instead, which was selected as no prior exposure, although it was untested by the ChemoID® assay. Patient progressed on sixth-line chemotherapy and expired under inpatient management under management for supportive care.

In our case study patient enjoyed 7 and one-half year survival after initial poor prognosis diagnosis with aggressive cytoreduction and persistent efforts at chemotherapy. Patient status was KPS 90 ECOG 0 or 1 throughout her disease except for 1 month in June 2015 prior to ChemoID® and in last 2 months of life. ChemoID® guided chemotherapy selection afforded 9-10 month fifth-line chemotherapy response with KPS 90 and ECOG 0-1 status with symptomatic control of effusions facilitated by use of standard drugs. Drugs selected with current chemotherapy-induced nausea and vomiting (CINV) agents available caused less than grade-1 nausea, no vomiting, and no recurrent peripheral neuropathy at dosages selected.

Discussion

Despite tremendous advances in surgery, primary chemotherapy, and novel treatments for recurrent disease, the diagnosis of advanced epithelial ovarian cancer in 2016 remains ultimately fatal in the majority of cases. The main goals of chemotherapy for recurrent/refractory ovarian cancer are the palliation of disease-related symptoms, and improvement of quality and quantity of life.[37] Unfortunately, the impact of palliative chemotherapy on survival, quality of life and cost in advanced ovarian cancer are largely still unknown as there have been no studies comparing palliative treatment with best supportive care. There is some contradicting evidence to suggest that chemotherapy has a role in palliation of symptoms with an apparent improvement in quality of life[37] as well as recent reports of lack of survival benefit of patients who are treated with empirically chosen chemother-

apy within 3 months from their end of life.[38] When ovarian cancer progresses, goals change from cure to prolongation of life with the best possible quality for the patient. Goals of palliative chemotherapy for cancer after fourth line are mainly directed at controlling symptoms, preventing secondary burdens of toxicities, providing ease of access, limiting impact on patients' quality of time, avoid hospitalizations as far as possible, reducing economic burden of testing, and permitting access to testing at point of service.

The introduction of platinum-based drugs and paclitaxel has been a landmark development in the treatment of ovarian cancer. However, there has been little progress in long-term survival improvement since the last 30 years.

Available reported options for fourth line chemotherapy or beyond are limited to the data provided by the Aurelia trial of low dose taxol and low dose avastin, or by administration of PARP inhibitors in case of BRCA ½ positive testing. Cells with defective BRCA proteins are deficient in the repair of double-stranded DNA breaks by homologous recombination (HR) and rely on other pathways to repair DNA damage, notably the PARP pathway that detects single DNA strand breaks and activates a number of effector proteins to initiate repair.

Administration of ineffective anticancer therapy is associated with unnecessary toxicity and the development of more aggressive cancer cell clones that are resistant to subsequent therapies.[7,8] Anticancer drugs have a high rate of failure and cell culture chemotherapy testing have been used to identify which drugs are more likely to be effective especially against those of gynecological origin.[10,11] However, this approach has been hindered by the chemotherapy testing only being performed on bulk of tumor cells derived from cancer biopsies.[12-21] It is known that cancer stem cells (CSCs) contribute to tumorigenesis, treatment resistance and tumor recurrence[22-29] in ovarian cancer.

ChemoID® is a functional test that uses patient's live tumor cells to indicate which chemotherapy or combination will kill not only the bulk tumor cells, but more importantly the cancer stem cells (CSCs) that are known to cause cancer to recur. This constitutes an important advantage of ChemoID® approach over other cell culture testing methods. Our recent clinical studies showed that patient derived CSCs from primary cancer cell cultures can be used in a drug response assay.[9,30-36] We have optimized the enrichment of CSCs from tumor biopsies and have developed the ChemoID® chemotherapy response assay, which meas-

ures the sensitivity of CSCs and bulk of tumor cells to chemotherapy to determine the most effective combination of anticancer drugs for solid tumors.[9,30-36]

Conclusions

The current case demonstrates the utility of ChemoID® guidance to commonly available drugs with identified toxicity profiles and predictable cost profile available at POS, along with rapid response in correcting symptomatic disease features, and minimal treatment burden from toxicities. We observed in the reported case the survival and symptom management benefits of ChemoID® guided therapy even after plateau of response (9-10 months similar to prior regimens after first line platinum response). Further studies are indicated to increase the clinical adoption of ChemoID® for gynecological malignancies in the palliative setting.

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