Medical Policy

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Title: In Vitro Chemoresistance and Chemosensitivity Assays

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DESCRIPTION

In vitro chemoresistance and chemosensitivity assays have been developed to provide information about the characteristics of an individual patient's malignancy to predict potential responsiveness of their cancer to specific drugs. Thus, these assays are sometimes used by oncologists to select treatment regimens for an individual patient. Several assays have been developed that differ with respect to processing of biological samples and detection methods. However, all involve similar principles and share protocol components including: 1) isolation of cells and establishment in an in vitro medium (sometimes in soft agar); 2) incubation of the cells with various drugs; 3) assessment of cell survival; and 4) interpretation of the result.

Background

A variety of chemosensitivity and chemoresistance assays have been clinically evaluated in human trials. All assays use characteristics of cell physiology to distinguish between viable and non-viable cells to quantify cell kill following exposure to a drug of interest. For the Oncotech Extreme Drug Resistance Assay (EDR®; Exiqon Diagnostics, Tustin, CA); and the ChemoFX® assay (Precision Therapeutics; Pittsburgh, PA) premarket approval from the U.S. Food and Drug Administration (FDA) is not required when the tests are performed in a laboratory licensed by the Clinical Laboratory Improvement Act (CLIA) for high-complexity testing.

With few exceptions, drug doses used in the assays are highly variable depending on tumor type and drug class. But all assays require drug exposures ranging from several-fold below physiological relevance to several-fold above physiological relevance.

Although a variety of assays exist to examine chemosensitivity or chemoresistance, only a few are commercially available and currently used in the clinic periodically.

The DiSC assay uses dye exclusion by live cells (1)

- The Differential Staining Cytotoxicity (DiSC) Assay involves mechanical disaggregation of cells from surgical or biopsy specimens by centrifugation. Cells are then established in culture and treated with the drugs of interest at 3 dose levels; the middle dose is that which could be achieved in therapy; 10-fold lower than the physiologically relevant dose; and, 10-fold higher. Exposure time ranges from 4-6 days; then, cells are restained with fast green dye and counterstained with hematoxylin and eosin (H&E). The fast green dye is taken up by dead cells, and H&E can differentiate tumor cells from normal cells. The intact cell membrane of a live cell precludes staining with the green dye. Drug sensitivity is measured by the ratio of live cells in the treated samples to the number of live cells in the untreated controls.

Ex-vivo analysis of programmed cell death (EVA/PCD™) assay

- The EVA/PCD™ assay (available from Rational Therapeutics) measures both apoptotic and non-apoptotic cell death markers in tumor samples exposed to chemotherapeutic agents. Tumor specimens obtained through biopsy or surgical resection are disaggregated using DNAse and collagenase IV to yield tumor clusters of the desired size (50-100 cell spheroids). Because these cells are not proliferated, these micro-aggregates are believed to more closely approximate the human tumor micro-environment. These cellular aggregates are treated with the dilutions of the chemotherapeutic drugs of interest and incubated for 3 days. After drug exposure is completed, a mixture of Nigrosin B & Fast Green dye with glutaraldehyde-fixed avian erythrocytes are added to the cellular suspensions. (2) The samples are then agitated and cytospin-centrifuged and, after air drying, are counter-stained with H&E. The endpoint of interest for this assay is cell death as assessed by observing the number of cells differentially stained due to changes in cellular membrane integrity. (3)

Several methods measure incorporation of radioactive precursors by macromolecules in viable cells.

- Tritiated thymine incorporation measures uptake of tritiated thymidine by DNA of viable cells. Using proteases and DNAse to disaggregate the tissue, samples are seeded into single cell suspension cultures on soft agar. They are then treated with the drug(s) of interest for 4 days. After 3 days, tritiated thymidine is added. After 24 hours of additional incubation, cells are lysed and radioactivity is quantified and compared to a blank control consisting of cells that were treated with sodium azide. Only cells that are viable and proliferating will take up the radioactive thymidine. Therefore, there is an inverse relationship between update of radioactivity and sensitivity of the cells to the agent(s) of interest. (4)
- The Extreme Drug Resistance assay (EDR®) (5) (commercially available at Exiqon Diagnostics, Tustin, CA) is methodologically similar to the thymidine incorporation assay, using metabolic incorporation of tritiated thymidine to measure cell viability; however, single cell suspensions are not required, so the assay is simpler to perform. Small tissue samples are incubated with the drug(s) of interest for 5 days at doses ranging from 5-fold below to 80-fold above concentrations that would reflect physiological relevance. Subsequently, tritiated thymidine is added to the culture and uptake is quantified after various incubation times. Only live (resistant) cells will incorporate the compound. Therefore, the level of tritiated thymidine incorporation is directly related to chemoresistance. The interpretation of the results is unique in that resistance to the drugs is evaluated as opposed to evaluation of responsiveness. Tumors are considered to be highly resistant when thymidine incorporation is at least 1 standard deviation (SD) above reference samples.
- The Histoculture Drug Resistance Assay (HDRA), available online at: http://www.anticancer.com/HDRA_ref.html, a commercially available by AntiCancer, Inc. (San Diego, CA) tests tissue fragments 1 to 2 millimeters in size. Samples are placed on a collagen matrix so they can grow 3 dimensionally and maintain signaling pathways mediated by cadherins and integrins, which control cell-cell and cell-matrix contact, respectively. After 24 hours, explants are incubated with drug for 3 days. Subsequently, they are fixed in formalin and embedded in paraffin. Radioactivity is quantified in slide sections using autoradiography.

Drug sensitivity is evaluated by quantification of cell growth in the 3-dimensional collagen matrix. There is an inverse relationship between the drug sensitivity of the tumor and cell growth. Concentrations of drug and incubation times are not standardized and vary depending on drug combination and tumor type.

- The Adenosine Triphosphate (ATP) Bioluminescence Assay relies on measurement of ATP to quantify the number of viable cells in a culture. Single cells or small aggregates are cultured, then exposed to drugs. Following incubation with drug the cells are lysed and the cytoplasmic components are solubilized under conditions that will not allow enzymatic metabolism of ATP. Luciferin and firefly luciferase are added to the cell lysis product. This catalyzes the conversion of ATP to adenosine di- and monophosphate and light is emitted proportionally to metabolic activity. This is

quantified with a luminometer. From the measurement of light, the number of cells can be calculated. A decrease in ATP indicates drug sensitivity, whereas no loss of ATP suggests that the tumor is resistant to the agent of interest.

- Precision Therapeutics (Pittsburgh, PA) commercially markets ChemoFX®, which uses this technology (available online at: http://www.chemofx.com/index.html. While the firefly luciferase and luciferin catalyze reduction of ATP and emitted light is quantified using a luminometer; cells must be grown in a monolayer rather than in a 3-dimensional matrix.

The rationale for chemosensitivity assays is strongest where there are a variety of therapeutic options and there are no clear selection criteria for any particular regimen in an individual patient.

POLICY

A. In vitro chemosensitivity assays, including, but not limited to, the histoculture drug response assay or a fluorescent cytoprint assay, are considered **experimental / investigational.**

B. In vitro chemoresistance assays, including, but not limited to, extreme drug resistance assays, are considered **experimental / investigational.**

RATIONALE

This policy is based on a 2002 Technology Evaluation Center (TEC) Assessment (6) and a 2004 *Journal of Clinical Oncology* systematic review, (7) supplemented by additional searches of MEDLINE and online site clinicaltrials.gov searches for the years 2004 through March 2013. Compiled data from the previous updates and the recent literature and clinical trial review suggest that evidence is insufficient to support use of chemosensitivity and chemoresistance assays for guiding choice of therapy regimen in cancer patients.

A variety of studies have reported a correlation between in vitro prediction or response and clinical response. While these studies may have internal validity, they cannot answer the question of whether patients given assay-guided therapy or empiric therapy have different outcomes. For example, if one group of patients is treated based on assay results and demonstrates an overall response rate of 75%, it is possible that a similar group of patients matched for important prognostic factors and given a uniform empiric chemotherapy regimen could achieve the same overall response rate. However, even if response rates are the same for both groups, the assay-guided group may experience more adverse effects from treatment or may have lower overall survival (OS). To determine whether assay-guided treatment results in overall different outcomes than empiric treatment, it is important to take into account response rates, survival, adverse effects, and quality of life. These effects may be assessed indirectly, for example, using decision analysis, or directly with comparative trials.

Both the 2002 BCBSA TEC Assessment and the 2004 systematic review (6, 7) recommend validating chemotherapy sensitivity and resistance assays with direct evidence gathered from prospective trials comparing patients treated empirically to patients treated with assay-directed therapy. In this way, not only can response rates and survival be taken into account, but also adverse events (e.g., from the toxic effects of an ineffective drug or delay or loss of benefits of an effective drug) and quality of life. Few such trials have been published, and none to date have provided sufficient evidence.

Chemoresistance Assays

Overview and Previous Evidence

The 2002 TEC Assessment identified one nonrandomized retrospective comparative study using the extreme drug resistance (EDR®) assay, published by Loizzi et al. in 2003. (8) While this study of patients with recurrent ovarian cancer found a significantly higher overall response rate, better progression-free survival (PFS), and higher OS among platinum-sensitive patients receiving assay-guided therapy, it was not designed to adequately address potential biases and confounding. Since the Loizzi et al. paper appeared, no additional comparative studies of assay-guided therapy versus physician-directed therapy have appeared for chemoresistance assays.

Chemoresistance assays are used to deselect potential chemotherapeutic regimens. The negative predictive value (NPV) is a key statistical measure. Unless the NPV is high, there is a chance that clinical decision making based on a chemoresistance assay could inappropriately exclude an effective therapy. The NPV will vary according to the prior probability of chemoresistance, as well as the assay's sensitivity and specificity. The TEC Assessment (6) concluded that chemoresistance assays have the highest clinical relevance in tumors with low probability of response. However, it is still unclear how this information will affect clinical decision making and whether health outcomes are improved as a result.

The EDR assay was specifically designed to produce a very high NPV (>99%), such that the possibility of inappropriately excluding effective chemotherapy is remote in all clinical situations. (9) However, clinical data are still inadequate to determine whether the use of EDR assays to deselect ineffective chemotherapies results in improved health outcomes. While the relevant clinical outcome in chemosensitivity assays focuses on improved survival, the relevant outcome associated with chemoresistance assays is more controversial. Advocates of the EDR assay point out that avoidance of the toxicity of ineffective drugs is the relevant outcome, while others point out that this represents an intermediate outcome and that improved patient survival is the relevant outcome for chemoresistance assays.

The bulk of the literature regarding EDR assays have focused on correlational studies that correlate results from predictive in vitro assays with observed outcomes of chemotherapy. However, in these studies, the patients do not receive assay-guided chemotherapy regimens. As discussed in the 2004 systematic review, (7) correlational studies are inadequate for several reasons. First, such studies often aggregate patients with different tumor types, disease characteristics, chemotherapy options, and probabilities of response. This process is problematic since the accuracy of each assay used to predict in vivo response probably varies across different malignancies and patient characteristics. Second, the method by which assay results are translated into treatment decisions is not standardized. Without knowing the rules for converting assay findings into treatment choices, it is impossible to determine the effects of assay-guided

treatment on health outcomes. Third, it is important to consider not only response but also survival, quality of life, and adverse effects. The overall value of assay-guided therapy depends on the net balance of all health outcomes observed after treatment for all patients subjected to testing, regardless of the assay results or the accuracy of its predication for response. Examples of some of the earlier published correlation studies of the EDR assay include those by Eltabbakh and colleagues, (10, 11) Mehta et al., (12) Holloway and co-workers, (13) and Ellis et al. (14) An updated literature search through 2013 did not identify prospective comparative studies focusing on the use of the EDR to guide therapy versus physician-directed therapy.

Comparative studies testing outcome with assay-directed therapy versus physician-chosen therapy
None identified.

Correlational Studies

Prospective
A study by Tiersten et al. (15) was designed to use the Oncotech EDR assay to examine whether chemotherapy resistance was an independent predictor of progression-free survival (PFS) in patients with ovarian cancer treated with neoadjuvant chemotherapy and surgical cytoreduction followed by intraperitoneal chemotherapy. Fifty-eight eligible women were prospectively enrolled in this study; however, results from the EDR assay were not used to direct therapy. Evaluable EDR assay results were available for 22 of the 58 patients. No difference in PFS was reported. Follow-up has not been sufficient to measure OS. These data do not provide support for use of the EDR assay in predicting outcome and guiding patient management.

A 2006 review published by Nagoury et al. included 21 non-comparative studies using ex-vivo programmed cell death assays. The authors of these studies correlated the drug susceptibility findings of the ex-vivo assay with objective clinical response (complete or partial) compared to non-responders for 659 total patients. The authors obtained aggregate positive values by site of primary cancer: breast (82.9%), colon (80%), non-small-cell lung cancer (66.7%), gynecologic (77%), and small-cell lung cancer (50%).(3). A 2012 study by this same investigator prospectively assessed 98 patients with non-small-cell lung cancer treated between 2003 and 2010.(2) Only 41 were found to be eligible for inclusion and were tested with the EVA/PCD™ assay to determine which chemotherapeutic drugs to use. A further 10 patients were excluded (5 due to insufficient cellular yield, 3 for resistance to all drugs tested, and 2 due to physician's choice) yielding only 31 patients who received the assay-recommended treatment. The authors compared the results of these 31 patients treated with assay-directed chemotherapy to historic controls (not described) on the outcome of observed objective response rate (complete response and partial response). The objective response rate for the study was 64.5% (95% confidence interval [CI]: 46.9-78.9%) which was significantly greater than the stated historic standard of 30% objective response ($p<0.0001$).

Retrospective
In 2010, Matsuo et al. published a study examining the relevance of EDR in epithelial ovarian carcinomas. (16) Two-hundred fifty-three records from the Oncotech database were identified for women with advanced stage ovarian cancer and from whom samples were collected at the time of the primary surgery. Tissue samples were cultured and tested for response to primary drugs (4 platinum- or taxane-based) and secondary drugs (e.g., gemcitabine, topotecan, doxorubicin,

etoposide, 5-fluorouracil (5-FU). Paclitaxel showed the highest resistance rate. Other agents had a resistance rate of less than 20%. There was only one (0.4%) tumor that showed complete resistance to all drugs tested, and 25% of tumors showed no resistance to any of the drugs. There was no statistical correlation between assay results and response to initial chemotherapy. The investigator acknowledges that the study, due to its retrospective and noncomparative design, is not sufficiently strong to validate use of this assay in managing therapy. Potential confounding factors, as described by the investigator, may have included tumor heterogeneity and the variations in resistance between primary tumor and metastases.

Another study by the same group (17) evaluated the role of the EDR assay to platinum- and taxane-based therapies for management of advanced epithelial ovarian, fallopian, and peritoneal cancers. From the Oncotech database, 173 cases were identified. For all cases, tissue was collected at the time of cytoreductive therapy. The EDR assay was performed on all samples, and tumors were classified as having low drug resistance (LDR), intermediate drug resistance (IDR), or extreme drug resistance (EDR). The 58 patients (33.5%) whose tumors had LDR to both platinum and taxane showed statistically improved PFS and OS compared to the 115 patients (66.5%) who demonstrated IDR or EDR to platinum and/or taxane (5-year OS rates, 41.1% vs. 30.9%, respectively; p=0.014). The 5-year OS rates for the 28 (16.2%) cases that had optimal cytoreduction with LDR to both platinum and taxane was significantly improved over the 62 (35.8%) cases that were suboptimally cytoreduced with IDR or EDR to platinum and/or taxane (54.1% vs. 20.4%, respectively; p<0.001). Although the EDR assay was predictive for survival, it is of interest that assay results did not indicate response to therapy with either taxane or cisplatin. The investigators conclude that the EDR assay may be an independent predictor of PFS and OS; however, a prospective, randomized trial would be required to further assess its clinical utility in predicting response to taxane or platinum therapies.

A smaller study by Matsuo et al. testing the EDR assay for prediction of uterine carcinosarcoma response to taxane and platinum was also conducted. (18) Of 51 cases, 31 (60.8%) received postoperative chemotherapy with at least a single agent; and 17 (33.3%) received combination chemotherapy with platinum and taxane modalities. Overall response rate for the 17 combination chemotherapy cases was 70.6%. Presence of EDR to either platinum or taxane showed a significantly lower PFS (1-year PFS rate, 28.6% vs. 100%, respectively; p=0.01) and lower OS (5-year OS rate, 26.9% vs. 57.1%, respectively; p=0.033). These data indicate that use of an in vitro drug resistance assay may be predictive of response to chemotherapy response and survival outcome in advanced ovarian and uterine carcinosarcoma. However, larger, prospective, randomized clinical trials (RCTs) would be required to validate use of this assay for directing chemotherapy regimens.

Matsuo and colleagues also completed a study examining the rates of EDR after cytoreductive therapy and neoadjuvant chemotherapy versus the rates of ERD after postoperative chemotherapy. (19) The goal of this study was not to test whether the EDR assay could direct therapeutic regimens. The findings suggested that platinum resistance was most common after neoadjuvant chemotherapy, while paclitaxel resistance was more prevalent after postoperative chemotherapy.

Karam and colleagues conducted a retrospective review of 377 patients with epithelial ovarian cancer to examine the effect of EDR assay-guided therapy on outcomes in the primary and recurrent setting. (20) The primary endpoints were time to progression (TTP), OS, and survival

after recurrence (RS). The patient population was heterogeneous, with a median age of 59 years (median 24-89), tumor completely resected in 30% of patients, and varying tumor stages (Federation of Gynecologists and Obstetricians [FIGO] stages I, II, III, and IV in 7%, 4%, 78%, and 11%, respectively). Sixty-four percent of patients underwent a secondary cytoreductive surgery. Patients had an EDR assay sent either at the time of their primary cytoreductive surgery (n=217) or at the time of disease recurrence (n=160). Predictors of survival included increasing age and greater volume of residual disease after cytoreductive surgery. EDR assay results analyzed for single agents or combinations of chemotherapies failed to independently predict patient outcomes regardless of whether the assay was performed at the time of the primary surgery or at recurrence.

Hetland et al. conducted a study to identify primary platinum resistance in epithelial ovarian cancer patients with FIGO stage III-IV disease. (21) Eighty-five biopsies from 58 patients were included in the study. Resistance was assessed with a modified drug-response assay including ATP-based tumor-chemosensitivity and EDR assay. Samples were tested for response to platinum, paclitaxel and the combination of the drugs. Results from the assay were combined, and tumors were classified using a resistance index, which summarized the percentage of tumor growth inhibition for each drug concentration tested. All patients received a primary chemotherapy treatment of carboplatin, paclitaxel or a combination of the two drugs. Platinum resistance, as defined by the risk index, was associated with significantly poorer PFS (p=0.03) with a median value of 3.9 months (95% CI: 3.2-4.7) compared with the platinum sensitive group with a median PFS of 8.1 months (95% CI: 3.7-12.4). Patients who had partial response, stable disease or progressive disease were more resistant to platinum based on risk index score than those with a complete response (p=0.02). In a sub-group analysis of metastatic tumors, platinum resistance was not associated with PFS or clinical response. Response to paclitaxel or carboplatin/paclitaxel was not associated with PFS or clinical response. In vitro response was not associated with overall survival in any group.

In summary, studies do not support use of the EDR assay for directing therapy or for prediction of outcome. Weaknesses in the studies have included retrospective design, noncomparative design, and small sample size. Furthermore, tissue samples are often not sufficient to achieve evaluable results. Large, randomized, prospective clinical studies would be required to justify use of the EDR assay in these patient populations. The studies would have to compare outcomes between assay-directed therapy versus physician-directed therapy. Initial response to assay-directed therapy and TTP would be interesting endpoints; however, evaluation of overall and disease-specific survival, quality of life, and adverse events would be critical to validate the clinical utility of this assay.

Chemosensitivity Assays

Overview and Previous Evidence

As for the chemoresistance assays, the critical type of evidence needed to establish the effectiveness of chemosensitivity assays would come from comparative studies of assay-guided therapy versus physician-directed therapy. Relevant outcomes would include OS and disease-specific survival, as well as quality of life and adverse events.

Since the 1990s, enthusiasm for chemosensitivity assays, in general, has diminished due to the poor positive predictive values (PPVs), which indicate the likelihood that drugs shown to be

effective in vitro may not produce a positive clinical response. (22-24) A meta-analysis of 54 different retrospective correlational studies by Von Hoff et al. reported a PPV of only 69%. (25) The poor result may, in part, have been related to a variety of host factors, such as tumor vascularity, poor quality of data, or tumor sampling bias.

The 2002 TEC Assessment (6) and 2004 systematic review (7) identified 9 comparative studies, 2 of which were randomized. (22-24, 26-31) One randomized trial is irrelevant because it used an in vivo rather than an in vitro assay. (32) One randomized crossover trial published by Von Hoff et al. in 1990 (24) addressed patients with diverse types of advanced cancer. These authors reported that significant advantages for assay-guided therapy in terms of tumor response did not translate into survival differences. Response rate differences seen in other nonrandomized comparative studies may be attributable to bias or confounding, and survival outcomes were rarely reported.

Comparative studies testing outcome with assay-directed therapy versus physician-chosen therapy

In a case-control study, Moon and colleagues retrospectively compared adenosine triphosphate (ATP) assay-based guided chemotherapy with empirical chemotherapy in unresectable non-small-cell lung cancer. (33) All of the patients who received ATP-assay-guided platinum-based doublet chemotherapy as first-line therapy received platinum-based chemotherapy combined with a nonplatinum drug, regardless of their in vitro platinum sensitivity; 14 patients had platinum-sensitive disease and 13 were platinum-resistant. Ninety-three matched controls (matched for performance status, stage, and chemotherapy regimen) were selected from a retrospective review of a database. In the empirical group, a nonplatinum drug was chosen, depending on physicians' discretion, along with a platinum agent determined by renal function and performance status. The primary endpoint was clinical response rate, assessed every 2 cycles of chemotherapy by the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. The secondary endpoints were PFS and OS. The response rate and survival in both groups were not statistically different. The platinum-sensitive subgroup by ATP assay showed a higher response rate than the empirical group (71% vs. 38%, respectively; p=0.02), but there was no statistical significance between PFS or OS.

In a small nonrandomized comparative study (n=64), Iwahashi and colleagues (34) reported on outcomes of chemosensitivity-guided chemotherapy (CSC) compared to standard chemotherapy and no chemotherapy in patients with advanced gastric cancer. In some subsets, survival was improved in the CSC subgroup. However, given the small sample, additional studies are needed to confirm these findings and to extend them to other malignancies.

Cree and colleagues (35) reported on a prospective, randomized trial of chemosensitivity assay-directed chemotherapy versus physician's choice in patients with recurrent platinum-resistant ovarian cancer. The primary aim of this randomized trial was to determine response rate and PFS following chemotherapy in patients who had been treated according to an ATP-based tumor chemosensitivity assay in comparison with the physician's choice. A total of 180 patients were randomized to assay-directed therapy (n=94) or physician-choice chemotherapy (n=86). Median follow-up at analysis was 18 months; response was assessable in 147 (82%) patients: 31.5% achieved a partial or complete response in the physician-choice group compared with 40.5% in the assay-directed group (26% vs. 31% by intention-to-treat [ITT] analysis, respectively). Intention-to-treat analysis showed a median PFS of 93 days in the physician's-choice group and

104 days in the assay-directed group (hazard ratio 0.8, not significant). No difference was seen in OS between the groups, although 12 of 39 patients (41%) who crossed over from the physician's-choice arm obtained a response. Increased use of combination therapy was seen in the physician's-choice arm during the study as a result of the observed effects of assay-directed therapy in patients. The authors concluded that this small RCT documented a trend toward improved response and PFS for assay-directed treatment and that chemosensitivity testing might provide useful information in some patients with ovarian cancer. They also noted that the ATP-based tumor chemosensitivity assay remains an investigational method in this condition.

Correlational studies

Prospective

Kim et al. reported the results of a prospective, multicenter clinical trial designed to define the accuracy of the ATP-based chemotherapy response assay in gastric cancer patients receiving paclitaxel and cisplatin chemotherapy, by comparing clinical response and the ATP-assay results. (36) The primary endpoint of the study was to assess accuracy of the ATP-assay results, and the secondary endpoint was to find the best method of defining in vitro chemosensitivity. Forty-eight patients with chemotherapy-naïve locally advanced or metastatic gastric cancer were treated with combination chemotherapy after a tissue specimen was obtained for the ATP assay. Tumor response was assessed by World Health Organization (WHO) criteria using a computed tomography (CT) scan after every 2 cycles of chemotherapy. Both laboratory technicians and physicians were blinded to the assay or clinical results. Thirty-six patients were evaluable for both in vitro and in vivo responses. Using a chemosensitivity index method, the specificity of the ATP assay was 95.7% (95% CI]: 77.2-99.9%), sensitivity 46.2% (95% CI: 19.2-74.9%), PPV 85.7% (95% CI: 42.1-99.6%) and NPV was 75.9% (95% CI: 55.1-89.3%). Median PFS was 4.2 months (95% CI: 3.4-5.0) and median OS was 11.8 months (95% CI: 9.7-13.8). The in vitro chemosensitive group showed a higher response rate (85.7% vs. 24.1%, respectively; p=0.005) compared to the chemoresistant group. The authors concluded that the ATP assay could predict clinical response to paclitaxel and cisplatin chemotherapy with high accuracy in advanced gastric cancer and that the study supported the use of the ATP assay in further validation studies.

In a European study, Ugurel and colleagues reported on a nonrandomized, prospective, Phase II study of 53 evaluable patients with metastatic melanoma. (37) All 53 received assay-directed therapy. This study found a 36% response rate in patients with chemosensitive tumors compared with 16% in those with chemoresistant tumors. Based on these preliminary results, a Phase III study is to follow. While these studies begin to provide needed information about the impact of these assays on clinical outcomes, the data are still limited.

Retrospective

Gallion et al. conducted a retrospective study (38) that evaluated the association of ChemoFX® test results with the treatment response of 256 patients with ovarian or peritoneal cancer who had been treated with at least one cycle of postsurgical chemotherapy. A subset of 135 patients had an exact match between drugs assayed and received; the rest had only a partial match. Predictive values were not reported nor were they calculable. For the subset of 135, in a multivariable analysis, ChemoFX® was an independent significant predictor (p=0.006) of PFS along with 2 other clinical variables. Hazard ratio (HR) for resistant versus sensitive was 2.9 (95% CI: 1.4–6.30) and was 1.7 (95% CI: 1.2–2.5) for resistant versus intermediate. The

median progression-free interval was 9 months for the resistant group, 14 months for the intermediate group, and had not been achieved for the sensitive group.

Herzog et al. included 147 patients from the above study by Gallion et al. (38) and reported on a total of 192 women with advanced-stage primary ovarian cancer, 175 of whom had tumors that were tested for in vitro chemosensitivity to platinum therapy using ChemoFX. (39) Tumors were classified as responsive, intermediately responsive, or nonresponsive to chemotherapy. Seventy-eight percent were categorized as responsive or intermediately responsive, and 22% were nonresponsive. Median OS was 72.5 months for patients with tumors categorized as responsive, 48.6 months for intermediately responsive, and 28.2 months for nonresponsive ($p=0.03$; HR: 0.70; 95% CI: 0.50-0.97). The authors concluded that the result of chemosensitivity testing with a drug response marker for therapy was predictive of OS in patients with primary ovarian cancer.

Lee et al. conducted a retrospective study of the histoculture drug response assays (HDRA) assay in 79 patients with ovarian cancer. (40) Tissue samples were assessed for 11 chemotherapeutic agents and found the highest inhibition rates in carboplatin (49.2%), topotecan (44.7%), and belotecan (39.7%). These inhibition rates were higher than in cisplatin (34.7%), the traditional drug used to treat epithelial ovarian cancer. A subset of 37 patients with FIGO stage II/IV stage III or IV epithelial ovarian serous adenocarcinoma who had been treated with at least 3 cycles of carboplatin chemotherapy was assessed to compare outcomes between carboplatin-sensitive and -resistant patients. Multiple comparison and regression analyses established a cut-off value of 40% inhibition rate in response to 50 ug/mL carboplatin to determine sensitivity or resistance. This selected cut-off had a disease-free survival of 23.2 months (95% CI: 6.3-55.3) and 13.8 months (95% CI: 4.9-35.6) in the carboplatin-sensitive and carboplatin- resistant groups respectively ($p<0.05$). Overall survival between the 2 groups did not differ significantly, with carboplatin-sensitive patients having a mean 60.4 months and carboplatin-resistant patients having 37.3 months ($p=0.621$).

ChemoFX® assay is commercially available for breast and ovarian cancer treatment. The ChemoFX® website lists ongoing clinical trials of ChemoFX® for both breast cancer and ovarian cancer. However, none of the ongoing studies would satisfy the criteria to assess validity and clinical utility with a prospective comparative design (available online at: http://www.chemofx.com/cancer-treatment/drug-trials.html).

Summary

Through March 2013, there have been no studies published with a randomized, prospective, design to evaluate this testing. Therefore to date, the clinical utility of chemoresistance and chemosensitivity assays has not been determined, and data are insufficient to determine whether use of the test to select chemotherapy regimens for individual patients will improve outcomes. Most studies have been relatively small correlational designs that evaluated the association between assay results and already known patient outcomes; and most acknowledge that larger studies are needed. Furthermore, unexpected limitations have arisen including sampling bias due to heterogeneity of tumors and insufficient biospecimen processing resulting in unevaluable data. Therefore, this testing is considered investigational.

Practice Guidelines and Position Statements

National Comprehensive Cancer Network (NCCN) Guidelines
The 2012 NCCN guidelines for the treatment of epithelial ovarian cancer, fallopian tube cancer, and primary peritoneal cancer (v 1.2013) state that chemotherapy/resistance assays are used in some NCCN centers to help select chemotherapy when multiple equivalent chemotherapy options are available; the current level of evidence (category 3) is not sufficient to supplant standard-of-care chemotherapy. The panel believes that in vitro chemosensitivity testing to help choose a chemotherapy regimen for recurrent disease situations should not be recommended because of the lack of demonstrable efficacy for this approach.

The American Society of Clinical Oncology (ASCO) also does not recommend use of chemotherapy sensitivity and resistance assays, unless in a clinical trial setting.(41)

2013 National Cancer Institute (NCI) Clinical Trials Database (PDQ®) and online Clinicaltrials.gov
A Phase III trial was identified for chemosensitivity testing to assign treatment for patients with stage III or IV ovarian cancer. Patients are stratified according to tumor size after debulking surgery and stage. Within 14 days after undergoing debulking surgery, patients will be randomized to 1 of 2 treatment arms. Arm 1 patients will receive 1 of 6 treatment regimens. Arm 2 patients are assigned a treatment regimen based on a chemosensitive assay of tumor specimens collected after debulking surgery. Approximately 300 patients will be accrued over 6 years (study start date July 1996). The study status has been verified as completed in May 2012, but no results have been reported. (NCT00003214).

A Phase III trial investigating whether individual chemosensitivity-direct chemotherapy, as assessed by ATP-based chemosensitivity assay, is superior to the standard dacarbazine therapy for surgically unresectable metastatic melanoma (NCT00779714). All patients are being treated for the first time for metastatic disease, but may have received chemotherapy prior to metastatic growth. The estimated enrollment is 360 patients and a completion date of April 2013.

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

CPT/HCPCS

87230	Toxin or antitoxin assay, tissue culture (eg, Clostridium difficile toxin)
88104	Cytopathology, fluids, washings or brushings, except cervical or vaginal; smears with interpretation
88305	Level IV - Surgical pathology, gross and microscopic examination
88313	Special stain including interpretation and report; Group II, all other (eg, iron, trichrome), except stain for microorganisms, stains for enzyme constituents, or immunocytochemistry and immunohistochemistry
88358	Morphometric analysis; tumor (eg, DNA ploidy)

89050 Cell count, miscellaneous body fluids (eg, cerebrospinal fluid, joint fluid), except blood
89240 Unlisted miscellaneous pathology test

- The extreme drug resistance assay is a multistep laboratory procedure that might be identified by the above CPT codes.

DIAGNOSIS
Experimental / investigational for all diagnoses related to this policy.

REVISIONS

09-20-2011	Policy added to the bcbsks.com web site.
06-29-2012	Description section updated.
	In Coding section: Coding nomenclature updated
	Rationale section added.
	References section updated.
09-25-2013	Description section updated
	Rationale section updated
	References updated

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