Kansas City

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In Vitro Chemoresistance and Chemosensitivity Assays

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Policy

Blue Cross and Blue Shield of Kansas City (Blue KC) will not provide coverage for In Vitro Chemoresistance and Chemosensitivity Assays. This is considered investigational.

When Policy Topic is covered

Not Applicable.

When Policy Topic is not covered

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

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

Description of Procedure or Service

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 they 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.

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. 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. Available assays are outlined as follows:

Methods using differential staining/dye exclusion:

- The Differential Staining Cytotoxicity assay.(1) This assay relies on dye exclusion of live cells after 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 to

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 then 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.

- The EVA/PCD™ assay (available from Rational Therapeutics). This assay relies on ex vivo analysis of programmed cell death, as measured by differential staining of cells after apoptotic and nonapoptotic 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 microaggregates are believed to more closely approximate the human tumor microenvironment. 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 is added to the cellular suspensions.(2) The samples are then agitated and cytospin-centrifuged and, after air drying, are counterstained with H&E. The end point 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)

<u>Methods using incorporation of radioactive precursors by macromolecules in viable cells:</u>

- 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 with 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 physiologic 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 above reference samples.

<u>Methods to quantify cell viability by colorimetric assay:</u>

- The Histoculture Drug Resistance Assay (HDRA; AntiCancer Inc., San Diego, CA).(6) This assay evaluates cell growth after chemotherapy treatment based on a colorimetric assay that relies on mitochondrial dehydrogenases in living cells. 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.

Methods using incorporation of chemoluminescent precursors by macromolecules in viable cells:

- The Adenosine Triphosphate (ATP) Bioluminescence assay. This assay relies on measurement of ATP to quantify the number of viable cells in a culture. Single cells or small aggregates are cultured, and 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.
- ChemoFX® (Precision Therapeutics, Pittsburgh, PA).(7) This assay also relies on quantifying ATP based on chemoluminescence. Cells must be grown in a monolayer rather than in a 3-dimensional matrix.

Methods using differential optical density:

- Microculture Kinetic (MiCK) assay (Diatech Oncology, Franklin, TN).(8) Similar to the EVA/PCD assay, this assay relies on measures of programmed cell death. In the assay, tumor cells are exposed to multiple concentrations of drugs and cultured. The optical density of the cells is measured over time, to create a density-by-time curve. A sudden increase in optical density is associated with cell apoptosis; the extent of drug-induced apoptosis is a measure of the cell's sensitivity to that agent.

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

Regulatory Status

Commercially available chemosensitivity and chemoresistance assays are laboratory developed tests for which approval from the U.S. Food and Drug Administration is not required when the tests are performed in a laboratory licensed by the Clinical Laboratory Improvement Act (CLIA) for high-complexity testing. Such tests must meet the general regulatory standards of CLIA.

Rationale

This policy was originally based on a 2002 Technology Evaluation Center (TEC) Assessment(9) and a 2004 *Journal of Clinical Oncology* systematic review,(10) which concluded that evidence is insufficient to support use of chemosensitivity and chemoresistance assays for guiding choice of therapy regimen in cancer patients.

The policy has been updated with periodic literature reviews, most recently through March 6, 2014.

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. 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(9,10) recommend validating chemotherapy sensitivity and resistance assays with direct evidence gathered from prospective trials comparing patients treated empirically with patients treated with assay-directed therapy. In this way, not only can response rates and survival be taken into account, but also adverse events (eg, from the toxic effects of an ineffective drug or delay or loss of benefits of an effective drug) and quality of life.

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 2002 TEC Assessment(9) concluded that chemoresistance assays have the highest clinical relevance in tumors with low probability of response.

The extreme drug resistance (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.(11)

To determine whether chemoresistance assays have value in clinical decision making, studies comparing outcomes for patients managed with chemoresistance assays with those managed with routine care would be ideal. Potential relevant clinical outcomes include improved survival and avoidance of toxicity (as an intermediate outcome).

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, patients do not receive assay-guided chemotherapy regimens. Correlational studies are limited for several reasons. As discussed in the 2004 systematic review,(10) 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 because 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 et al,(12,13) Mehta et al,(14) Holloway et al,(15) and Ellis et al.(16)

The 2002 TEC Assessment identified 1 nonrandomized retrospective comparative study using the EDR® assay, by Loizzi et al in 2003.(17) While this study of patients with recurrent ovarian cancer found a significantly higher overall response rate, better progression-free survival (PFS), and higher overall survival (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, no additional comparative studies of assay-guided therapy versus physician-directed therapy have appeared for chemoresistance assays.

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

None identified.

Correlational studies

Prospective. A study by Tiersten et al(18) was designed to use the Oncotech EDR assay to examine whether chemotherapy resistance was an independent predictor of 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 noncomparative 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 with nonresponders 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% to 78.9%) which was significantly greater than the stated historic standard of 30% objective response ($p<0.001$).

Retrospective. In 2010, Matsuo et al published a study examining the relevance of EDR in epithelial ovarian carcinomas.(19) 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 (eg, gemcitabine, topotecan, doxorubicin, etoposide, 5-fluorouracil. Paclitaxel showed the highest resistance rate. Other agents had a resistance rate of less than 20%. There was only 1 (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(20) 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 EDR. The 58 patients (33.5%) whose tumors had LDR to both platinum and taxane showed statistically improved PFS and OS compared with 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.(21) 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 would be required to validate use of this assay for directing chemotherapy regimens.

Matsuo et al also completed a study examining the rates of EDR after cytoreductive therapy and neoadjuvant chemotherapy versus the rates of ERD after postoperative chemotherapy.(22) 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 et al 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.(23) The primary end points were time to progression (TTP), OS, and survival after recurrence. 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.(24) 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 2 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 to 4.7) compared with the platinum sensitive group with a median PFS of 8.1 months (95% CI, 3.7 to 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 subgroup 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 OS in any group.

Section 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 end points; however, evaluation of OS and disease-specific survival, quality of life, and adverse events would be critical to validate the clinical utility of this assay.

Chemosensitivity Assays

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.(25-27) A meta-analysis of 54 different retrospective correlational studies by Von Hoff et al reported a PPV of only 69%.(28) 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(9) and 2004 systematic review(10) identified 9 comparative studies, 2 of which were randomized.(25-27,29-34) 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 et al retrospectively compared adenosine triphosphate (ATP) assay-based guided chemotherapy with empirical chemotherapy in unresectable non-small-cell lung cancer.(35) 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 end point was clinical response rate, assessed every 2 cycles of chemotherapy by the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. The secondary end points 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 et al(36) reported on outcomes of chemosensitivity-guided chemotherapy compared with standard chemotherapy and no chemotherapy in patients with advanced gastric cancer. In some subsets, survival was improved in the chemosensitivity-guided chemotherapy subgroup. However, given the small sample, additional studies are needed to confirm these findings and to extend them to other malignancies.

Cree et al(37) 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). ITT analysis showed a median PFS of 93 days in the physician-choice group and 104 days in the assay-directed group (hazard ratio [HR], 0.8, *NS*). No difference was seen in OS between the groups, although 12 of 39 patients (41%) who crossed over from the physician-choice arm obtained a response. Increased use of combination therapy was seen in the physician-choice arm during the study as a result of the observed effects of assay-directed therapy in patients. The authors concluded that this small randomized controlled trial 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.(38) The primary end point of the study was to assess accuracy of the ATP-assay results, and the secondary end point 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 criteria using a computed tomography 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% to 99.9%), sensitivity 46.2% (95% CI, 19.2% to 74.9%), PPV 85.7% (95% CI, 42.1% to 99.6%), and NPV was 75.9% (95% CI, 55.1% to 89.3%). Median PFS was 4.2 months (95% CI, 3.4 to 5.0) and median OS was 11.8 months (95% CI, 9.7 to 13.8). The in vitro chemosensitive group showed a higher response rate (85.7% vs 24.1%, respectively; p=0.005) compared with 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 et al reported on a nonrandomized, prospective, phase 2 study of 53 evaluable patients with metastatic melanoma.(39) 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 3 study is to follow.

Rutherford et al reported results from a prospective, noninterventional, multicenter cohort study that was designed to assess whether the ChemoFX assay was predictive of outcomes among women with histologically confirmed epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer.(40) Three hundred thirty-five patients were enrolled and treated with 1 of 15 study protocols, with treating physicians blinded to the ChemoFX assay result. Two hundred sixty-two patients (78.2% of total) had both available clinical follow up data and a ChemoFX result. Cancer cells were classified based on the ChemoFX result as sensitive, intermediate, or resistant to each of several chemotherapeutic agents. Patients treated with an assay-sensitive regimen had a PFS of median 8.8 months, compared with 5.9 months for those with assay-intermediate or -resistant regimens (HR=0.67, p=0.009). Mean overall survival was 37.5 months for patients treated with an assay-sensitive regimen, compared with 23.9 months for those with assay-intermediate or -resistant regimens (HR=0.67, p=0.010). Strengths of this study include its prospective design with physicians blinded to the assay results, which reduces the risk of bias in patient selection or measurement of outcomes. However, because the selection of chemotherapeutic agent was, by design, not influenced by the ChemoFX assay, the impact on health outcomes cannot be determined.

In a similar study design, Salom et al conducted a prospective, noninterventional, multicenter cohort study to assess whether the Microculture Kinetic (MiCK) assay was predictive of outcomes among women with epithelial ovarian cancer.(41) Data from 150 women with any stage of cancer with specimens suitable for MiCK assay were included. Chemosensitivity was expressed as kinetic units following each dose of drug in the MiCK assay and reported as mean, minimum, and maximum. For each patient, the "best" chemotherapy was defined as any single drug or combination of drugs in the patient's MiCK assay that had the highest kinetic units. Patients' regimens were at the discretion of their treating physicians, who were blinded to the MiCK assay results. OS stage III or IV disease was longer if patients received a chemotherapy that was considered "best" by the MiCK assay, compared with shorter survival in patients who received a chemotherapy that was not the best. (HR=0.23, p<0.01).

Jung et al conducted a single-center prospective study to determine whether sensitivity to paclitaxel and carboplatin, determined by using the Histoculture Drug Resistance Assay (HDRA), was predictive of outcomes among women with advanced epithelial ovarian cancer.(42) The study included 104 patients with epithelial ovarian cancer, all of whom had undergone initial surgery and were treated with paclitaxel and carboplatin therapy. Tumor cells' sensitivity to the chemotherapy agents was classified as sensitive, intermediate, or resistant to paclitaxel, carboplatin, or both, based on the HDRA. Patients whose tumors were sensitive to both drugs had a lower recurrence rate than those who had resistance to both drugs (29.2% vs 69.8%, p=0.02) and had a longer PFS (35 months vs 16 months, p=0.025).

While these studies establish that the results of chemosensitivity assays are correlated with outcome, they do not evaluate how the test may alter clinical decision making and whether changes in management based on the test improve outcomes.

Retrospective. Gallion et al conducted a retrospective study(43) 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 1 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. HR for resistant versus sensitive was 2.9 (95% CI, 1.4 to 6.30) and was 1.7 (95% CI, 1.2 to 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 study by Gallion et al(43) 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.(44) 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 to 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.

In a smaller study, Grigsby et al conducted a retrospective analysis to assess the association of pretreatment chemosensitivity to cisplatin with clinical outcomes among 33 women with cervical cancer.(45) Tumor cell sensitivity to cisplatin was categorized as responsive, intermediately responsive, or nonresponsive with the ChemoFX assay. Patients with responsive or intermediately responsive tumors had a 2-year recurrence free survival of 87%, compared with 58% for those with nonresponsive tumors (p=0.036).

Lee et al. conducted a retrospective study of the HDRA in 79 patients with ovarian cancer.(46) 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 cutoff value of 40% inhibition rate in response to 50 µg/mL carboplatin to determine sensitivity or resistance. This selected cutoff had a disease-free survival of 23.2 months (95% CI, 6.3 to 55.3) and 13.8 months (95% CI, 4.9 to 35.6) in the carboplatin-sensitive and carboplatin-resistant groups respectively ($p<0.05$). OS 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 ($p=0.621$).

Strickland et al conducted a retrospective evaluation of the association between chemosensitivity to anthracyclines, measured by the drug-induced apoptosis MiCK assay, among 109 patients with adult-onset acute myelogenous leukemia.(47) Patients were treated with a “7 plus 3” chemotherapy regimen. Chemosensitivity was expressed as maximal kinetic units following each dose of drug in the MiCK assay. Receiver-operator characteristic curve analysis and logistic regression were used to determine the optimal cutoff for chemosensitivity response to discriminate between chemoresponder and nonresponder. Patients determined to be chemoresponders to idarubicin were more likely to have complete response to chemotherapy (72%) than those who were nonresponders ($p=0.01$). Data for the patient cohort were collected over a 14 year period from 1996-2010, which may limit the generalizability of the results to currently-used chemotherapy regimens. In addition, the MiCK assay is limited by lack of standardized cutoffs to discriminate responders from nonresponders.

Ongoing and Unpublished Clinical Trials

A search of website ClinicalTrials.gov in March 2014 identified no ongoing comparative studies evaluating the use of in vitro chemosensitivity/chemoresistance assays.

At least 2 phase 3 trials were identified previously from searches of the National Cancer Institute Clinical Trials Database (PDQ®) and online ClinicalTrials.gov. The current status of these trials is uncertain:

A phase 3 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 3 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.

Summary

There are only a few comparative studies that evaluate use of a chemosensitivity assay to select chemotherapy versus standard care, and these studies do not report significant differences in outcomes between groups. A larger number of studies have used correlational designs that evaluate the association between assay results and already known patient outcomes. These studies report that results of chemosensitivity and chemoresistance assays are predictive of outcomes. However, these studies do not evaluate whether these assays lead changes in management and whether any changes in management lead to improved outcomes. In addition, interpretation of these studies is limited by heterogeneity in test methodology, tumor type, patient population, and chemotherapeutic agents. As a

result, 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. Therefore, this testing is considered investigational.

Practice Guidelines and Position Statement

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 1.2014) 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.(48)

The American Society of Clinical Oncology Clinical Practice Guideline Update on the Use of Chemotherapy Sensitivity and Resistance Assays, 2011 also does not recommend use of chemotherapy sensitivity and resistance assays, unless in a clinical trial setting.(49) .

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Billing Coding/Physician Documentation Information

88358 Morphometric analysis; tumor (eg, DNA ploidy)
88305 Level IV Surgical pathology, gross and microscopic examination Abortion
88104 Cytopathology, fluids, washings or brushings, except cervical or vaginal; smears with interpretation
87230 Toxin or antitoxin assay, tissue culture (eg, Clostridium difficile toxin)
88313 Special stains (List separately in addition to code for primary service); Group II, all other, (eg, iron, trichrome), except immunocytochemistry and immunoperoxidase stains, each
89050 Cell count, miscellaneous body fluids (eg, cerebrospinal fluid, joint fluid), except blood;

The extreme drug resistance assay is a multistep laboratory procedure identified by the CPT codes listed above.

Additional Policy Key Words

N/A

Policy Implementation/Update Information

10/1/88 New policy.
10/1/00 No policy statement changes.

10/1/01	No policy statement changes.
10/1/02	No policy statement changes.
10/1/03	No policy statement changes.
10/1/04	No policy statement changes.
10/1/05	No policy statement changes.
10/1/06	No policy statement changes.
10/1/07	No policy statement changes.
10/1/08	No policy statement changes.
10/1/09	No policy statement changes.
10/1/10	No policy statement changes.
10/1/11	No policy statement changes.
10/1/12	No policy statement changes.
10/1/13	No policy statement changes.
10/1/14	No policy statement changes.
