# Medical Policy

## 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. 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 nonviable 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
Differential Staining Cytotoxicity Assay
The Differential Staining Cytotoxicity 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 (relevant) dose which could be achieved in therapy; a 10-fold lower dose than the physiologically relevant dose; and a 10-fold higher dose. 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 differentiates 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.

EVA/PCD Assay
The EVA/PCD assay (Rational Therapeutics, Long Beach, CA) 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 and fast
green dye with glutaraldehyde-fixed avian erythrocytes are added to the cellular suspensions. The samples are then agitated and cytopin-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.

**Fluorometric Microculture Cytotoxicity Assay**
The fluorometric microculture cytotoxicity assay is another cell viability assay that relies on the measurement of fluorescence generated from cellular hydrolysis of fluorescein diacetate to fluorescein in viable cells. Cells from tumor specimens are incubated with cytotoxic drugs; drug resistance is associated with higher levels of fluorescence.

**Methods Using Incorporation of Radioactive Precursors by Macromolecules in Viable Cells**

**Tritiated Thymine**
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 uptake of radioactivity and sensitivity of the cells to the agent(s) of interest.

**Extreme Drug Resistance Assay**
The Extreme Drug Resistance assay (EDR, Exiqon Diagnostics, Tustin, CA; no longer commercially available) 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. Tritiated thymidine is added to the cultures of tumor cells, 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.

**Methods That Quantify Cell Viability Using Colorimetric Assay**

**Histoculture Drug Resistance Assay**
The Histoculture Drug Resistance Assay (HDRA; AntiCancer, Inc. San Diego, CA) 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
relation 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

Adenosine Triphosphate Bioluminescence Assay
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 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 Assay
The ChemoFX (Helomics Corp., previously called Precision Therapeutics, Pittsburgh, PA) assay also relies on quantifying ATP based on chemoluminescence. Cells must be grown in a monolayer rather than in a 3-dimensional matrix.

Methods That Use Differential Optical Density

CorrectChemo Assay
Similar to the EVA/PCD assay, the CorrectChemo (previously called the Microculture Kinetic [MiCK]) assay; DiaTech Oncology, Franklin, TN) 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. As of March 2016, DiaTech no longer offers the CorrectChemo assay commercially.

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
Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Chemosensitivity and chemosensitivity assays discussed in this review are available under the auspices of...
CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

**POLICY**

A. In vitro chemosensitivity assays, including, but not limited to, the Histoculture Drug Response Assay, a fluorescent cytoprint assay, the ChemoFX assay, or the CorrectChemo 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 evidence review was originally-based on a 2002 TEC Assessment\(^{10}\) and a 2004 systematic review,\(^{11}\) which concluded collectively that evidence is insufficient to support use of chemosensitivity and chemoresistance assays for guiding choice of therapy regimen in cancer patients.

This evidence review has been updated with periodic literature reviews of the MEDLINE database, most recently through June 14, 2016.

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 whether patients given assay-guided therapy or empiric therapy have different outcomes. To determine whether assay-guided treatment results in overall different outcomes than empirical treatment, it is important to take into account response rates, survival, adverse effects, and quality of life (QOL). These effects may be assessed indirectly (eg, using decision analysis) or directly with comparative trials. Both the 2002 TEC Assessment and the 2004 systematic review\(^{10,11}\) recommended 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 QOL.

**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\(^{10}\) 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.\(^{12}\)
To determine whether chemoresistance assays have value in clinical decision making, studies comparing outcomes for patients managed with chemoresistance assays to 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 on EDR assays have focused on correlational studies assessing results from predictive in vitro assays and observed outcomes of chemotherapy. However, in these studies, patients do not receive assay-guided chemotherapy regimens. As discussed in the 2004 systematic review, correlational studies are inadequate to demonstrate the clinical utility of chemoresistance assays 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. Third, it is important to consider not only response but also survival, QOL, 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 assay results or the accuracy of its predicted response. Examples of some of the earlier published correlation studies of the EDR assay include those by Eltabbakh et al, Mehta et al, Holloway et al, and Ellis et al.

The 2002 TEC Assessment identified 1 nonrandomized retrospective comparative study using the EDR assay (Loizzi et al, 2003). 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 article, no additional comparative studies of assay-guided therapy versus physician-directed therapy have appeared for chemoresistance assays.

**Analytic Validity**

The analytic validity of the various chemosensitivity and chemoresistance assays is expected to vary across the different individual assay types.

**Clinical Validity**

**Prospective Studies**

A study by Tiersten et al used the Oncotech EDR assay (Exiqon Diagnostics, Tustin, CA) 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 patients. No difference in PFS was reported. Follow-up was not sufficient to measure OS. These data do not support use of the EDR assay in predicting outcome and guiding patient management.

A 2006 review published by Nagourney included 21 noncomparative studies using ex vivo programmed cell death assays. Selected studies correlated drug susceptibility findings for the ex vivo assay for those with an objective clinical response (complete or partial) and nonresponders.
(total 659 N=patients). Nagourney 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%). A 2012 study by this same investigator prospectively assessed 98 patients with non-small-cell lung cancer treated between 2003 and 2010. Only 41 were eligible for inclusion and tested with the EVA/PCD assay to determine which chemotherapeutic drugs to use. Another 10 patients were excluded (5 due to insufficient cellular yield, 3 for resistance to all drugs tested, 2 due to physician’s choice), yielding only 31 patients who received the assay-recommended treatment. The authors compared results for these 31 patients treated with assay-directed chemotherapy to historical 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 Studies

In 2010, Matsuo et al published a study examining the relevance of EDR in epithelial ovarian carcinomas. 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%. Only 1 (0.4%) tumor 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. Investigators acknowledged that the study, due to its retrospective and noncomparative design, was not sufficiently strong to validate use of this assay in managing therapy. Potential confounding factors, described by investigators, may have included tumor heterogeneity and variations in resistance between primary tumor and metastases.

Another study by the same group evaluated the potential of the EDR assay to guide selection of platinum- and taxane-based therapies for management of patients with 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 (33.5%) patients whose tumors had LDR to both platinum and taxane showed statistically improved 5-year PFS and OS compared with the 115 (66.5%) patients 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 rate for the 28 (16.2%) cases who had optimal cytoreduction with LDR to both platinum and taxane (54.1%) was significantly better than that for the 62 (35.8%) cases who were suboptimally cytoreduced with IDR or EDR to platinum and/or taxane (20.4%; p<0.001). Although the EDR assay was predictive for survival, it is noteworthy that assay results did not indicate response to therapy with taxane or cisplatin. The investigators concluded 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.

In a smaller study (N=51) by Matsuo et al testing predictive value of the EDR assay for uterine carcinosarcoma response to taxane and platinum was also conducted, 31 (60.8%) patients 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 1-year PFS rate (28.6% vs 100%, respectively; p=0.01) and lower 5-year OS rate (26.9% vs 57.1%, respectively; p=0.033). These data would 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 are required to validate use of this assay for directing chemotherapy regimens.

Matsuo et al also examined the rates of EDR after cytoreductive therapy and neoadjuvant chemotherapy versus the rates of EDR after postoperative chemotherapy. 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. The primary end points were time to progression, OS, and survival after recurrence. The patient population was heterogeneous, with a median age of 59 years (range, 24-89 years), and 30% of patient with completely resected tumors, and enrolled at 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 whether or not the assay was performed at the time of the primary surgery or at recurrence.

Hetland et al studied primary platinum resistance in epithelial ovarian cancer patients with FIGO stage III or IV disease. Eighty-five biopsies from 58 patients were included. Resistance was assessed with a modified drug-response assay including adenosine triphosphate (ATP)-based tumor-chemosensitivity and EDR assay. Samples were tested for response to platinum, paclitaxel, and a 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 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.

**Clinical Utility**

No studies evaluating outcomes with assay-directed therapy, compared with physician-chosen therapy were identified.
Section Summary: Chemoresistance Assays
Some retrospective and prospective studies have suggested that chemoresistance assays, particularly the EDR assay, may be associated with chemotherapy response. However, prospective studies do not consistently demonstrate that chemoresistance assay results are associated with survival. Furthermore, no comparative studies were identified that compare outcomes between patients managed with assay-directed therapy and those managed with physician-directed therapy.

Large, randomized, prospective clinical studies comparing outcomes, including OS and disease-specific survival, QOL, and adverse events, between assay-directed therapy and physician-directed therapy, are needed.

Chemosensitivity Assays
Chemosensitivity assays are designed to select the most appropriate chemotherapy regimens for a given tumor type, and would therefore ideally be associated with high positive predictive values (PPVs) for clinical response. 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 QOL and adverse events.

The 2002 TEC Assessment and 2004 systematic review identified 9 comparative studies, 2 of which were randomized. Selected studies 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.

Clinical Validity
Prospective Studies
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. 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-naive 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. 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 was 46.2% (95% CI, 19.2% to 74.9%), PPV was 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%) than the chemoresistant group (24.1%; p=0.005). 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. 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.

Rutherford et al reported results from a prospective, noninterventional, multicenter cohort study 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. Three hundred thirty-five patients were enrolled and treated with 1 of 15 study protocols; treating physicians were blinded to the ChemoFX assay result. Two hundred sixty-two (78.2% of total) patients 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 median PFS of 8.8 months compared with 5.9 months for those with assay-intermediate or -resistant regimens (HR=0.67, p=0.009). Mean OS was 37.5 months for patients treated with an assay-sensitive regimen and 23.9 months for those with assay-intermediate or -resistant regimens (HR=0.67, p=0.010).

In a follow-up analysis, Tian et al evaluated whether the ChemoFX assay could predict PFS by comparing the association when the assayed therapy matched the administered therapy (match) and the association when the assayed therapy was randomly selected (mismatch). The authors generated a simulation in which the average prognostic value of assay results for multiple different therapies was generated using the assay results for mismatch, in which the assay result for 1 treatment was randomly selected from the (up to) 15 designated therapies with equal probability for each patient. Based on 3000 repeated simulated resamplings, the mean HR for cases of mismatch was 0.81 (reported as 95% range, 0.66 to 0.99), which the authors suggested indicated that patients with a mismatch had less benefit when treated with an assay-sensitive therapy. Strengths of this study include its prospective design with physicians blinded to the assay results, which reduced the risk of bias in patient selection and 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.

Krivak et al reported results from a subsequent prospective, observational, multicenter study to determine whether sensitivity to carboplatin and/or paclitaxel is associated with disease progression among patients with primary epithelial ovarian cancer following initial treatment with a platinum/taxane regimen. A total of 462 patients were enrolled, with 276 evaluable for analysis. Assay results for carboplatin and paclitaxel were available for 231 and 226 patients, respectively, with 44 (19.1%) patients identified as carboplatin-resistant and 49 (21.7%) identified as paclitaxel resistant. Carboplatin-resistant patients were at a higher risk of disease progression than nonresistant patients (HR=1.87; 95% CI, 1.29 to 2.70; p<0.001).

In a similar study design, Salom et al conducted a prospective, noninterventional, multicenter cohort study to assess whether the Microculture Kinetic (MiCK) assay (now called CorrectChemo) was predictive of outcomes among women with epithelial ovarian cancer. 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 treating physicians, who were blinded to the MiCK assay results. OS for stage III or IV disease was longer if patients received a chemotherapy that was considered “best” by the MiCK assay, compared with shorter OS for 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 104 women with advanced epithelial ovarian cancer.41 All patients had undergone initial surgery and were treated with paclitaxel and carboplatin therapy. Tumor cell 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 with 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 correlate 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 Studies**
A number of retrospective studies have evaluated the association between various chemosensitivity assays and clinical outcomes in several tumor types, most commonly epithelial ovarian cancer. Some representative studies are discussed next.

In 2016, Tanigawa et al published a retrospective study evaluating the association between in vitro chemosensitivity results and relapse-free survival (RFS) in 206 gastric cancer patients.42 The collagen gel droplet embedded culture drug sensitivity test is commercially available as a kit in Japan. All patients underwent surgery and were then treated with S-1 (tegafur/gimeracil/oteracil) chemotherapy. In vitro sensitivity of resected tumor specimens to fluorouracil was used as a surrogate of in vitro sensitivity to S-1 (this approach had been previously validated by the research group). Tumors were categorized as in vitro sensitive (responders) or in vitro insensitive (nonresponders). Median length of follow-up from the time of surgery was 3.2 years. Three-year RFS was significantly higher in the in vitro sensitive (responder) group (82.9%; 95% CI, 74.4% to 91.3%) than in the in vitro insensitive (nonresponder) group (63.4%; 95% CI, 54.7% to 72.1%; p=0.001).

Gallion et al retrospectively evaluated the association between ChemoFX test results and the treatment response of 256 patients with ovarian or peritoneal cancer treated with at least 1 cycle of postsurgical chemotherapy.43 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 or 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 PFS 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 Gallion study43 and reported on 192 women with advanced-stage primary ovarian cancer, 175 of whom had tumors tested for in vitro
chemosensitivity to platinum therapy using ChemoFX. 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 retrospectively analyzed the association between pretreatment chemosensitivity to cisplatin and clinical outcomes in 33 women with cervical cancer. 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 HDRA in 79 patients with ovarian cancer. 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. Outcomes for 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 were compared 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). Mean OS between the 2 groups did not differ significantly, with carboplatin-sensitive patients having of 60.4 months and carboplatin-resistant patients having 37.3 months (p=0.621).

Strickland et al retrospectively evaluated the association between chemosensitivity to anthracyclines, measured by the drug-induced apoptosis MiCK assay, among 109 patients with adult-onset acute myelogenous leukemia. 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 14 years, from 1996 to 2010, which may limit the generalizability of the results to currently used chemotherapy regimens.

Other retrospective studies have evaluated chemosensitivity results as measured by other assay types. Von Heideman et al evaluated the semi-automated fluorometric microculture cytotoxicity assay in 112 patients (125 samples) with ovarian cancer and concluded that samples from patients with clinical response were more sensitive to most drugs than samples from nonresponding patients.
Clinical Utility
A small number of nonrandomized studies have evaluated differences in outcomes for patients treated with assay-directed therapy compared with 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.49 All 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. Response rates and survival in both groups did not differ statistically. By ATP assay, the platinum-sensitive subgroup (71%) showed a higher response rate than the empirical group (38%; p=0.02), but PFS and OS differences between groups were not statistically significant.

In a nonrandomized comparative study (N=64), Iwahashi et al reported on outcomes of chemosensitivity-guided chemotherapy compared with standard chemotherapy and no chemotherapy in patients with advanced gastric cancer.50 In some subsets, survival was improved in the chemosensitivity-guided chemotherapy subgroup.

Cree et al reported on a prospective, randomized trial of chemosensitivity assay-directed chemotherapy versus physician’s choice in patients with recurrent platinum-resistant ovarian cancer.51 The primary aim of this RCT was to determine response and PFS rates following chemotherapy in patients treated according to an ATP-based tumor chemosensitivity assay and treated by physician 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; p=NS). No difference was seen in OS between groups, although 12 (41%) of 39 patients 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 RCT suggested 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 would still remain an investigational method in this condition.

Section Summary: Chemosensitivity Assays
The most direct evidence related to the effectiveness of chemosensitivity assays in the management of patients with cancer comes from several studies that compare outcomes for patients managed with an ATP-based tumor chemosensitivity assay with those managed with standard care, including 1 RCT. Although some improvements in tumor response were noted, no
differences between OS or PFS were seen. A number of retrospective and prospective studies of
several different chemosensitivity assays, including the ATP-based tumor chemosensitivity assay,
the CorrectChemo assay, and the ChemoFX assay, have suggested that patients whose tumors
have higher chemosensitivity have better outcomes. However, additional studies to determine
whether the clinical use of in vitro chemosensitivity testing leads to better outcomes are needed.

Ongoing and Unpublished Clinical Trials
Some currently unpublished trials that might influence this review are listed in Table 1.

Table 1. Summary of Key Trials

<table>
<thead>
<tr>
<th>NCT No.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
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<tr>
<td>NCT02580253</td>
<td>Adjuvant Chemotherapy Based on the Adenosine Triphosphate Tumor Chemosensitivity Assay for Hepatocellular Carcinoma After Liver Transplantation</td>
<td>300</td>
<td>Nov 2018</td>
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NCT: national clinical trial.

a Denotes industry-sponsored or cosponsored trial.

Summary of Evidence
For individuals who have cancer who are initiating chemotherapy who receive chemosensitivity
assays, the evidence includes correlational observational studies. Relevant outcomes are overall
survival, disease-specific survival, test accuracy and validity, and quality of life. Some
retropective and prospective correlational studies have suggested that chemoresistance assays
may be associated with chemotherapy response. However, prospective studies do not
consistently demonstrate that chemoresistance assay results are associated with survival.
Furthermore, no studies were identified that compared outcomes for patients managed with
assay-directed therapy to those managed with physician-directed therapy. Large, randomized,
prospective clinical studies comparing clinical outcomes are needed. The evidence is insufficient
to determine the effects of the technology on health outcomes.

For individuals who have cancer who are initiating chemotherapy who receive chemosensitivity
assays, the evidence includes 1 randomized controlled trial (RCT) and correlational observational
studies. Relevant outcomes are overall survival, disease-specific survival, test accuracy and
validity, and quality of life. The most direct evidence on the effectiveness of chemosensitivity
assays in the management of patients with cancer comes from several studies comparing
outcomes for patients managed with a chemosensitivity assay to those managed with standard
care, including 1 RCT. Although some improvements in tumor response were noted, there were
no differences in survival outcomes. A number of retrospective and prospective studies of several
different chemosensitivity assays have suggested that patients whose tumors have higher
chemosensitivity have better outcomes. Currently, additional studies to determine whether the
clinical use of in vitro chemosensitivity testing leads to better outcomes are needed. The
evidence is insufficient to determine the effects of the technology on health outcomes.

Practice Guidelines and Position Statements
National Comprehensive Cancer Network
The 2016 National Comprehensive Cancer Network (NCCN) guidelines for the treatment of
epithelial ovarian cancer, fallopian tube cancer, and primary peritoneal cancer (v.1.2016) state...
the following: “Chemosensitivity/resistance and/or other biomarker assays are being used in some NCCN Member Institutions. The current level of evidence is not sufficient to supplant standard-of-care chemotherapy. (category 3)”

American Society of Clinical Oncology
The 2011 American Society of Clinical Oncology clinical practice guideline update on the use of chemotherapy sensitivity and resistance assays does not recommend use of chemotherapy sensitivity and resistance assays, unless in a clinical trial setting.

U.S. Preventive Services Task Force Recommendations
Not applicable.

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
81535 Oncology (gynecologic), live tumor cell culture and chemotherapeutic response by DAPI stain and morphology, predictive algorithm reported as a drug response score; first single drug or drug combination
81536 Oncology (gynecologic), live tumor cell culture and chemotherapeutic response by DAPI stain and morphology, predictive algorithm reported as a drug response score; first single drug or drug combination each additional single drug or drug combination (List separately in addition to code for primary procedure)
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

- There are no specific CPT codes for these tests. The Extreme Drug Resistance assay is a multistep laboratory procedure that might be identified by the following CPT codes: 87230, 88104, 88305, 88313, 88358, 89050.
- There are specific CPT codes for ChemoFX®: 81535, 81536.

DIAGNOSIS
Experimental / investigational for all diagnoses related to this policy.
In Vitro Chemoresistance and Chemosensitivity Assays

REVISIONS

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<tr>
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<td>Rationale section added.</td>
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<td>▪ In Item A removed “or” and added “the ChemoFx assay, or the CorrectChemo assay,” to read “In vitro chemosensitivity assays, including, but not limited to, the Histoculture Drug Response Assay, a fluorescent cytoprint assay, the ChemoFx assay, or the CorrectChemo assay, are considered experimental / investigational.”</td>
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<td>▪ Added CPT codes: 81535, 81536 (Effective January 1, 2016)</td>
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<td>▪ Updated Coding notations.</td>
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REFERENCES


