In Vitro Chemoresistance and Chemosensitivity Assays

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 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.

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. 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 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, Long Beach, CA). 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 DNAs 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. The samples are then agitated and cytopsin-centrifuged and, after air drying, are counterstained with H&E.
In Vitro Chemoresistance and Chemosensitivity Assays

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.

• The fluorometric microculture cytotoxicity assay (FMCA) 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 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.

• The Extreme Drug Resistance assay (EDR®) (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. 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 to quantify cell viability by colorimetric assay:

• The Histoculture Drug Resistance Assay (HDRA; AntiCancer Inc., San Diego, CA). 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® (Helomics Corporation, 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 using differential optical density:

• CorrectChemo® (previously called the Microculture Kinetic (MiCK) assay (Diatech Oncology, Franklin, TN). Similar to the EVA/PCD 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...
In Vitro Chemoresistance and Chemosensitivity Assays

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 is no longer offering 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**

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.

***Note: This Medical Policy is complex and technical. For questions concerning the technical language and/or specific clinical indications for its use, please consult your physician.***

**Policy**

In Vitro Chemoresistance and Chemosensitivity Assays are considered investigational for all applications. BCBSNC does not provide coverage for investigational services or procedures.

Some patients may be eligible for coverage under Clinical Trials. Refer to the policy on Clinical Trial Services.

**Benefits Application**

This medical policy relates only to the services or supplies described herein. Please refer to the Member's Benefit Booklet for availability of benefits. Member's benefits may vary according to benefit design; therefore member benefit language should be reviewed before applying the terms of this medical policy.

When In Vitro Chemoresistance and Chemosensitivity Assays are covered

Not applicable.

When In Vitro Chemoresistance and Chemosensitivity Assays are not covered

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

**Policy Guidelines**

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 retrospective 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
In Vitro Chemoresistance and Chemosensitivity Assays

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.

Billing/Coding/Physician Documentation Information

This policy may apply to the following codes. Inclusion of a code in this section does not guarantee that it will be reimbursed. For further information on reimbursement guidelines, please see Administrative Policies on the Blue Cross Blue Shield of North Carolina web site at www.bcbsnc.com. They are listed in the Category Search on the Medical Policy search page.

Applicable codes: 81535, 81536

The extreme drug resistance assay is a multistep laboratory procedure that might be identified by the following CPT codes: 88358, 88305, 88104, 87230, 88313, and/or 89050. Providers may use 89240 for this service.

BCBSNC may request medical records for determination of medical necessity. When medical records are requested, letters of support and/or explanation are often useful, but are not sufficient documentation unless all specific information needed to make a medical necessity determination is included.

Scientific Background and Reference Sources

TEC 12/95
In Vitro Chemoresistance and Chemosensitivity Assays

BCBSA Medical Policy Reference Manual [Electronic]. 2.03.01, 2/14/08


Senior Medical Director - 4/2009


Blue Cross and Blue Shield Association Technology Evaluation Center (TEC). Chemotherapy Sensitivity and Resistance Assays. TEC Assessments 2002; Volume 17, Tab 12.


Policy Implementation/Update Information

8/83 Original policy
In Vitro Chemoresistance and Chemosensitivity Assays

11/84 Reaffirmed

11/87 Evaluated: Investigational

2/96 Evaluated: Added NCDR

3/96 Reaffirmed: National Association reviewed 3/96

3/97 Reaffirmed


6/99 Reformatted, Medical Term Definitions added.

11/99 Archived

6/00 Reactivated due to investigational status of assay


12/00 Revised description.

5/01 Changes in formatting.

2/02 Coding format change.


4/04 Benefits Application and Billing/Coding sections updated for consistency.


4/10/06 Specialty Matched Consultant Advisory Panel review 3/15/06. Added current terminology, "chemoresistance and chemosensitive assay" where appropriate. Referenced medical policy, "Clinical Trial Services for Life-Threatening Conditions, MED1093" in the "Description" section. Added the following statement to the "Policy" section; "Some patients may be eligible for coverage under Clinical Trials. Refer to the policy on Clinical Trial Services for Life-Threatening Conditions." Rationale added to the "Policy Guidelines" section. References added.

6/2/08 Specialty Matched Advisory Panel review 3/17/08. No change to policy statement. Updated the "Policy Guidelines" section. References added.

4/27/09 Policy name changed from "Human Tumor Stem Cell Drug Sensitivity Assay" to "In Vitro Chemoresistance and Chemosensitivity Assays". Reviewed with Senior Medical Director 4/7/09. "Description" section revised. "In vitro chemoresistance 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." Added CPT code 89240 to "Billing/Coding" section. Notice given 4/27/09. Effective date is 8/3/09. (btw)

6/22/10 Policy Number(s) removed. (amw)

7/6/10 Specialty Matched Consultant Advisory Panel review 5/24/2010. Updated “Description” section. No changes to policy statement. Added the following statement to the “Billing/Coding” section; “The extreme drug resistance assay is a multistep laboratory procedure that might be identified by the following CPT codes: 88358, 88305, 88104, 87230, 88313, and/or 89050. Providers may use 89240 for this service. References added. (btw)
In Vitro Chemoresistance and Chemosensitivity Assays


6/21/11 Reference added. (btw)

5/15/12 Specialty Matched Consultant Advisory Panel review 4/18/2012. Updated Policy Guidelines. No change to policy intent. Reference added. (btw)

4/30/13 Specialty Matched Consultant Advisory Panel review 4/17/2013. No change to policy. (btw)


5/27/14 References added. Description and Policy Guideline sections extensively revised. No changes to Policy Statements. Reference policy title changed from “Clinical Trial Services for Life Threatening Conditions” to “Clinical Trial Services”. (mco)

5/26/15 Updated Description section to add ChemoFX and CorrectChemo assays. Under “When Not Covered” section: added ChemoFX and CorrectChemo assays to the “including but not limited to” list of investigational indications. Specialty Matched Consultant Advisory Panel review 4/29/2015. Reference added. (lpr)

12/30/15 Added CPT codes 81535 and 81536 to Billing/Coding section for effective date 1/1/2016. (lpr)

5/31/16 Specialty Matched Consultant Advisory Panel review 4/27/2016. No change to policy statement. (lpr)

8/30/16 Updated Description and Policy Guidelines sections. Reference added. No change to policy statement. (lpr)

5/26/17 Specialty Matched Consultant Advisory Panel review 4/26/2017. No change to policy statement. (lpr)

Medical policy is not an authorization, certification, explanation of benefits or a contract. Benefits and eligibility are determined before medical guidelines and payment guidelines are applied. Benefits are determined by the group contract and subscriber certificate that is in effect at the time services are rendered. This document is solely provided for informational purposes only and is based on research of current medical literature and review of common medical practices in the treatment and diagnosis of disease. Medical practices and knowledge are constantly changing and BCBSNC reserves the right to review and revise its medical policies periodically.