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 biologic 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.

Summary of Evidence

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 correlative 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 to 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.

Policy

In vitro chemosensitivity assays, including, but not limited to, the Histoculture Drug Response Assay, or a fluorescent cytoprint assay, the ChemoFx assay, or the CorrectChemo assay, are considered investigational.

In vitro chemoresistance assays, including, but not limited to, Extreme Drug Resistance Assays, are considered investigational.
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 physiologic relevance to several-fold above physiologic 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. 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 three 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 four to six 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 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 three 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 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.

- 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 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 four days.

  After three 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.
• The Extreme Drug Resistance assay (EDR®)⁶ (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 one standard deviation above reference samples.

Methods to quantify cell viability by colorimetric assay:
• The Histoculture Drug Resistance Assay (HDRA; AntiCancer Inc., San Diego, CA).⁷ 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 three-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 the 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 Corp., previously called Precision Therapeutics, Pittsburgh, PA).⁸ This assay also relies on quantifying ATP based on chemoluminescence. Cells must be grown in a monolayer rather than in a three-dimensional matrix.

Methods using differential optical density:
• CorrectChemo® (previously called the Microculture Kinetic [MiCK]) assay; DiaTech Oncology, Franklin, TN).⁹ 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.
Services that are the subject of a clinical trial do not meet our Technology Assessment Protocol criteria and are considered investigational. For explanation of experimental and investigational, please refer to the Technology Assessment Protocol.

It is expected that only appropriate and medically necessary services will be rendered. We reserve the right to conduct prepayment and postpayment reviews to assess the medical appropriateness of the above-referenced procedures. Some of this Protocol may not pertain to the patients you provide care to, as it may relate to products that are not available in your geographic area.

References

We are not responsible for the continuing viability of web site addresses that may be listed in any references below.


