Protocol

In Vitro Chemoresistance and Chemosensitivity Assays

(20301)

Medical Benefit		**Effective Date**: 10/01/12	**Next Review Date**: 01/15
Preauthorization	No	**Review Dates**: 01/07, 03/08, 03/09, 01/10, 01/11, 01/12, 07/12, 01/13, 01/14	

The following Protocol contains medical necessity criteria that apply for this service. It is applicable to Medicare Advantage products unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. ***Preauthorization is not required but is recommended if, despite this Protocol position, you feel this service is medically necessary; supporting documentation must be submitted by the ordering physician to Utilization Management.*** *Please note that payment for covered services is subject to eligibility and the limitations noted in the patient's contract at the time the services are rendered.*

Description

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

Background

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

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

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

<u>The DiSC assay uses dye exclusion by live cells (1)</u>

- The Differential Staining Cytotoxicity (DiSC) Assay involves mechanical disaggregation of cells from surgical or biopsy specimens by centrifugation. Cells are then established in culture and treated with the drugs of interest at 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 differentiate tumor cells from normal cells. The intact cell membrane of a live cell precludes staining with the green dye. Drug sensitivity is measured by the ratio of live cells in the treated samples to the number of live cells in the untreated controls.

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

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

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

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

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

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

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

Policy (Formerly Corporate Medical Guideline)

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**.

Medicare Advantage

ChemoFx® assay may be **medically necessary** based on an individual consideration basis for those indications supported by peer-reviewed clinical literature and "generally accepted" in the medical community.

The number of assays eligible for reimbursement is based on individual tumor and member treatment options.

All other vitro chemosensitivity and chemoresistance assays are **investigational**.

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.

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