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## OVERVIEW

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. Oncologists may sometimes use these assays to select treatment regimens for a patient.

## MEDICAL CRITERIA

Not applicable

## PRIOR AUTHORIZATION

Not applicable

## POLICY STATEMENT

### Medicare Advantage Plans

In vitro chemosensitivity assays and chemoresistance assays are not covered as the evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

### Commercial Products

In vitro chemosensitivity assays and chemoresistance assays are considered not medically necessary as the evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

## COVERAGE

Benefits may vary between groups/contracts. Please refer to the appropriate section of the Benefit Booklet, Evidence of Coverage or Subscriber Agreement for applicable not medically necessary/not covered benefits/coverage.

## 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 cells killed 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. Examples of available assays are outlined below.

### 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 dose. Exposure time ranges from 4 to 6 days; then, cells are re-stained with fast green dye and counterstained with hematoxylin and eosin. The fast green dye is taken up by dead cells, and hematoxylin and eosin 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 the number of live cells in the treated samples to the number of live cells in the untreated controls.

- The Ex-Vivo Analysis of Programmed Cell Death (EVA/PCD<sup>®</sup>) assay (Rational Therapeutics) measures differential staining of cells after apoptotic and nonapoptotic cell death markers in tumor samples are 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 approximate the human tumor microenvironment more closely. 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 is added to the cellular suspensions. The samples are then agitated, cytospin-centrifuged, air-dried, and counterstained with hematoxylin and eosin. The endpoint of interest for this assay is cell death, as assessed by 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 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 Oncotech Extreme Drug Resistance EDR<sup>®</sup> assay (Exiqon Diagnostics; 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 the evaluation of responsiveness. Tumors are considered to be highly resistant when thymidine incorporation is at least 1 standard deviation above reference samples.

#### **Methods Quantifying Cell Viability Using Colorimetric Assay**

- The Histoculture Drug Resistance Assay HDRA (AntiCancer) 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 Chemoluminescent Precursors by Macromolecules in Viable Cells**

- 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 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) assay also relies on quantifying ATP based on chemoluminescence. Cells must be grown in a monolayer rather than in a 3-dimensional matrix.

For individuals with cancer who are initiating chemotherapy and receive chemoresistance assays, the evidence includes correlational observational studies. Relevant outcomes are OS, 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 have not consistently demonstrated that chemoresistance assay results are associated with survival. Furthermore, no studies were identified that compared outcomes for patients managed using assay-directed therapy with those managed using physician-directed therapy. Large, randomized, prospective clinical studies comparing OS are needed. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with cancer who are initiating chemotherapy and receive chemosensitivity assays, the evidence includes a randomized controlled trial, nonrandomized studies, and correlational observational studies. Relevant outcomes are OS, 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, including a randomized controlled trial, comparing outcomes for patients managed using a chemosensitivity assay with those managed using standard care. Although some improvements in tumor response were noted in the randomized trial, there were no differences in survival outcomes. One small nonrandomized study reported improved OS in patients receiving chemosensitivity-guided therapy compared with patients receiving standard chemotherapy. 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 improvements in OS are needed. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

#### **CODING**

The following codes are not covered for Medicare Advantage Plans and not medically necessary for Commercial Products:

- 81535** Oncology (gynecologic), live tumor cell culture and chemotherapeutic response by DAPI stand 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 stand and morphology, predictive algorithm reported as a drug response score; each additional single drug or drug combination (List separately in addition to code for primary procedure)
- 0564T** Oncology, chemotherapeutic drug cytotoxicity assay of cancer stem cells (CSCs), from cultured CSCs and primary tumor cells, categorical drug response reported based on percent of cytotoxicity observed, a minimum of 14 drugs or drug combinations

There are no specific CPT codes for other assays. Claims should be filed with an unlisted code.

#### **RELATED POLICIES**

Genetic Testing Services

#### **PUBLISHED**

Provider Update, November 2021

Provider Update, September 2020  
Provider Update, October 2019  
Provider Update, August 2018  
Provider Update, June 2017

## REFERENCES

1. Bird MC, Godwin VA, Antrobus JH, et al. Comparison of in vitro drug sensitivity by the differential staining cytotoxicity (DiSC) and colony-forming assays. *Br J Cancer*. Apr 1987;55(4):429-431. PMID 3580265
2. Nagourney RA, Blitzer JB, Shuman RL, et al. Functional profiling to select chemotherapy in untreated, advanced or metastatic non-small cell lung cancer. *Anticancer Res*. Oct 2012;32(10):4453-4460. PMID 23060572
3. Nagourney RA. Ex vivo programmed cell death and the prediction of response to chemotherapy. *Curr Treat Options Oncol*. Mar 2006;7(2):103-110. PMID 16455021
4. Csoka K, Larsson R, Tholander B, et al. Cytotoxic drug sensitivity testing of tumor cells from patients with ovarian carcinoma using the fluorometric microculture cytotoxicity assay (FMCA). *Gynecol Oncol*. Aug 1994;54(2):163-170. PMID 7520407
5. Yung WK. In vitro chemosensitivity testing and its clinical application in human gliomas. *Neurosurg Rev*. Jan 1989;12(3):197-203. PMID 2682352
6. Kern DH, Weisenthal LM. Highly specific prediction of antineoplastic drug resistance with an in vitro assay using suprapermeability drug exposures. *J Natl Cancer Inst*. Apr 4 1990;82(7):582-588. PMID 2313735
7. Anticancer Inc. Histoculture Drug Response Assay - HDRA. n.d.; [http://www.anticancer.com/HDRA\\_ref.html](http://www.anticancer.com/HDRA_ref.html). Accessed June 15, 2021.
8. Helomics. ChemoFx Chemoresponse Marker. n.d.; <https://www.helomics.com/chemoresponse-patients>. Accessed June 5, 2018.
9. Brower SL, Fensterer JE, Bush JE. The ChemoFx assay: an ex vivo chemosensitivity and resistance assay for predicting patient response to cancer chemotherapy. *Methods Mol Biol*. 2008;414:57-78. PMID 18175812
10. Samson DJ, Seidenfeld J, Ziegler K, et al. Chemotherapy sensitivity and resistance assays: a systematic review. *J Clin Oncol*. Sep 1 2004;22(17):3618-3630. PMID 15289487
11. Brown E, Markman M. Tumor chemosensitivity and chemoresistance assays. *Cancer*. Mar 15 1996;77(6):1020- 1025. PMID 8635118
12. Eltabbakh GH, Piver MS, Hempling RE, et al. Correlation between extreme drug resistance assay and response to primary paclitaxel and cisplatin in patients with epithelial ovarian cancer. *Gynecol Oncol*. Sep 1998;70(3):392- 397. PMID 9790793
13. Eltabbakh GH. Extreme drug resistance assay and response to chemotherapy in patients with primary peritoneal carcinoma. *J Surg Oncol*. Mar 2000;73(3):148-152. PMID 10738268
14. Mehta RS, Bornstein R, Yu IR, et al. Breast cancer survival and in vitro tumor response in the extreme drug resistance assay. *Breast Cancer Res Treat*. Apr 2001;66(3):225-237. PMID 11510694
15. Holloway RW, Mehta RS, Finkler NJ, et al. Association between in vitro platinum resistance in the EDR assay and clinical outcomes for ovarian cancer patients. *Gynecol Oncol*. Oct 2002;87(1):8-16. PMID 12468336
16. Ellis RJ, Fabian CJ, Kimler BF, et al. Factors associated with success of the extreme drug resistance assay in primary breast cancer specimens. *Breast Cancer Res Treat*. Jan 2002;71(2):95-102. PMID 11881914
17. Loizzi V, Chan JK, Osann K, et al. Survival outcomes in patients with recurrent ovarian cancer who were treated with chemoresistance assay-guided chemotherapy. *Am J Obstet Gynecol*. Nov 2003;189(5):1301-1307. PMID 14634558
18. Tiersten AD, Moon J, Smith HO, et al. Chemotherapy resistance as a predictor of progression-free survival in ovarian cancer patients treated with neoadjuvant chemotherapy and surgical cytoreduction followed by intraperitoneal chemotherapy: a Southwest Oncology Group Study. *Oncology*. Feb 2009;77(6):395-399. PMID 20130422
19. Matsuo K, Eno ML, Im DD, et al. Clinical relevance of extent of extreme drug resistance in epithelial ovarian carcinoma. *Gynecol Oncol*. Jan 2010;116(1):61-65. PMID 19840886

20. Matsuo K, Bond VK, Eno ML, et al. Low drug resistance to both platinum and taxane chemotherapy on an in vitro drug resistance assay predicts improved survival in patients with advanced epithelial ovarian, fallopian and peritoneal cancer. *Int J Cancer*. Dec 1 2009;125(11):2721-2727. PMID 19530239
21. Matsuo K, Bond VK, Im DD, et al. Prediction of chemotherapy response with platinum and taxane in the advanced stage of ovarian and uterine carcinosarcoma: a clinical implication of in vitro drug resistance assay. *Am J Clin Oncol*. Aug 2010;33(4):358-363. PMID 19875949
22. Matsuo K, Eno ML, Im DD, et al. Chemotherapy time interval and development of platinum and taxane resistance in ovarian, fallopian, and peritoneal carcinomas. *Arch Gynecol Obstet*. Feb 2010;281(2):325-328. PMID 19455347
23. Karam AK, Chiang JW, Fung E, et al. Extreme drug resistance assay results do not influence survival in women with epithelial ovarian cancer. *Gynecol Oncol*. Aug 2009;114(2):246-252. PMID 19500821
24. Bosserman L, Rogers K, Willis C, et al. Application of a drug-induced apoptosis assay to identify treatment strategies in recurrent or metastatic breast cancer. *PLoS One*. May 29 2015;10(5):e0122609. PMID 26024531
25. Kim JH, Lee KW, Kim YH, et al. Individualized tumor response testing for prediction of response to Paclitaxel and Cisplatin chemotherapy in patients with advanced gastric cancer. *J Korean Med Sci*. May 2010;25(5):684-690. PMID 20436702
26. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology: Gastric Cancer. Version 2.2020. [https://www.nccn.org/professionals/physician\\_gls/pdf/gastric.pdf](https://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf). Accessed June 2, 2021.
27. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Breast Cancer. Ver. 4.2020. Published May 8, 2020. [https://www.nccn.org/professionals/physician\\_gls/pdf/breast.pdf](https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf). Accessed June 2, 2021.
28. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Cutaneous Melanoma. Ver. 3.2020. Published May 20, 2020. [https://www.nccn.org/professionals/physician\\_gls/pdf/cutaneous\\_melanoma.pdf](https://www.nccn.org/professionals/physician_gls/pdf/cutaneous_melanoma.pdf). Accessed June 2, 2021.
29. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Non-Small Cell Lung Cancer. Ver. 6.2020. Published June 15, 2020. [https://www.nccn.org/professionals/physician\\_gls/pdf/nscl.pdf](https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf) Accessed June 2, 2021.
30. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology: Uterine Neoplasms. Version 1.2020. [https://www.nccn.org/professionals/physician\\_gls/pdf/uterine.pdf](https://www.nccn.org/professionals/physician_gls/pdf/uterine.pdf). Accessed June 2, 2021.
31. Burstein HJ, Mangu PB, Somerfield MR, et al. American Society of Clinical Oncology clinical practice guideline update on the use of chemotherapy sensitivity and resistance assays. *J Clin Oncol*. Aug 20 2011; 29(24): 3328-30. PMID 21788567

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