



Qualified Health Plans offered in North Carolina by CareSource North Carolina Co., d/b/a CareSource

In Vitro Chemoresistance and Chemosensitivity Assays

Policy Number: AHS – G2100 – In Vitro Chemoresistance and Chemosensitivity Assays	Prior Policy Name and Number, as applicable:
Effective Date: 10/01/2023	

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I. Policy Description

In vitro chemotherapy sensitivity and resistance assays refer to any in vitro laboratory analysis that is performed specifically to evaluate whether tumor growth is inhibited by a known chemotherapy drug or, more commonly, a panel of drugs (Hatok et al., 2009; Schrag et al., 2004).

II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request. Medical Policy Statements do not ensure an authorization or payment of services. Please refer to the plan contract (often referred to as the Evidence of Coverage) for the service(s) referenced in the Medical Policy Statement. If there is a conflict between the Medical Policy Statement and the plan contract (i.e., Evidence of Coverage), then the plan contract (i.e., Evidence of Coverage) will be the controlling document used to make the determination.

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient’s illness.

- 1) In vitro chemosensitivity assays, including, but not limited to, the histoculture drug response assay or a fluorescent cytoprint assay, **DO NOT MEET COVERAGE CRITERIA.**
- 2) In vitro chemoresistance assays, including, but not limited to, extreme drug resistance (EDR) assays, **DO NOT MEET COVERAGE CRITERIA.**

III. Table of Terminology

Term	Definition
5-FU	5-Fluorouracil

AML	Acute myelocytic leukemia
ASCO	American Society of Clinical Oncology
ATP-CRA	Adenosine triphosphate-based chemotherapy response assay
ATP-TCA	Adenosine triphosphate-tumor chemosensitivity
CDR	Cell death rate
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid
CR	Complete remission
CSC	Cancer stem cells
DISC	Differential staining cytotoxicity
EDR	Extreme drug resistance
FDA	Food and Drug Administration
HDRA	Histoculture drug response assay
HTCA	Human tumor cell assays
KU	Kinetic units
LCA	Local coverage article
LCD	Local coverage determination
LDT	Laboratory-developed test
MDR	Multiple drug resistance
MiCK	Microculture-kinetic
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
NCCN	National Comprehensive Cancer Network
OR	Odds ratio
OS	Overall survival
PFS	Progression-free survival
RGCC	Regulator of Cell Cycle
RPPA	Reverse phase protein array
TMZ	Temozolomide

IV. Scientific Background

Chemotherapy treatment recommendation has long been based on carefully designed clinical studies in large patient populations and provide an individual patient with a probability for response based on clinically observed response rates. This approach has led to major progress in clinical oncology and has helped to identify successful therapeutic regimens for patients with many cancers. However, the response rates are relatively low, and there are still many cancers for which there is only marginal treatment. Tumor cells isolated from these patients often are

resistant to a wide range of anticancer drugs. In addition, it is becoming clear that each individual patient's tumor is genotypically and phenotypically different (Hatok et al., 2009).

Chemotherapy sensitivity and resistance assays were developed to determine if a patient with cancer might be resistant or sensitive to a specific chemotherapy treatment prior to use. A chemosensitivity assay detects the effects (cytotoxic, apoptotic, and so on) of a given chemotherapeutic agent outside an organism. The assays vary, but typically they follow the same steps: cells from the patient are isolated, incubated with the chemotherapeutic agent, and assessed for cell survival and cell response (Hatok et al., 2009; Tatar et al., 2016). This assay allows clinicians to evaluate the effects of the chemotherapeutic agent without unnecessary exposure to cells. However, there are difficulties with these assays; for example, the potency of a chemotherapeutic agent may only be seen after time has elapsed. Many assays have been created to assess the potency of chemotherapeutic agents, including proprietary tests such as ChemoFX and ChemoINTEL, as well as non-proprietary assays such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), adenosine triphosphate-tumor chemosensitivity (ATP-TCA), and differential staining cytotoxicity (DISC) (Tatar et al., 2016).

Chemosensitivity assays typically rely on the use of cell cultures within the presence of the anticancer agent(s). For example, the MTT procedure involves culturing tumor cells with anticancer agents, then adding MTT, which is reduced to a blue dye in the cell. The intensity of the uptake allows the user to estimate the drug resistance of the tumor cells. DISC cultures tumor cells in three different concentrations of the drug, incubates them for 6 days, then uses differential dye staining to identify viable cells (Hatok et al., 2009). Several proprietary assays exist, such as ChemoFX (from Helomics), which exposes tumor cells to increasing doses of chemotherapeutic drugs, and the number of live cells remaining post-treatment is counted. These counts are combined into a dose-response curve, which is used to categorize a tumor's response as "responsive," "intermediate response," or "non-responsive" (Brower et al., 2008). Another proprietary test is the Microculture-Kinetic (MiCK) assay (Pierian Biosciences) (Grendys et al., 2014; Pierian, 2021). This test relies on drug-induced apoptosis with the quantification of tumor cells' response to chemotherapeutic agents. This test is now branded as ChemoINTEL (Pierian, 2021). A third proprietary test comes from RGCC, titled "Onconomics". This test evaluates both molecular markers and viability assessments to determine efficacy of certain drugs. However, this test does follow the same pattern as the previously discussed tests; developing cell cultures and examining effects of chemotherapeutic agents on their population (RGCC, 2021). Other proprietary assays include human tumor cell assays (HTCA) and human tumor cloning assays.

Recent advances have led to new proprietary tests on the market, such as the KIYATEC Inc. ex vivo 3D cell culture technology, which predicts "in vivo cancer drug efficacy through precision ex vivo response profiling," by using live cancer cells from surgical and/or biopsy specimens to create a tumor specific to the patient genetic profile (KIYATEC, 2021). This manufactured tumor is then used to investigate the patient's potential responses to chemotherapy regimens or drugs. A second new proprietary test, from Theralink, uses a reverse phase protein array (RPPA) test to evaluate over 600 different protein and phosphoprotein targets on a cell's surface. The test is used to evaluate whether FDA-approved cancer therapies and investigational treatments will be effective based on cell surface proteins. Theralink's technology seeks to reduce exposure of

patients to cytotoxic treatments and therapies through analysis of drug-protein interactions that drive treatment responses (Theralink, 2021).

Clinical Utility and Validity

Tatar et al. (2016) conducted a study to assess three in vitro chemosensitivity assays in ovarian carcinoma. 26 patients with ovarian carcinoma contributed tumoral tissue, and three assays (the MTT assay, the ATP-TCA assay, and the DISC assay) were used to evaluate the chemosensitivity of paclitaxel, carboplatin, docetaxel, topotecan, gemcitabine, and doxorubicin. The authors stated that all three assays correlated reasonably well with each other and are “particularly useful for serous and advanced cancers.” However, they caution that “large prospective studies comparing standard versus assay-directed therapy with an endpoint of overall survival are required before routine clinical utilization of these assays” (Tatar et al., 2016).

Kwon et al. (2016) evaluated the usefulness of the in vitro adenosine triphosphate-based chemotherapy response assay (ATP-CRA) for prediction of clinical response to fluorouracil-based adjuvant chemotherapy in stage II colorectal cancer. Tumor specimens of 86 patients with stage II colorectal adenocarcinoma were tested for chemosensitivity to fluorouracil, and chemosensitivity was determined by cell death rate (CDR) of the drug-exposed cells. In total, 11 of the 86 patients had a recurrence, and the group with CDR $\geq 20\%$ was associated with better disease-free survival than the group under 20%. The authors concluded that “in stage II colorectal cancer, the in vitro ATP-CRA may be useful in identifying patients likely to benefit from fluorouracil-based adjuvant chemotherapy” (Kwon et al., 2016).

Krivak et al. (2014) conducted an observational study to evaluate if the ChemoFx assay can identify patients who are platinum-resistant prior to treatment. The study included 276 women with International Federation of Gynecology and Obstetrics stage III-IV ovarian, fallopian, and peritoneal cancer, and the responsiveness of their tumors was evaluated. All patients were treated with a platinum/taxane regimen following cytoreductive surgery. The authors found that the patients whose tumors were resistant to carboplatin were at increased risk of disease progression compared to those who were nonresistant. The authors stated that “assay resistance to carboplatin is strongly associated with shortened PFS among advanced-stage epithelial ovarian cancer patients treated with carboplatin + paclitaxel therapy, supporting use of this assay [ChemoFx] to identify patients likely to experience early recurrence on standard platinum-based therapy” (Krivak et al., 2014).

Rutherford et al. (2013) conducted a prospective study evaluating the use of ChemoFx assay in recurrent ovarian cancer patients. The study included 252 women with persistent or recurrent ovarian cancer and fresh tissue samples were collected for chemoresponse testing. Patients were treated with one of 15 protocol-designated treatments empirically selected by the oncologist, blinded to the assay results. Patients were prospectively monitored for progression-free survival (PFS) and overall survival (OS). Patients treated with an assay-sensitive regimen demonstrated significantly improved PFS and OS while there was no difference in clinical outcomes between intermediate and resistant groups. The researchers concluded that the “study demonstrated

improved PFS and OS for patients with either platinum-sensitive or platinum-resistant recurrent ovarian cancer treated with assay-sensitive agents” (Rutherford et al., 2013).

Hoffman (2018) conducted a study investigating the clinical correlation of histoculture drug response assay (HDRA) in 29 advanced gastric and colon cancer patients. The authors revealed that all 29 were being treated with drugs considered “ineffective” by the HDRA. However, nine patients were also being treated with drugs identified as “effective” by the HDRA, and these patients showed response or arrest of disease progression. The authors investigated another subset of 32 patients treated with mitomycin C and 5-fluorouracil (5-FU) and whom had advanced gastric cancer. Ten patients were identified as “sensitive” to these drugs, and their survival rates were higher than the other 22 whose tumors were “insensitive.” A separate 128-patient subset had their tumors evaluated by the HDRA, and the overall and disease-free survival rate was higher for the sensitive group compared to the resistant group. Overall, both “sensitive” groups experienced higher survival rates (Hoffman, 2018).

Strickland et al. (2013) evaluated the correlation of the MiCK assay with patient outcomes in initial treatment of adult acute myelocytic leukemia (AML). 109 patients with untreated AML contributed samples for the MiCK assay. The amount of apoptosis was measured over 48 hours and standardized to “kinetic units” of apoptosis (KU). The authors observed that complete remission (CR) was “significantly” higher in patients with high idarubicin-induced apoptosis (>3 KU) compared to patients with <3 KU. A multivariate analysis indicated the only significant variable to be idarubicin-induced apoptosis. The authors concluded, “Chemotherapy-induced apoptosis measured by the MiCK assay demonstrated significant correlation with outcomes and appears predictive of complete remission and overall survival for patients receiving standard induction chemotherapy” (Strickland et al., 2013).

Howard et al. (2017) developed and assessed a “chemopredictive” assay (ChemoID), which was intended to identify the most effective chemotherapy out of a panel of selected treatments. ChemoID evaluates the efficacy of chemotherapies using a patient’s live tumor cells, as well as the cancer stem cells (CSC) that are purported to cause recurrence in patients. The study included 42 glioblastoma patients who were treated with standard of care temozolomide (TMZ). Clinical outcomes such as “tumor response, time to recurrence, progression-free survival (PFS), and overall survival (OS). Odds ratio (OR) associations of 12-month recurrence, PFS, and OS outcomes” were estimated. The authors found that for every 5% increase in CSC kill by TMZ, 12-month patient response (defined as “nonrecurrence of cancer”) increased by 2.2-fold. The authors also identified a less significant association with the bulk tumor cells; a 5% increase in bulk tumor cell kill corresponded with a 2.75-fold increase in nonresponse ($p = .07$). At >40% cell kill for CSC and >55% cell kill for bulk tumor cells, the area under curve was 0.989. Median recurrence time was 20 months for patients with a positive (defined as >40%) CSC test, compared to three months for patients with a negative test. Similarly, median recurrence time was 13 months for patients with a positive bulk tumor cell test (>55%), compared to 4 months for a negative test. Finally, the ChemoID CSC results were found to “potentially” identify more optimal treatments in 34 patients, while the bulk tumor results may have resulted in more optimal treatments in 27 patients. Overall, the authors concluded that “the ChemoID CSC drug response

assay has the potential to increase the accuracy of bulk tumor assays to help guide individualized chemotherapy choices” (Howard et al., 2017).

Chen et al. (2018) evaluated in vitro chemosensitivity and multiple drug resistance (MDR) using an ATP-based tumor chemosensitivity assay (ATP-TCA). The authors evaluated 120 lung cancer patients’ chemosensitivity to eight single drug chemotherapies and 291 lung cancer patients’ chemosensitivity to seven chemotherapy regimens. Additionally, 284 lung adenocarcinoma patients and 90 lung squamous cell carcinoma patients were evaluated for chemosensitivity to both single-drug and chemotherapy regimens. Authors found that “PTX (51.7%), TXT (43.3%), GEM (12.5%), PTX+DDP (62.5%), TXT+L-OHP (54.3%) and VP-16+DDP (16.2%) had the highest in vitro chemosensitivity rates.” Additionally, approximately 37.1% of patients developed resistance to eight single-drug chemotherapies; 25.8% showed resistance to all seven chemotherapy regimens. In conclusion, testing for drug sensitivity before chemotherapy could assist in preventing the “occurrence of primary drug resistance and inappropriate drug treatment” (Chen et al., 2018).

V. Guidelines and Recommendations

American Society of Clinical Oncology (ASCO)

The 2011 clinical practice guideline update states that: “The use of chemotherapy sensitivity and resistance assays to select chemotherapeutic agents for individual patients is not recommended outside of the clinical trial setting. Oncologists should make chemotherapy treatment recommendations on the basis of published reports of clinical trials and a patient’s health status and treatment preferences. Because the in-vitro analytic strategy has potential importance, participation in clinical trials evaluating these technologies remains a priority” (Burstein et al., 2011).

National Comprehensive Cancer Network

The NCCN Practice Guidelines in Oncology for Ovarian Cancer (NCCN, 2021b) state that: “chemosensitivity/resistance and/or other biomarker assays are being used at some NCCN Member Institutions for decisions related to future chemotherapy in situations where there are multiple equivalent chemotherapy options available. The current level of evidence is not sufficient to supplant standard of care chemotherapy.” This is a category 3 recommendation (based on any level of evidence but reflects major disagreement).

Chemosensitivity/resistance testing is not mentioned in the guidelines for gastric, colon, or prostate cancers (NCCN, 2021a).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Procedure codes appearing in medical policy documents are only included as a general reference. This list may not be all inclusive and is subject to updates. In addition, codes listed are not a guarantee of payment.

CPT	Code Description
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 Proprietary test: ChemoFX® Lab/manufacturer: Helomics, Corp
81536	Oncology (gynecologic), live tumor cell culture and chemotherapeutic response by DAPI stain 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) Proprietary test: ChemoFX® Lab/manufacturer: Helomics, Corp
88104	Cytopathology, fluids, washings or brushings, except cervical or vaginal; smears with interpretation
88199	Unlisted cytopathology procedure
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
0083U	Oncology, response to chemotherapy drugs using motility contrast tomography, fresh or frozen tissue, reported as likelihood of sensitivity or resistance to drugs or drug combinations Proprietary test: Onco4D™ Lab/manufacturer: Animated Dynamics, Inc.
0248U	Oncology (brain), spheroid cell culture in a 3D microenvironment, 12 drug panel, tumor-response prediction for each drug Proprietary test: 3D Predict Glioma Lab/Manufacturer: KIYATEC®, Inc

0249U	Oncology (breast), semiquantitative analysis of 32 phosphoproteins and protein analytes, includes laser capture microdissection, with algorithmic analysis and interpretative report Proprietary test: Theralink® Reverse Phase Protein Array (RPPA) Lab/Manufacturer: Theralink® Technologies, Inc
0285U	Oncology, response to radiation, cell-free DNA, quantitative branched chain DNA amplification, plasma, reported as a radiation toxicity score Proprietary test: RadTox™ cfDNA test Lab/Manufacturer: DiaCarta Clinical Lab/DiaCarta Inc
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 (Reported for ChemoID®)

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IX. Revision History

Revision	Summary of Changes
01/01/2023	Initial Effective Date
04/04/2023	Policy reviewed at least 3 times in detail over the past 12 months. In addition, the professional society guidelines have been reviewed and, to our knowledge, there have been no new updates or guidance. Added CPT codes 0249U, 0285U, 0324U, and 0325U Committee approved 4/4/2023



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06/12/2023	Off-cycle coding only updates: Removed deleted PLA codes 0324U, 0325U (effective 4/1/2023) Committee approved 06/12/2023
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