

Cell Culture Drug Resistance Testing (CCDRT) Predicts for Patient Survival in Ovarian Cancer

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Introduction

30 years' worth of clinical trials have produced no important progress in the chemotherapy of advanced (Stage III, IV, and recurrent/refractory) ovarian cancer. Such trials have not benefited ovarian cancer patients; they have produced no progress whatsoever in the treatment of ovarian cancer as a disease; they have not benefited cancer research in general. These issues are discussed in detail elsewhere on this website.

We have produced an entire generation of investigators in clinical oncology who believe that the only valid form of clinical research is to perform "well-designed," prospective, randomized trials in which patients are randomized to receive one empiric drug combination versus another empiric drug combination. The problem is not with using the prospective, randomized trial as a research instrument. The problem comes from applying this (time and resource-consuming) instrument to address hypotheses of trivial importance (i.e. do most cancers prefer Pepsi Cola or Coca Cola?). As once stated by Dr. Martin Appel (in another context): "any experiment which has failed 1,000 consecutive times should be viewed with suspicion." Clinical

research into the chemotherapy of advanced ovarian cancer has produced no progress dating back to the 1960s. To this date, no form of first-line chemotherapy has been proven superior to the single agent alkylators (including orally-administered agents) which were utilized in the 1960s. Government and academic clinical investigators have failed to support the individualization of chemotherapy through laboratory testing, in favor of attempts to identify "one size fits all treatments" through trial and error testing which has consumed tens of thousands of human lives. This entire effort has been a colossal failure and a colossal waste of human and financial resources.

As discussed elsewhere on this website, cell culture drug resistance testing (CCDRT, also known as "chemosensitivity testing," "drug resistance testing," and "drug response testing") has been shown to correlate strongly (and non-controversially) with patient response to chemotherapy and with long-term patient survival after treatment with chemotherapy. What has been missing until most recently are data to show that basing treatment decisions on the results of CCDRT improves clinical outcomes. This is simply because

the investigators who control the clinical trials system have been entirely non-supportive of clinical trials to compare CCDRT-directed chemotherapy to "standard," one-size-fits all chemotherapy. Some day this will change. The failure of 30 years' worth of clinical trials research into "one size fits all" therapy will eventually force a consideration of new approaches. But it will be years before the results of such trials are available. Too late for treatment decision

which must be made today.

There is no proof beyond reasonable doubt for any approach to treating advanced ovarian cancer today. There is only the bias of clinical investigators as a group and as individuals. My own bias, based on the preponderance of evidence as outlined on this web site, is to make extensive use of cell culture drug resistance testing in treatment decisions.

Research

There have now been 5 different studies of the relationship between the results of cell culture drug resistance testing (CCDRT) and patient survival in ovarian cancer, and all 5 studies show significant correlations between CCDRT and patient survival.

Konecny, et al tested the tumors of 38 previously-untreated FIGO stage III patients who received treatment (independent of assay results) with one of several platinum-based regimens. Drugs were tested as combinations (e.g. cisplatin + cyclophosphamide, cisplatin + Taxol, etc.). With cut-offs determined retrospectively, using a method to optimize separation of survival curves, 29 assay-"sensitive" patients had a significantly-longer progression-free survival (median 28.5 vs 12.6 months, $P=0.033$) and overall survival (median 46 vs 18 months, $P=0.03$), compared with

9 patients classified as "resistant" by CCDRT. (*Gynecol Oncology* 77:258-73,'00).

The ATP endpoint was used in this study.

Taylor, et al tested the tumors of 93 previously-untreated FIGO stage III and IV patients who received treatment (independent of assay results) with either a platinum-based regimen (71 patients) or with a single agent alkylator (22 patients). Drugs were tested as single agents, with the tumor classified as "sensitive" if at least one drug used in subsequent treatment was active in CCDRT and as "resistant" if all drugs were inactive in CCDRT. Cut-offs were determined on the basis of prior, "training-set" assays. 51 assay-"sensitive" patients had a median survival of 23 months, while 42 assay-"resistant" patients had a median survival of 19 months (n.s.). However, the three year

survival of the "sensitive" group was 36%, compared to 16% in the "resistant" group, while the five year survivals for the "sensitive" and "resistant" groups were 24% versus 12%, respectively (P=0.033). (Eur J Gynaecol Oncol 22:278-82,' 01). The MTT endpoint was used.

In the best study correlating patient outcomes with CCDRT in ovarian cancer published to date, **Holloway, et al** tested the tumors of 79 previously-untreated patients with optimally debulked stage III/IIc disease (63 cases) and suboptimally debulked stage III or stage IV disease (16 cases). Patients were treated with platinum/Taxol if CCDRT did not show resistance to Taxol, or else cyclophosphamide/platinum, cyclophosphamide/doxorubicin/platinum, or platinum alone if CCDRT showed resistance to Taxol. Cut-offs were determined objectively, with assay "resistance" defined as an assay result one standard deviation more resistant than the median in training set assays. Median progression-free survival was 6 months in 17 cases with platinum "resistance" in the assay, compared to 24 months for 62 cases exhibiting "sensitivity" to platinum (hazards ratio = 3.78, 95% confidence interval 1.82 - 7.83). Median overall survival for the "resistant" group was 24 months, but was not reached in the "sensitive" group. Estimated 5 year survival was 19% in the "resistant" group, compared to 68% in the "sensitive" group (hazards ratio = 2.32, 95%

confidence interval 1.06 - 5.07). On multivariate analysis including stage, debulking status, tumor grade, and in vitro response to cyclophosphamide, assay "resistance" to platinum was the most powerful determinant of outcome (Gynecol Oncology 87:8-16,' 02). The ³H-thymidine endpoint was used.

Nagourney, et al tested the tumors of 17 previously-treated ovarian cancer patients with the combination of gemcitabine + cisplatin. Assay results were cut at the median IC50 value for the drug combination. Patients with "sensitive" tumors had an 85% progression-free survival probability at 6 months, compared to approximately 28% for assay-"resistant" tumors, and Kaplan-Meier progression-free survival curves were superior in the assay-"sensitive" group (P2=0.012). Overall survival was also superior in the assay-"sensitive" group (P2=0.05). On multivariate analysis, neither number of prior therapies nor time since last platinum therapy were independent predictors of progression-free survival. Assay results remained significant when adjusted for number of prior therapies (P2=0.027) but lost significance when adjusted for time since last platinum therapy (P2=0.14). Gynecol Oncology 88:35-39,' 02. The DISC assay endpoint (delayed loss of cell membrane integrity) was used.

Weisenthal, et al correlated the results of CCDRT to both cisplatin and carboplatin with

long-term, overall patient survival in ovarian cancer. The in vitro activity of cisplatin and carboplatin was determined through the concurrent application of two different cell death endpoints (cell membrane dye exclusion/DISC assay and mitochondrial metabolism/MTT assay) following 96 hour culture of 3 dimensional microclusters of tumor cells.

"Sensitive"/"Intermediate"/"Resistant" cut-offs were defined by calculating means and standard deviations of training set assays performed on a wide variety of human tumors (including non-ovarian tumors) and were reported prospectively. These cut-offs were also re-calculated retrospectively, based only on the datasets of ovarian cancer assays.

Results: Specimens from previously-treated patients were significantly more resistant to platinum than were specimens from untreated patients, and this

difference was most pronounced in the case of poorly-differentiated tumors. Well-differentiated tumors had significantly greater platinum resistance than poorly-differentiated tumors. In untreated patients (n = 115) resistance to cisplatin and (separately) to carboplatin correlated significantly with long-term survival, as reported prospectively. This relationship was strongest in the case of poorly-differentiated tumors (hazards ratio "resistant" versus "sensitive" = 3.23, 95% confidence interval 1.61 - 25.6, for assay results reported prospectively and hazards ratio = 4.54, 95% C.I. 2.13 - 23.3, for cut-offs objectively calculated retrospectively, based on only the ovarian cancer dataset). There was no significant relationship between platinum resistance and patient survival in previously-treated patients (n = 327)..

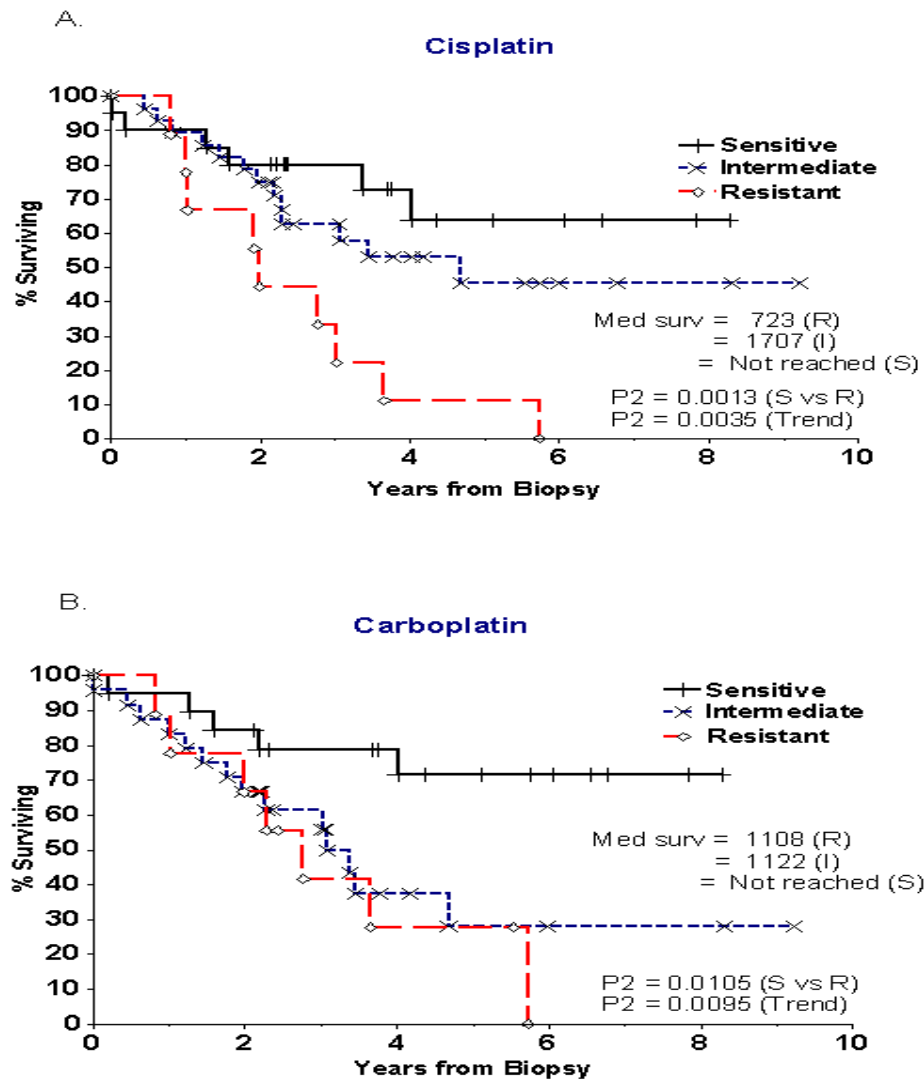
Platinum Resistance Determined by Cell Culture Drug Resistance Testing (CCDRT) Predicts for Patient Survival in Ovarian Cancer

(Weisenthal, et al, submitted for publication)

Representative findings:

Conclusions: There is a clear relationship between platinum resistance and tumor differentiation. Poorly-differentiated tumors are, on average, significantly more sensitive to platinum than are well-differentiated tumors. In contrast, Taxol resistance is not related to tumor differentiation. In the absence of CCDRT, single agent platinum may be the most appropriate first line chemotherapy for poorly-differentiated tumors, while platinum/Taxol may be more appropriate for well-differentiated tumors.

Figure 7: Correlations between platinum resistance and patient survival in poorly-differentiated tumors.



Conclusions: Platinum resistance determined by CCDRT predicts for long-term patient outcome. Copyright 2003 Weisenthal Cancer Group