Patient-specific tumor biology-based selection of ovarian cancer therapy

"Early detection of stage I disease could transform the field, where 95% of such patients enjoy long-term survival after surgical resection. Discovery of early-detection biomarkers is therefore of great importance."

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Epithelial ovarian cancer accounts for 5% of cancer deaths in women in the USA [1]. Owing to the lack of validated cancer-specific proteins for early detection and the clinically silent nature of its growth, more than 70% of ovarian cancer patients present with incurable stage III or IV disease. Unfortunately, owing to the lack of cancer-specific proteins for early screening, current treatment is predominantly directed toward postoperative unresectable disease [2]. Early detection of stage I disease could transform the field, where 95% of such patients enjoy long-term survival after surgical resection. Discovery of early-detection biomarkers is therefore of great importance.

Chemotherapy is initially effective for most patients with advanced disease, yielding response rates of 70% from platin-based treatments [3–6]. However, patients have significantly diminished response rates after relapse and eventually develop treatment-resistant disease, suggesting that resistant subsets are selected for during initial therapy [7]. *In vitro* assay results derived from tests performed on a patient's tumor could potentially improve outcomes by assisting in the selection of the most appropriate second-line agent [8–11]. Resistance to such second-line treatment does not appear to depend on the initial chemotherapy, suggesting that assay results from initial biopsies may continue to be accurate after failure of first-line treatment [12]. Recent development of monoclonal antibodies that target angiogenesis, such as bevacizumab, and small molecules that attack other cancer-related pathways are poised to offer additional incremental benefit [13–15]. Relevant *ex vivo* tests of a patient's living cancer should be designed to provide guidance to the treating physician for the selection of targeted therapeutics, such as bevacizumab. Our current understanding of tumor biology needs to be incorporated into the design of patient-specific assays.

Over the past 10 years, various publications have indicated that *in vitro* assay results obtained from tumor specimens have generally been predictive of response rates and overall survival (Table 1) [16–20]. While not all series have demonstrated significant relationships in multivariate analysis, the general trend indicates that patients who received platin-based therapy that was inactive *ex vivo* experienced poorer overall survival. Owing to variations in optimal debulking and chemotherapy administration, it is not possible to perform a meta-analysis on these data sets. Owing to low rates of extreme drug resistance (EDR) to cisplatin (7–20%) [16–18], large sample sizes to evaluate the impact of this finding and the clinical value of making alternative treatment choices are needed to definitively determine its impact on outcomes, as described by Matsuo *et al.* [19]. In one series where EDR to cisplatin was seen in 21% of patients [18], a significant association between EDR and outcomes was seen, while in another study at MD Anderson, University of Texas (TX, USA), the frequency of EDR to cisplatin was 6.9%, and was not significantly associated with overall survival in multivariate analysis. A large, appropriately powered, prospective, randomized, multi-institutional trial will be needed to determine the relationship between the use of assay-directed therapy and improved outcomes. The cooperative groups offer the most appropriate setting for such a trial. Since there is strong level II evidence supporting the view that *in vitro* assays identify inactive agents before their use, these technologies will continue to be employed [21]. Are there weaknesses in the current methodology that should be considered before such a trial moves forward?

Insights into the biological basis of how drugs actually kill cancer cells and mechanisms of drug resistance have grown enormously since these assay platforms were initially developed. The old view that DNA damage directly causes cell death has undergone a significant tectonic shift over the past 30 years. New insights have demonstrated the role of the mitochondria and

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Table 1. Recent *in vitro* testing results for ovarian cancer (n > 40) reported with clinical outcomes.

Study	Assay type	Patients (n)	Result	p-value	Ref.
Holloway *et al.* (2002)	EDR	79	PFS EDR = 6 months PFS LDR = 24 months	0.011	[16]
Gallion *et al.* (2006)	ChemoFx	84 chemonaive	HR progression resistant vs sensitive = 2.9	<0.01	[17]
Matsuo *et al.* (2009)	EDR	173	HR (% 5-year OS) LDR (41.1) HR = 3.32 DR + EDR (30.9)	0.014	[18]
Matsuo *et al.* (2010)	EDR	253	Number of EDR agents and OS (univariate) Proportion of EDR and OS (multivariate)	0.024 0.13	[19]
Kim *et al.* (2009)	EDR	43	Mean OS: EDR cisplatin = 29.2 months Non-EDR = 33 months Mutlivariate OS EDR vs non-EDR	0.032 >0.05	[20]

DR: Drug resistance; EDR: Extreme drug resistance; HR: Hazard ratio; LDR: Low drug resistance; OS: Overall survival; PFS: Progression-free survival.

reactive oxygen species signaling in apoptotic programmed cell death [22]. Apoptosis was an unknown process in 1980, and cisplatin was thought to enter cells by diffusion and cause cell death by DNA unwinding [23]. Today, it is understood that cisplatin enters cells via copper carriers; DNA damage response proteins, such as BRCA1 and ERCC1, are associated with cisplatin activity; and regulation of apoptosis is a critical pathway in platin sensitivity and resistance [24–28]. Gene profiling using cell lines and tumor specimens collected from various centers worldwide has led to the delineation of expression signatures associated with ovarian cancer response to carboplatin and paclitaxel [29]. Such signatures need to be validated, but their association with outcomes will eventually be strengthened as new techniques and bioinformatics approaches are explored. Unfortunately, extracting tumor tissue from patients is likely to alter the endogenous gene expression related to low intratumor oxygen levels. In addition, mixing mRNA extracted from the tumor as a whole obliterates the capability of identifying critical subsets of cells that exist in heterogenous microenvironments within the tumor [30]. Such heterogeneous domains are likely to be critical for the determination of drug resistance and the emergence of resistant disease [31,32].

One of the fundamental aspects of our new biological insight into cancer cell metabolism over the past 10 years is the role of hypoxia and hypoxia inducible factor (HIF). HIF plays a central role in promoting cell survival in solid tumors and it exhibits a heterogeneous distribution within a tumor mass as a result of variations in oxygen delivery and angiogenesis [33]. Variations in vascular distribution have been detected by dynamic enhanced imaging and found to correlate with chemotherapy response [34]. The role of HIF in upregulating genes associated with drug resistance may be fundamental to treatment failure over time and is critically dependent on hypoxia for its manifestation [35–37]. Targeting HIF protein function is an emerging area made relevant by the ability of topotecan to inhibit HIF protein accumulation in ovarian cancer [38].

Platform design of *in vitro* drug response assays needs to be informed by these recent insights into cancer biology. 3D spheroids are a newer *in vitro* model of cancer phenotype that capture the element of heterogeneity and HIF expression as a determinant of drug resistance [39,40]. Interrogation of gene expression patterns associated with resistance in ovarian cancer spheroids would be expected to yield more accurate results than those obtained from cell lines grown in monolayer under normoxic conditions. Growth of tumor specimens freshly collected and grown in spheroids in suspension matrices may provide a more appropriate model of drug response than current systems that depend on disaggregation of tumor populations into either single cells or small clusters. Furthermore, tumor cell cultures in the commercially available assays are carried out at normal oxygen tensions that preclude HIF stabilization seen *in vivo* and activation of other concordant systems, such as mammalian targets of rapamycin, and so lack important resistance pathways [28,41]. If the accuracy of *in vitro* assays is to improve, testing platforms will need to incorporate hypoxia and the complexity of cancer matrices and cross-talk with other cellular elements such as vascular endothelial cells [31,32]. Inclusion of these variables into the next generation of *in vitro* testing platforms is critical if they are to remain relevant. Attempts are currently

underway to model these multicellular interactions in the laboratory and to then use their data to build computer simulations that predict drug response [42,43]. The future where small tumor cores may be obtained and injected onto the assay multiplexer, which then provides a patient with a specific list of targeted therapies, is just around the corner. One wonders how our current concepts of tumor biology and therapeutics will be viewed 30 years hence.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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