

Chemosensitivity and chemoresistance testing in ovarian cancer

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Purpose of review

Sensitivity testing in ovarian cancer to individualize therapy remains an active area of interest and this has been renewed recently by results from several groups. The clinical results of assay-directed therapy are invariably better than would be expected from empirical treatment, but it has proved difficult to get these tests into practice.

Recent findings

Several recent studies suggest that cellular individualized tumour response tests, particularly the ATP-based tumour chemosensitivity assay, can improve the chance of response and probably survival for individual patients. Most tumour response tests show excellent correlation with clinical resistance, but vary in their ability to predict sensitivity. Molecular assays of sensitivity and resistance are less developed in ovarian cancer than in breast cancer, but those using multiple gene signatures show considerable promise.

Summary

Individualized therapy has the ability to guide the use of chemotherapy as well as newer agents. The development of companion diagnostics for drugs used in ovarian cancer has considerable potential for the future and such assays are already proving useful where there is clinical evidence of equivalence between possible treatments.

Keywords

assay, chemoresistance, chemosensitivity, ovarian cancer

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Concept of tumour heterogeneity and consequences

The selection of a chemotherapy regimen for an individual tumour is usually based on tumour histology and evidence from randomized clinical trials of different treatments in patients with the same diagnosis. However, individuals with the same histology often respond differently to the same chemotherapy regimen due to tumour heterogeneity. Cancers are just as individual as their hosts, and arguably more so, given that the effect of the acquired genetic alterations that give rise to them are influenced by the genotypic and phenotypic differences between patients. Despite widespread recognition of this heterogeneity, oncology remains a largely empirical discipline, on the basis of randomized clinical trials that treat all patients with one cancer type as though they were identical. Large numbers of patients are, therefore, required to show relatively small differences.

Individualized therapy takes a different approach. It requires information about the individual patient and his or her tumour provided by a pathology laboratory to allow the oncologist to choose between available treatment options.

Although evidenced-based and individualized approaches may seem to be at variance with one another, it is possible to combine the two, benefiting both patient treatment and drug development. Evidence from randomized trials often suggests that several approaches have similar efficacy, such that larger or further trials are necessary to decide between them. The difference in expected efficacy is often so small as to be academic. Yet the apparent inferiority of one regimen may be false for an individual patient, as evidenced by crossover effects within many trials showing that those patients who respond to one treatment may not be the same patients that would respond to another. Predictive testing is an attractive option in deciding which of several permissible regimens of similar toxicity to use in an individual patient. The next question is of course how to do this, and that is where the debate becomes difficult for individualized medicine, which has historically operated on a tiny budget compared with that poured into randomized trials of new drugs.

It is also important to distinguish between existing and newer agents. Newer agents often have a well-defined molecular target and definition of responsive patients may be relatively easy on the basis of this information. There may also be a financial incentive for pharmaceutical

companies and healthcare organizations to develop companion predictive diagnostic tests for such agents. However, the same does not apply to existing cytotoxic drugs, which have more general mechanisms of action and require a broader approach to assay design.

This review of recent progress in individualized testing for ovarian cancer is based on an Entrez-Pubmed literature search for papers published in 2007 and 2008 using the terms listed as keywords above. Reference is given to several other seminal papers in the field to provide a context for the comments made. Cell line papers were excluded from consideration as these are not directly relevant to patient care.

Cellular methods

The first methods to be designed for individualized therapy tested drugs directly against tumour-derived cells. This is an attractive option in gynecologic malignancies where it is often possible to obtain the large amounts of material these assays need. The initial ITRTs were 'clonogenic assays', and were derived from colony-forming unit assays used successfully with bacteria and bone marrow stem cells [1]. The various methods available have been reviewed by Fruehauf [2]. Stem cells are making a come-back [3,4], but clonogenic assays proved less robust than the original enthusiasm suggested. However, others took up the challenge, developing assays with a cell death endpoint, such as the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or ATP assay, or a proliferation endpoint such as the incorporation of tritiated thymidine, the basis of the extreme drug resistance (EDR) assay [5]. All of these assays produced encouraging results and many are still in use. The EDR assay tests single agents for resistance to high concentrations of drugs likely to indicate resistance and is not able to comment on sensitivity. Furthermore, it is only applicable to single agents and combination effects cannot be studied. The EDR assay is commercially available and quite widely used by oncologists in the USA. Several trials of this assay are in progress and results are awaited. Pathologists like to look at the cells they are studying, and several groups decided that it would be better to observe the effects of drugs directly. Development of the differential staining cytotoxicity assay and its derivatives ensued, but despite progress with image analysis, these are time-consuming tests and not easily automated [2].

However, many such assays ignored the problem that tumours contain large numbers of normal cells, and researchers continue to grow cells in serum-containing media, conveniently ignoring the fact that this can lead to the growth of nonneoplastic cells (usually less chemosensitive), whereas increasing the growth of neoplastic

cells that would then appear to be more sensitive to agents to which they were resistant [6]. Growth of adherent cells can also be problematic, particularly if steps are not taken to limit the growth of nonneoplastic cells [7,8].

The recent development of the ChemoFX assay provides a further variation on the ITRT concept. This assay is not strictly a primary culture, since it relies on sub-culture of cells growing out of explants. However, it needs relatively few cells, uses multiple concentrations and employs image analysis of stained cells to provide a viable cell count. It is a relatively slow assay, needing three weeks to produce results, but has been applied to ovarian cancer with initial studies providing evidence of efficacy [9]. This ITRT is now being validated in a large prospective study.

Our group took a different approach, and developed a serum and adherence-free cellular sensitivity and resistance assay with a sensitive ATP endpoint on the basis of luciferin-luciferase detection of biomass. It is important to distinguish this assay from others using an ATP endpoint, which use serum-containing media and are still in use by some centres [10^{*}]. The ATP-based tumour chemosensitivity assay (ATP-TCA) proved robust and reproducible and was in part developed using ovarian cancer samples. The heterogeneity of chemosensitivity of ovarian cancer to different drugs using this assay matched expectations, and it has since been used for the development of new drugs and regimens for ovarian cancer, for which it still has an important role.

Clinical validation of the ATP-based tumour chemosensitivity assay

Clinical application of the assay by Dr Christian Kurbacher in Cologne was delayed by a number of factors, but the first data in recurrent ovarian cancer (ROC) were published in 1998 [11]. They showed a spectacular difference in response rate and progression-free survival between an assay-directed treatment group, who received mainly doublet chemotherapy and a group treated empirically by another team in the same hospital with single agent chemotherapy [11]. The next step was difficult – the ensuing randomised trial of assay-directed therapy was the first of its type to be completed in ovarian cancer. It was seriously delayed by funding and insurance issues and these limited its size, but it was powered to answer the question whether the difference observed in the 1998 paper was due to the drugs or the test. If the former, then no difference was expected, but if both were influential, we expected a small, nonsignificant difference. The trial results [12^{**}] show exactly that and, therefore, suggest that both drug and test influenced the outcome in individual patients, though of course a

much larger study would be required to show this conclusively.

The trial produced several other take-home messages [12^{••}]. The first was that we only found complete resistance in the assay to all drugs tested in 5% of our platinum-resistant ROC patients. Most oncologists would expect this figure to be much higher, and in the past at least, many patients would have been offered best supportive care or hormonal treatment rather than further chemotherapy, as response rates around 10% with limited survival were common. The data provide hard scientific evidence that chemotherapy can work in this scenario, and should be offered to patients with some confidence.

The second message was that oncologists who use the ATP-TCA alter their preferred treatment when they see good results in patients treated with assay-directed therapy. Given the fact that the guidance issued to oncologists in the United Kingdom was that combination chemotherapy was ineffective in this setting, this was unexpected. It was equally unexpected that this switch to combinations designed using the assay, but chosen by the oncologist, would result in a highly statistically significant improvement in survival within the physician's choice group, though it did not match the response rate or survival in the assay-directed group.

The fact is that that any chemosensitivity assay is only as good as the drugs used, and to expect an assay to improve survival *per se* is clearly fallacious. Individualized treatment involves the combination of predictive testing (often on multiple occasions) with access to a wide range of drugs, allowing the oncologist to continue to obtain responses long after best guess treatment has failed to benefit the patient. The response obtained to any one course of assay-directed chemotherapy may not translate into improved survival, as they are unlikely to be greater or longer lasting than those obtained by empirical treatment, but multiple treatments may achieve much, as shown in a separate study which included some patients from the TCA Ovarian Cancer Trial [13]. This study showed that use of the ATP-TCA on multiple occasions to guide therapy could result in remarkably good survival, far better than expected from published data using empiric treatment. Indeed, some patients in this study had up to six courses of chemotherapy, with multiple remissions.

During the period under review, the ATP-TCA has been used independently by several groups. A group from Tuebingen have recently shown good correlation between ATP-TCA results and outcome in primary ovarian cancer [14^{••}]. This confirmed earlier studies and would now be considered to have validated the method for use in primary

ovarian cancer using modern norms for predictive assays (<http://linus.nci.nih.gov/~brb/>).

Taken together, it is possible to draw the following conclusions from studies of the ATP-TCA in ovarian cancer:

- (1) Sensitivity testing using the ATP-TCA is a robust diagnostic method, which has been independently validated in multiple series of patients from different centres.
- (2) The ATP-TCA can be used to aid choice of treatment for primary ovarian cancer from multiple evidence-based regimens with equivalence in clinical trials.
- (3) The ATP-TCA provides a means to find potentially effective treatments for patients who relapse, even after diverse primary treatment.
- (4) Sensitivity testing can be used to study mechanisms of resistance and sensitivity, and to develop new regimens, without exposing patients to needless risk in clinical trials.

Despite these encouraging results, the ATP-TCA has yet to enter clinical practice in all but a handful of sites around the world. It is available as a kit from a company in Germany (DCS GmbH, Hamburg), but needs expertise lacking in most pathology departments, and an appropriate laboratory. Perhaps more importantly, there is a need to send fresh tissue to the pathology laboratory, something that few centres do routinely, though this is perhaps not as difficult or as expensive as some believe. There are, however, many patients in whom the assay would require surgery to obtain tissue, and this is difficult to justify. There is, therefore, a need for tests, which do not require fresh tissue. Recent data suggest that molecular assays may offer a way forward.

Molecular methods

The most obvious molecular assays are based on single markers of potential significance such as the targets of doxorubicin (topoisomerase II α) or the putative target of taxanes (tubulin beta III) [15[•],16]. Although it is known that microtubules are the targets of taxanes, it is difficult to know exactly what proteins influence this, as a multitude of microtubule associated proteins may be involved, before one starts to consider other resistance mechanisms such membrane transporters of which MDR1/Pg-P is probably the most widely studied [17[•]]. The p53 molecule is much studied in this regard and still producing controversial papers [18], but is probably of limited use in predicting response. Such studies are reported when they produce positive results, but often fail to do so, and languish in filing cabinets.

The ability to look at multiple mechanisms by gene expression or immunohistochemistry has revolutionized the search for molecular predictive assays. Efforts have diverged between those taking a hypothesis-driven approach to design assays on the basis of small numbers of biologically relevant genes [19], and those taking a pan-genomic screening approach to find gene signatures correlated with outcome. An example of the former approach is the use of immunohistochemistry for a combination of VEGF, MDR1 and CD31 (microvessel density) to predict sensitivity [19], though as the authors admit, using just three markers ignores many of equal or greater significance. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) can also be used for this purpose, and the recent article by Nainwa *et al.* [20**] is a good example of the use of the hypothesis-driven approach. These authors looked at a combination of MDR1, MRP1, topoisomerase I and II, with considerable success, judged by Receiver Operator Characteristic curves, which provide the most robust assessment of a diagnostic test, but again ignored other genes of potential importance. The pan-genomic approach is expensive, but is popular, and successful [21,22*]. It can of course generate gene signatures, which can then be transferred to more robust platforms such as qRT-PCR or immunohistochemistry [22*] for clinical application.

For some agents, however, one gene may be enough to justify treatment. Tuefferd *et al.* [23] have recently reported that human epidermal growth factor receptor 2 is expressed in 6.6% of ovarian cancer patients, and amplified, raising the possibility that such patients could benefit from trastuzumab treatment.

Requirements for introduction

Whether molecular or cellular, all such tests need to be based in pathology departments for clinical application. The introduction of new tests to pathology requires the same degree of rigor whether it is aimed at hypertension, infection or cancer. There is a need for independent validation of any test in multiple centres, with a consideration of the need for external quality assurance to ensure that assay results in one centre are as robust as those elsewhere. There is a need to demonstrate that the test alters clinical management, but it is inappropriate to subject tests to clinical trials: these compare treatments or differing management models, not tests, though test results may influence the choice of treatment and can be used to improve the efficiency of clinical trials. Sargent *et al.* [24] published an important review of this topic, and suggested that an 'enrichment design' could be used to allow small trials to produce large returns, and higher response rates.

Conclusion

Clearly one size does not fit all when it comes to cancer treatment, but individualized therapy needs predictive tests to tailor treatment to individual patients. The requirement for such information is new to many pathologists, who tend to think that the job has been done when the diagnosis has been made, and many of the tests available have been developed by motivated researchers working in a variety of settings. It is also difficult to persuade healthcare managers and politicians of the value of expensive new drugs unless these are matched by considerable improvements in patient survival. Targeting drugs makes good economic sense and patients avoid the side effects of drugs from which they are unlikely to benefit.

The problem of which test to use is still an issue. For some patients, obtaining the amount of tissue required for cellular sensitivity testing requires surgery, which may not be otherwise indicated. Molecular methods may require less cancer tissue, but are often (currently) limited to the assessment of a relatively small number of drugs.

The need for predictive tests is greatest when there is a need to make a treatment decision. In the past, when in some countries guidance only allowed platinum chemotherapy for the treatment of primary ovarian cancer, there was little point in testing such tumours. Chemotherapy leads to adaptation of surviving cells to the drugs used, and cross-resistance to other drugs, depending upon which mechanisms of resistance the cancer cell uses [25]. The problem then becomes one of selecting second-line therapy – and the oncologist has a number of roughly equivalent options from which to choose. Any assistance at this point is usually welcome, and a predictive assay would be very useful.

The failure of the GOG-0182/ICON5 trial to show selective advantage between five arms using different regimens means that primary ovarian cancer is now ready for a clinical trial of sensitivity testing. The question to be answered is whether all those who responded in one arm of the GOG-0182/ICON5 trial would also have responded in another. Crossover data from many previous trials tends to suggest that this is unlikely, and chemoresponse testing provides strong scientific evidence to support the idea that responders in one arm might not have done so well in another arm of the study. It is, therefore, likely that patients will do better if chemoresponse testing is used to provide evidence of the individual likelihood of response to any treatment proposed.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 103).

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