

**ATP-based Chemosensitivity Testing: a New Direction for Chemotherapy**

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*This introduction to the ATP-TCA is intended for doctors, healthcare professionals, and others with some background knowledge.*

**Introduction**

While some types of cancer remain localised for many years or virtually never spread to other organs, others can be regarded as a systemic disease at the time the patient first becomes aware of the primary tumour. While localised tumours can be dealt with by surgeons with increasingly successful results, tumours which have spread (metastasised) to other organs are often impossible to remove and will ultimately kill the patient unless they respond to systemic treatment. This usually means chemotherapy with drugs designed to kill tumour cells, although immunotherapy (persuading the patient's immune system to kill the tumour) and anti-angiogenic therapy (targeting the blood vessels needed by the tumour to grow) are now viable options for some tumours. The much-hyped possibility of gene therapy is as yet just that - a possibility for the future.

However, the causes of cancer are now understood. Cancer arises when cells lose the molecular mechanisms that control their growth and death - the result is that they proliferate out of control and form tumours. These molecular mechanisms are controlled by genes encoded in the DNA of the cell, and acquired mutations or other changes in these genes lead to loss of control. Cancers are therefore genetic diseases, but only rarely familial. Each individual tumour, even those of the same general type (e.g. breast cancer) will have different mutations, the consequences of which may be altered by the tissue environment, which can in turn be influenced by the external environment. The result is that cancers are even more different from each other than the individuals affected. This heterogeneity means that a particular drug will rarely be effective against all tumours of a particular type, and that the degree of efficacy will vary between patients too. Not only that, but as a result of their original mutations, many tumour cells acquire the ability to adapt rapidly to changes in their environment, sometimes by further mutation, often by molecular changes which induce resistance to the drugs used to treat them. Further courses of chemotherapy then select for resistant cells, and the treatment eventually fails to control the tumour.
The Challenge

It has long been the goal of oncologists to be able to tailor chemotherapy to individual patients, to overcome the problems of heterogeneity and prevent rapid cellular adaptation. This can theoretically be done by testing the tumour cells to see if they are susceptible to particular drugs before giving them to the patient. The concept is widely acknowledged to be sound, but the development of such tests has proved difficult and many oncologists have given up hope that this idea will ever succeed. Many hope that molecular tests may hold the key to success, particularly as more specific drugs are designed to hit the molecular changes that are responsible for the uncontrolled growth of cancer cells in the first place. There are some indications that this approach may work - testing breast cancer for the presence of hormone receptors and more recently over-expression of growth factor receptors is of proven benefit to patients. However, most drugs cannot be looked at in this way, and even the tests that are in use have limited predictive accuracy (usually around 65%).

The Solution

So how about exposing cancer cells to the drug and testing their effect? Previous tests (assays) failed for a number of technical reasons, mainly because they needed large amounts of tumour material and could not determine whether all of the cells present were killed. In the early 1990s, Peter Andreotti and I started working with a new assay based on our knowledge of luminescence, the production of light from biochemical reactions. We were excited by the potential of ATP assays based on the firefly luciferin-luciferase reaction to measure the presence of very small numbers of bacterial cells in fluids, on surfaces and even in milk. Dead cells contain no ATP (it is used up in seconds following cell lysis by enzymatic degradation).

It was a small step to design a chemosensitivity assay using an ATP endpoint (fig 1), but we also had to find a way of exposing the cancer cells to the drugs without altering their behaviour from the original tumour. It is not possible to remove the non-cancer cells from the tumour without doing this. We therefore developed a selective culture method to get rid of the non-cancer cells before the end of the 6-day culture period used in the assay, as we just want to measure the degree to which the cancer cells themselves survive. Clinically achievable drug concentrations in tumours are rarely known with any accuracy, so we have used achievable plasma concentrations for most of the drugs we test, altered to take account of protein-binding and tissue distribution data. The result is an assay with the following characteristics:

- Needs relatively small amounts of tumour material (allowing histological and molecular analysis), though needle biopsies are not suitable.
- Measure multiple drugs and combinations.
- Measures 6 concentrations of 4 drugs in triplicate on one 96 well microplate.
- High evaluability rate (>95%).
- Clearly defined endpoint with criteria for analysis of results and interpretation.
- Good relevance to the clinical situation.
The ATP-based tumour chemosensitivity assay (ATP-TCA) has now been tested in a large variety of different solid cancers and has a remarkable track record. Within two years, the assay had produced evidence of heterogeneity similar to that seen in patients for both ovarian and breast cancer. Correlation with outcome proved to be 70-80\% in both breast and ovarian cancer, leading to trials of assay-directed treatment in ovarian cancer, breast cancer, and melanoma. The results in recurrent ovarian cancer are mature and were recently published, showing an increase in both progression-free survival and response rate. This has recently been confirmed in a second laboratory in a group of patients from multiple centres. A randomised phase II trial comparing assay-directed drug selection with physician’s choice from the same panel tested in the assay is complete and preliminary analysis of the data is encouraging.

The ATP-TCA has also shown itself to be capable of improving current therapy by aiding the development of new regimens and the assessment of new drugs (Kurbacher et al., 1994; Cree & Kurbacher, 1999). Many tumours are treated with combinations rather than single agents, and the ATP-TCA data on single agents has already proved invaluable for studies of the molecular basis of both resistance and sensitivity.
Figure 2. Assay results for the combination of cisplatin and gemcitabine in a recurrent ovarian carcinoma. There is resistance to cisplatin, with just 40% inhibition at the 100 test drug concentration, which equates to the maximum clinically achievable concentration. There is also resistance to gemcitabine, but in combination there is considerable synergy, and the result indicates likely activity of the combination.

The Future

The ATP-TCA has already established itself as the most promising chemosensitivity assay for clinical use in breast and ovarian cancer, and is being used in a variety of other settings. It is particularly appropriate for patients with rare tumours, or tumours of unknown type, for which there is little or no trial-based data available. In these instances, it has produced some spectacular results with regimens the oncologist would not otherwise have considered. The contribution of this assay to new drug and regimen development is set to grow considerably in the next few years. It has already contributed to the molecular understanding of chemosensitivity and resistance, and this too is likely to become even more important.
Publications


58. Whitehouse PA, Mercer SJ, Knight LA, Di Nicolantonio F, O’Callaghan A, Cree IA, on behalf of the Portsmouth Upper GI and Colorectal Cancer Multidisciplinary


