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## Review paper

# ATP-based tumor chemosensitivity testing: assisting new agent development

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**Chemotherapy of cancer based on cytotoxic agents has proved successful in the treatment of many cancers. The number of agents available to the oncologist has grown steadily and drug combinations are in widespread use. The perceived success of these combinations makes the introduction of new agents difficult. For any new agent, multiple phase II and III trials are likely to be needed. Since phase II/III trials usually only address single issues, the cost of introducing a new agent is substantial. Multiple studies are required with different tumor types to define the activity profile of a new drug, followed by adjusted combinations to define the role of the new drug in conjunction with older ones. Recent advances in the understanding of cancer at a molecular level are already leading to new agent design. The next problem is how to introduce and use these agents. One possible approach is to trial the drugs with tumor cells *ex vivo*, using a chemosensitivity assay such as the ATP-based chemosensitivity assay which is designed to mimic the situation within the tumor accurately enough to examine issues of dose response, sequence and timing in many different tumors. The avoidance of cell lines ensures relevance and the sensitivity of some of these methods allows large numbers of mechanistically logical permutations to be tested with material from small numbers of patients. The results may be used to choose the most effective combinations for clinical testing in a limited number of subsequent phase II/III trials, saving money and time, while permitting new agents to be introduced faster. [© 1999 Lippincott Williams & Wilkins.]**

*Key words*: ATP, chemosensitivity, chemotherapy, development, new drugs, regimen.

## Introduction

Cancer is usually a systemic disease by the time of diagnosis and as such requires systemic treatment. Medical oncology has had several notable successes, particularly in the treatment of certain lymphoproliferative diseases, testicular tumors and choriocarcinoma, all of which were previously rapidly fatal. The pharmaceutical industry has provided oncologists with many drugs, working through a number of different mechanisms. Recent developments in the molecular understanding of cancer are leading to the development of yet more drugs with differing mechanisms of action, some of which will be more specific and may act at a genomic level.[1] However, this wealth brings its own problems.

Evidence-based reviews are increasingly used to direct treatment and the majority of patients now receive combinations of drugs which have been extensively (and expensively) tested in clinical trials. Any new agent has a problem: it must look good enough in preclinical and limited phase I/II trials (often in heavily pretreated patients who may not be representative) for the investment in further phase II/III trials to be justified.[2] It is becoming increasingly difficult and costly to introduce new chemotherapeutic agents. Emphasis on managed health care has lead to a requirement for drugs to demonstrate cost effectiveness as well as efficacy. Even effective drugs such as paclitaxel are not available for use in parts of the UK for this reason.[3] The bottleneck in drug development is now not just pre-clinical, but in clinical development too. It is hardly suprising that many drugs fall at the clinical hurdle.

## Heterogeneity of chemosensitivity

In addition, the current approach to drug development completely ignores the differences between tumors of the same type. In any clinical trial, drug A may look better than drug B, but the those patients responding

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to drug A may not be the same as those responding to drug B, which might be a very useful agent in its own right (Figure 1). This heterogeneity is well recognized and is now known to exist at a molecular level as well as a clinical level.[4,5] Few tumors have the same molecular phenotype or indeed the same mutations within a single gene.[4] There is increasing evidence that such differences are reflected clinically in differing responses to treatment.[6]

One of the best examples of this is melanoma, where 20% response rates have been reported for multiple single agents, particularly vincristine, DTIC, cisplatin and paclitaxel. However, there is no clinical evidence that these patients are the same. Rare patients even respond to anthracyclines.[7,8] There is molecular heterogeneity too—30% of primary cutaneous melanomas show loss of p16 expression, while 20-40% have p53 mutations and about 1% express MDR1.[9-11] In breast cancer, the picture is similar with somewhat higher response rates to individual agents, particularly taxanes and anthracyclines. Cross-over results from phase II/III trials suggest that patients show considerable heterogeneity and that some may benefit from drugs which would not be the physician's first choice for the majority.[12-15]

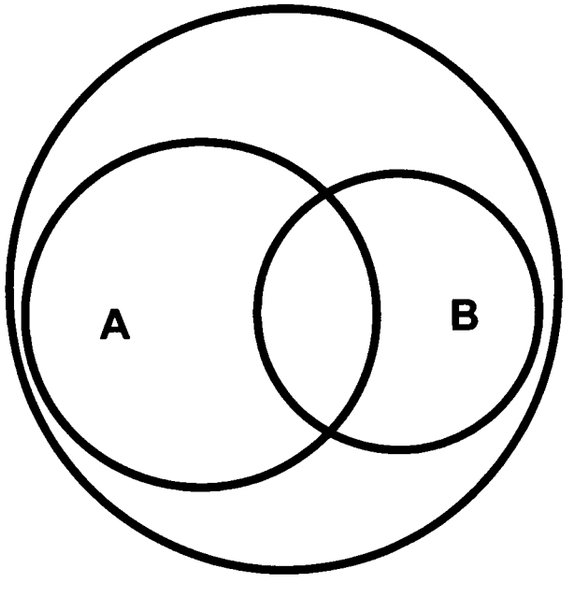


**Figure 1.** A Venn diagram summarizing the results of a hypothetical clinical trial. In this example, drug A does better than drug B and is therefore preferred for treatment of those with the tumor, represented by the larger circle. However, those responding to drug A and drug B are not all the same patients. The ability to determine which patients should receive drug A and which should get drug B would improve the overall response rate.

One way round the problem of heterogeneity is to individualize patient therapy. This may be done by molecular methods (e.g. estrogen receptor assay) or by *ex vivo* chemosensitivity testing to examine the sensitivity of tumors to different agents before treating the patient.[16-18] Even with increased agent specificity, it is unlikely that many drugs will have single molecular determinants of sensitivity and resistance because these molecules do not act alone, but as part of complex interacting pathways within the cell.[19] Furthermore, cells adapt to their changing environment: measuring aspects of the molecular phenotype before exposing cells to drugs may not be sufficient. While prediction of chemosensitivity on the basis of multiple molecular measurements may prove feasible in the long term, in the interim *ex vivo* chemosensitivity testing at the cellular level is beginning to show considerable promise.[18,20]

Previous attempts to use chemosensitivity testing have run into considerable technical difficulties, but several laboratories have persisted with the clonogenic stem cell or other assays (reviewed by Bellamy,[16] Bosanquet,[17] and Cree and Kurbacher[18]). We have been working with the ATP-based tumor chemosensitivity assay (ATP-TCA), a cytotoxicity assay which has now been tested extensively in comparison with outcome, showing 76-80% correlation in breast and ovarian cancer series.[21,22] This compares well with estrogen receptor assays and microbiological antibiotic sensitivity testing. Further, assay-directed therapy has showed improved response rate and progression-free survival in recurrent ovarian cancer.[20] A randomized phase III controlled trial of assay-directed therapy versus physician's choice is now in progress in platinum-resistent recurrent ovarian cancer[23] and further studies in metastatic melanoma are in progress. If trials confirm the clinical utility of this approach, it might be feasible to introduce widespread pre-chemotherapy testing.

Several studies have now shown that the ATP-TCA can also be used to aid the development of new regimens and to provide therapeutic options for rare tumors in which phase II trials are less common.[24,25] It has also already shown considerable utility in evaluating new agents[26] and regimen design.[14,15,24,25] In another role, the ATP-TCA has been shown to facilitate examination of molecular mechanisms of chemosensitivity and resistance.[27,28] In these studies, the individual agent data can be compared with molecular analysis of the tumor, allowing the relative contribution of individual molecular determinants of sensitivity/resistance to be assessed.

## Tumors or cell lines?

Batteries of cell lines are commonly tested during preclinical drug development and the results used to give some indication of which tumors might be sensitive to the drug in question.[29] They can be used with median effect analysis to show whether certain combinations are synergistic or antagonistic.[30] In chemosensitivity assays, cell lines are often found to be poorly representative of the behavior of 'real' tumor cells.[31] The reasons for this are complex, but relate partly to phenotypic drift as the cells adapt, often by mutation, to survive in cell culture medium. Media used for cell culture often provide considerable growth stimulation, either by the addition of serum or artificial supplements. Cells in culture have a much faster growth rate and doubling time than the parent tumors.[31] This suggests that testing of tumor-derived cells in serum-free media providing less growth support could have a number of advantages in targeting drugs to the most appropriate tumors.

A few laboratories have persisted in the use of primary tissue with clonogenic or other assays to provide the pharmaceutical industy with information on the efficacy of new agents *ex vivo* for clinical trial planning.[32] The San Antonio experience with taxanes is a good example of how such planning can help—although this has not stopped very large numbers of trials of paclitaxel being conducted.[33] The utility of this approach has been demonstrated again for a new platinum agent, oxaliplatin, which has been targeted towards particular tumors based partly on this data.[34]

Despite the apparent success of the clonogenic and other *ex vivo* assays for clinical trial planning, the use of primary tumor tissue does have its problems. Many methods require separation of neoplastic cells from normal cells (such as fibroblasts) in the tumor.[17] However, this procedure often leads to differences in the behavior of the cultured cells whose phenotype is partly determined by the presence of other cells which provide a specific microenvironment differing considerably from that in culture.[31] In the ATP-TCA, a serum-free medium is part of the process by which neoplastic cells are selectively enriched, but the normal cells are not immediately removed. In this context, since environmental conditions differ between tissues, it is likely that occasional cases will be found in which a biopsy from one source will show differential sensitivity with another (e.g. bone and skin metastasis). Such differences in the chemosensitivity of metastases in different tissues have long been recognized clinically. However, our experience to date suggests that major differences are relatively rare and may be specific to certain drug-tumor pairings (Cree *et al.*, unpublished).

## A new approach

A new approach involving the use of chemosensitivity testing is outlined in Figure 2. The key decision point would continue to be at the preclinical/clinical development interface, but it would be possible to submit a drug to phase I testing and *ex vivo* evaluation at the same time, allowing concommitant assessment of its role in relation to exisiting drugs and safety testing. *Ex vivo* testing would provide a wealth of data, including efficacy in different tumor types, cross-resistance with existing agents and possible combinations. The results can be correlated with molecular analysis of the tumor using multi-analyte methods such as 'DNA chips' or *in situ* methods such as immunohistochemistry and *in situ* hybridization. This allows rational regimen design.

With sufficient resources, it should be possible for every new drug to be tested with more than 100 tumors of the common types within a year alongside molecular analysis. Combinations can be designed using this and preclinical data, and tested in further chemosensitivity assays before the expense of phase II trials is attempted. Chemosensitivity assays have been used to examine issues of scheduling with considerable success, again reducing the number and complexity of required phase II trials. Such data may facilitate the switch from phase I to randomized trials (phase II or III) and reduce the number required, allowing more

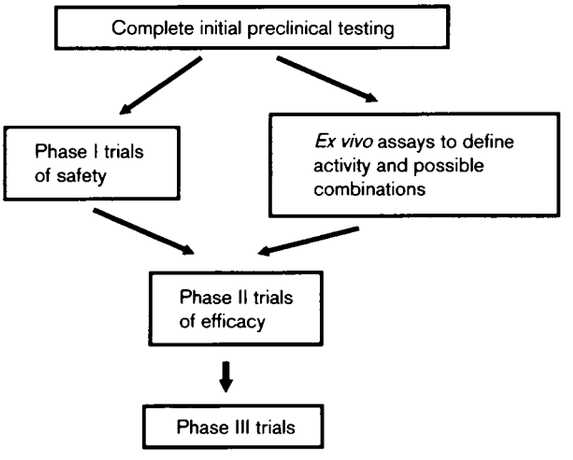


**Figure 2.** A scheme for drug development using chemosensitivity testing and standard phase I trials to direct the design of phase II trials.

rapid introduction of new agents at reduced cost. If the ATP-TCA or another chemosensitivity assay proves clinically applicable, then it may be possible to conduct phase II trials within chemosensitive subsets of tumor patient populations, as is already done for anti-microbiological agents. For agents acting at a molecular level, similar targeting of patients may be possible.

In conclusion, current methods of drug development are proving cumbersome and expensive, increasing drug costs, and impeding the introduction of new agents. Increased use of molecular and *ex vivo* chemosensitivity testing to evaluate new drugs and design combinations should limit the number of subsequent phase II/III trials required, saving money and time, while permitting new agents effective for subsets of cancer patients to be introduced faster.

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