Anti-Cancer Drugs 1997, **8**, pp. 541–548

Review paper

Individualizing chemotherapy for solid tumors — is there any alternative?

Ian A Cree and Christian M Kurbacher[1]
Department of Pathology, Institute of Ophthalmology, University College London, London EC1V 9EL, UK. Tel: (+44) 171 608-6808; Fax: (+44) 171 608-6862. [1]Labor fur Chemosensitivitatestungen, Universitats Frauenklinik, University of Cologne Medical Centre, Kerpenerstrasse 34, Cologne 50931 Germany

The burgeoning understanding of the molecular basis of carcinogenesis and tumor drug resistance is matched by an appreciation of the complexity of individual tumors. This complexity underlies the heterogeneity of response to treatment and is a major barrier to improving the outcome of solid tumor chemotherapy. While individualization of chemotherapy is theoretically attractive, past attempts to provide such information have produced many papers and little progress. However, the disparate molecular make-up of tumors of the same clinicopathologic type suggests that there may be no alternative and recent progress suggests that individualization of cancer therapy could have considerable benefits. In this review, we consider the alternative methods which might be employed and the requirements which need to be met before they are introduced. It will be some time before molecular analysis can predict chemosensitivity, although this may prove feasible for more specific agents than those currently in use. However, newly developed cellular chemosensitivity assays such as the ATP-tumor chemosensitivity assay allied to selected molecular measurement may already have the potential to select optimal therapy for patients. We need to develop a diverse series of acceptable and biologically logical regimens for each of the common tumor types, all of which can be tested *in vitro*.

Key words: ATP, chemosensitivity, chemotherapy, luminescence, *in vitro*, prediction.

Introduction

Despite many advances in understanding of carcinogenesis and an enlarged repertoire of anti-cancer agents, mortality rates from solid tumors remain stubbornly high. The lack of good response rates in metastatic or advanced disease is particularly problematic. For common tumors, vast resources are devoted to testing new regimens for adjuvant, palliative and neo-adjuvant treatment. The high cost of trials to examine small differences leads to a slow rate of new regimen development. Furthermore, as each new agent comes to market, the number of trials required increases.

Cancer is now known to be the result of multiple genetic defects within a cell leading to loss of growth control. However simple this concept may be, any cell requires multiple mutations before it can produce a tumor and probably several more before it can metastasize. The mutations are not always the same for the same tumor type, indeed even p53, the most commonly mutated gene in human cancers, is of wild-type in up to 60% of breast carcinomas.[1,2] Many tumors have an increased ability to mutate. This 'mutator phenotype' is mediated by mutation of one or more genes controlling the progression of genetically suspect cells through the cell cycle.[3] They include a series of growth suppressor genes, of which p53 and RB are the most well known, and DNA repair enzymes. The genetic instability which results from formation of a mutator phenotype underlies the acquisition of further mutations in growth genes resulting in tumor formation.[4] It also almost certainly underlies the ability of cancer cells to become rapidly resistant to cytotoxic agents, many of which are DNA-damaging mutagens which may paradoxically enhance this process.[5] However, the effect of these mutations is modulated by external influences from normal cells within the tissue affected and within the tumor once this is established. The phenotype of tumor cells can vary widely[6] and it is probable that this explains the observed differences in the influence of particular gene defects (e.g. p53) on the response to chemotherapy both *in vitro*[5,7] and *in vivo*.[8] Chemosensitivity is *context sensitive*. The point can be taken

This research is funded in part by the Guide Dogs for the Blind Association.

Correspondence to IA Cree

further: the degree of cellular stress will in part determine the ability of the cell to respond appropriately to chemotherapeutic agents, since stressed cells turn on p53 and other genes which prevent entry of damaged cells into the cell cycle[9]. Stress levels between cells vary, particularly within larger tumors, and there may therefore be heterogeneity of chemosensitivity *within* larger tumors of cells with the same genotype which will influence whether the patient has a sustained response.

Strategies for overcoming resistance

Current strategies for overcoming chemoresistance are based on the adjustment of dose and sequence to obtain an optimal effect. The use of drug combinations to prevent resistance is commonplace in both microbiology and oncology. The concept of treating the patient with two or more drugs to acheive enhanced tumor cell kill through additive or synergistic effects is attractive, and if the cells are resistant to one drug, they will probably be killed by the second.[10]

Since chemotherapy is too toxic to normal tissues to be given for long periods of time, it is usual to give cytotoxic agents at intervals, separated by an interval dependent upon the recovery time of the patient's bone marrow and other normally dividing cells. The use of granulocyte colony-stimulating factor (G-CSF) and cytoprotectants has allowed this interval to be compressed by up to 50% for a number of drugs.[11,12] Dose intensification of this type is given on the premise that the tumor recovers slowly and requires multiple cell divisions to acquire resistance. Hence the use of shorter intervals between cytocidal doses should prevent resistance developing in situations such as p53 mutation where rapid relapse is more common.[13–16]

Some drugs can also be given on the usual schedule, but at higher dose using G-CSF rescue, leading to higher intra-tumor drug concentrations and greater likelihood of tumor cell kill. The logical conclusion of this thinking is the use of stem cell rescue following the administration of very high-dose chemotherapy designed to destroy all tumor cells in breast and ovarian cancer.[17–19] Unfortunately, this rarely occurs. Such high doses are associated with high response rates, but partial responses are common — in solid tumors many cells are just not in growth phase and these are poorly susceptible to the agents used. It is debatable whether to exceed the minimum inhibitory concentration (MIC) by 10-fold is any better than exceeding it 1-fold. Since most cell lines and primary tumor cell cultures show sigmoid dose–response curves, it is often the case that a 2- to 3-fold increase in drug concentration will *not* result in 100% tumor cell death.[20–22]

Since resistant clones are likely to occur in situations where large numbers of tumor cells receive treatment, it is no surprise that adjuvant chemotherapy following removal of much of the tumor load is successful in reducing recurrence rates.[19] As an extension of this, Bonnadonna[23] proposed that following one therapeutic regimen with another to which the patient was unlikely to develop resistance would be even more successful. Such sequential therapy regimens are now undergoing clinical trial in both the USA and Europe.[24]

Individualization of chemotherapy

While the concept of combination chemotherapy is well-established, the use of several drugs, one or more of which may be ineffective, brings with it some inherent problems. It is often the case that the ineffective drug will contribute toxicity when it might have been possible to use the effective drug on its own at higher dose or with an effective alternative. The problem is knowing which tumors respond to which drug!

The heterogeneity of chemosensitivity of solid tumors has been apparent since the early days of chemotherapy. In a trial of two regimens (A and B), regimen A may produce a 60% response, while regimen B produces a 50% response. However, not all those given regimen B will respond to regimen A and if regimen A is then used for all patients with this tumor type, up to 40% may experience toxicity with no benefit.

Molecular studies confirm and clarify reasons behind this heterogeneity, since it is now apparent that tumors are genetically and phenotypically heterogeneous. To overcome this heterogeneity it is necessary to choose the drugs for each patient according to their likelihood of response. Recent evidence that the site of mutation within the p53 molecule can influence chemosensitivity[25,26] was predictable[5,27] given the pleotropic effects of this molecule. Different molecular pathways interact leading to a level of complexity from different mutations which may well explain the observed heterogeneity of chemotherapeutic sensivitivity in many tumors.[5,27]

Tumors are therefore heterogeneous at the mole-

cular, cellular and tissue level. The requirement for individualization of therapy is obvious and needs to be addressed to take advantage of the increasing number of agents available for treatment.

Potential methods for individualizing cancer therapy

Some molecular methods for individualizing therapy are already in use. It is now mandatory to measure the estrogen receptor (ER) status of breast tumors and this is often used as a guide for the use of tamoxifen or other hormonal drugs. ER positivity has a 70% or so correlation with response and this is felt to be acceptable guidance by many oncologists.[28] Other molecular/histopathological methods of individualizing therapy are being developed, but their track record is poor.[29] Few can guide treatment for more than one drug or choice of chemotherapy versus radiotherapy. While some have suggested that measurement of levels of expression of markers such as p53 and *bcl* series gene products may be able to predict individual tumor responses,[30] evidence for this is lacking.[5] We believe that the large number and complexity of such measurements that will be needed, together with the pleotropic nature of oncogenes[27] make it extremely unlikely that such approaches will succeed. However, the use of selected molecular measurements associated with use of new highly specific agents acting on oncogenes or intracellular pathways may well prove valuable.

The use of neoadjuvant therapy and the therapy of advanced tumors based on tumor marker levels are also forms of individualized therapy. One can see the tumor response and alter the regimen accordingly. However, often, the problem is knowing which of the drugs in any combination are effective and which should be dropped. Similar problems encountered by endocrinologists and immunologists in the whole organism or in organs have proved intractable in the past due to the immense complexity of these systems: the problems inside the cell appear even greater.

In vitro chemosensitivity assays in which tumor cells are exposed to drug also have a poor track record.[31–33] However, here the problems are mainly technical—in the past such assays have often had poor evaluability rates.[31–34] There were indications from the few clinical trial results available that such testing certainly did no harm to patients and indeed may have provided some benefit.[35,36] However, consistent failure has led many oncologists to abandon such assays despite the fact that there is no rational alternative in sight.[32,33,37]

Chemosensitivity assays

Clonogenic assays show the effect of anticancer agents on cell division (Table 1), while non-clonogenic assays (Table 2) show the effect on cell viability, usually by means of a measure of metabolic activity. Assays of both types continue to be used extensively with cell lines for drug screening, but their use with primary tumor cells has been plagued by technical difficulties. It should be emphasized here that drug screening has different requirements to *ex vivo* tumor chemosensitivity testing. Most such assays require isolation of cancer cells from others and then require the cells to grow or at least survive *in vitro* for a variable time. Isolation of tumor cells from the tissue is not only technically demanding, but often detrimental to expression of the 'real' phenotype of the cells *in vivo* which is determined to some extent by interaction with these cells. For instance, evidence from xenograft studies suggests that removal of fibroblasts can reduce expression of MDR1 in colonic cancer leading to spurious doxorubicin sensitivity.[38,39] If large cell numbers are required for *in vitro* assay, this causes clinical problems as the bulk of the tissue is often required for histopathology. It totally excludes patients in

Table 1. Clonogenic assays

Assay	Duration days	Evaluability rate (%)	Predictive accuracy (%)	Tumor types	References
Stem cell assay/ capillary stem cell assay	14–21	51–76	40–70	solid/hematogenous	34–36, 65–71
Thymidine incorporation	5	80	70	all	72–74

The figures given are estimated from the literature and only key references are quoted.

Table 2. Non-clonogenic assays

Assay	Duration days	Evaluability rate (%)	Predictive accuracy (%)	Tumor types	References
DiSC assay	4–6	86	83	all—especially hematogenous	48–49, 74–76
MTT assay	4–5	85	62	hematogenous, especially acute leukemia	31, 40–45, 78
ATP assay	6–7	> 97	75–85	solid tumors	5, 6, 12, 20, 22, 31, 41, 53–57, 63
Fluorescent cytoprint assay	4–5	91	87	solid tumors	47, 77
Histoculture drug response assay (HDRA)	7 days	96	92	solid tumors	46

DiSC = differential staining cytotoxicity, MTT = tetrazolium salt, ATP = adenosine triphosphate. The figures given are estimated from the literature and only key references are quoted.

whom surgical intervention is unlikely to be of benefit.

The ideal tumor chemosensitivity assay (TCA) would have the following characteristics:

- Ability to use small amounts of tumor material, always leaving sufficient for histological diagnosis and molecular analysis (e.g. ER status).
- Ability to measure multiple drugs and combinations using less than 5×10^6 cells.
- Provision of response information over a range of at least six concentrations.
- High evaluability rate (greater than 90%).
- Clearly defined criteria for analysis of results and interpretation.
- Good relevance to *in vivo* situation

Such criteria are only barely met by modern bacterological testing and TCAs have some way to go before they reach the same level of sophistication. Many of the methods used to date are technically demanding. It is unlikely that any successful assay will reach routine clinical practice unless it has good inter-laboratory reproducibility and can be performed routinely by relatively junior laboratory staff. The most successful assays according with these criteria at present are all non-clonogenic: the MTT assay, the DiSC assay and the ATP-TCA.

The MTT assay is the best known example of a number of assays based on detection of cell viability by conversion of an added substrate to a colored or fluorescent product by intracellular enzymes.[40] Loss of viability results in loss of the enzyme from the cell. The MTT assay compares favorably on price with the ATP-TCA, but requires larger numbers of cells and some drugs can interfere with the conversion of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) by mitochondrial succinate dehydrogenase.[40,41] There are a number of other problems, including limited dynamic range, the metabolic state and pH of the tumor cells, and reagent quality[31,42,43] as well as growth of normal cells.[44] Nevertheless, it has been shown that assay AUC values correlate well with clincally achievable AUC values for mitomycin C and nimustine–HCl.[45] The MTT has been applied as the end-point of micro-organ culture assays, including the histoculture drug response assay (HDRA).[46] The fluorescent cytoprint assay (FCA) uses a metabolic method resulting in fluorescein release from living cells in short-term agar cultures of small 'micro-organs' containing approximately 50 cells.[46]

The DiSC assay has proved particularly useful in hematogenous malignancy[48] and has been used prospectively to guide treatment in non-small cell lung cancer,[49] although it is technically demanding. It does not require single-cell suspensions or pure tumor cells and is not dependent upon cell division. Cells are imposed to cytotoxic drugs and then stained with fast green/nigrosin blue. Those cells which fail to exclude the dye are dead and the proportion of tumor cells killed is estimated by direct microscopy. This requires considerable cytological expertise for some tumors in which it can be difficult to distinguish normal and malignant cells. Several newer assays are also of interest, particularly those based on the detection of apoptosis in small numbers of cells,[50] the semiautomated fluorometric microculture cytotoxicity assay (FMCA)[51] and those measuring cell differentiation.[52]

Recently, we have been involved in further development of the ATP-TCA,[5,6,12,20,22,41,53–57] an assay based on the rapid loss of ATP from dead cells and the availability of extraordinarily sensitive luminescence assays using firefly luciferase,[58] which has been used by a number of groups.[59–62] This assay requires only 20 000 cells/well with a minimum of 20% tumor cells and can handle a variety of different samples (surgical, needle biopsy, effusion). Many technical problems associated with TCAs have been overcome by this assay.[6,22,55] In particular, normal cells do not interfere with the assay, but are not removed immediately.[55] This maintains the environment of the tumor cells more faithfully than assays requiring pure tumor cell populations[38] and is accomplished using a serum-free assay medium with polypropylene plates which do not support cell attachment.[55] Intra- and inter-assay variation is less than 10%, and the assay is both simple and objective.[55] The concentrations of each agent used in the assay have been adjusted to give the greatest discrimination between sensitive and resistant tumors.[55]

The ATP-TCA shows good evaluability (97% in one recent series of breast cancer biopsies)[63] and has good correlation with clinical outcome in both breast and ovarian cancer.[55,63] The ATP-TCA has already shown its ability to aid the development of new therapeutic regimens.[12] This is consistent with a requirement for different regimens which hit different aspects of tumor cell biology. ATP-TCA results can be used in conjunction with molecular analysis.[5,38] It can be used for preclinical drug assessment with real tumors,[56] not just cell lines, reducing the need for animal studies. Derivation of dose–response curves allows detailed examination of which drugs are suitable for dose intenstification[57] (Kurbacher *et al.*, unpublished).

Few TCAs have been subjected to clinical trial against physicians' choice: several trials have foundered on the inability of the assay to produce good evaluability rates with clinical samples.[31] However, recent experience in a case control study of recurrent ovarian cancer (Kurbacher *et al.*, unpublished) suggests that the ATP-TCA is worthy of such evaluation. In a prospective series of 25 patients and 30 controls, ATP-TCA directed therapy produced a 64% RR (37% in controls) and increased progression-free survival from 20 to 45 weeks. The majority of responses occurred with experimental combinations rather than the more commonly used single agents, but the diversity of different combinations used makes it likely that the ATP-TCA made a considerable contribution.

Conclusion

There is a need for a critical attitude to current chemotherapy success rates in solid tumors: the current level of success is still depressing despite the availability of new drugs.[33] There is overwhelming evidence of tumor heterogeneity at the molecular, cellular and tumor level. Some form of objective method is required to choose the best drug for each patient and it is necessary to avoid judging current success by past failures.[33,64] TCAs are only as useful as the drugs available, but with increasing diversity of cytotoxic agents, individualization of chemotherapy is biologically justified and provides a means to exploit advances in the understanding of cancer. The second generation of TCAs (particularly the ATP-TCA and DiSC assay) have good evaluability and have been designed to work with small tumor samples. Many can be used in conjunction with molecular analysis.

In the past, it may have been acceptable to introduce new surgical and diagnostic methods without prospective clinical trials. This is no longer the case and it is indeed up to the proponents of such assays to conduct randomized trials comparing empirical choice with TCA-guided therapy. Such trials are urgently required—a working TCA could be of immense benefit to the pharmaceutical industry, oncologists and their patients.

Acknowledgments

We are grateful to all those who have contributed to the development of many of the ideas expressed in this paper, particularly Drs Andreotti, Bruckner and Untch. Both authors are members of the Preclinical Therapeutic Models Group of the EORTC.

References

1. Mattieu M-C, Koscielny S, Le Bihan M-L, Spielman M, Arriagada R. p53 protein overexpression and chemoresistance in breast cancer. *Lancet* 1995; **345**: 1182.
2. Pietilainen T, Lipponen P, Aaltomaa S, Eskelinen M, Veli-Matti K, Syrjanen K. Expression of p53 protein has no independent prognostic value in breast cancer. *J Pathol* 1995; **177**: 225–32.
3. Loeb LA, Christians FC. Multiple mutations in human cancers. *Mutat Res* 1996; **350**: 279–86.
4. Kinzler KW, Vogelstein B. Gatekeepers and caretakers. *Nature* 1997; **386**: 761–3.
5. Petty RD, Sutherland LA, Hunter EM, *et al.* Expression of the p53 tumor suppressor gene product is a determinant of chemosensitivity. *Biophys Biochem*

Res Commun 1994; **199**: 264–70.
6. Hunter EM, Sutherland LA, Cree IA, Dewar JA, Preece PE, Andreotti PE. Heterogeneity of chemosensitivity in human breast carcinoma: use of an adenosine triphosphate (ATP) chemiluminescence assay. *Eur J Surg Oncol* 1993; **19**: 242–9.
7. Waldman T, Lengauer C, Kinzler KW, Vogelstein B. Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21. *Nature* 1996; **381**: 713–6.
8. Cote RJ, Esrig D, Groshen S, Jones PA, Skinner DG. p53 and treatment of bladder cancer. *Nature* 1997; **385**: 123–5.
9. Lane DP. p53: guardian of the genome. *Nature* 1992; **362**: 786–7.
10. Riggs CE, Bennett, JP. Combination Chemotherapy. In: Moosa AR, Schimpff SC, Robson MC, eds. *Comprehensive textbook of oncology*, 2nd edn. Baltimore: Williams & Wilkins 1991: 565–8.
11. Fennelly D, Wasserheit C, Schneider J, *et al.* Simultaneous dose escalation and schedule intensification of carboplatin-based chemotherapy using peripheral blood progenitor cells and filgrastim: a phase I trial. *Cancer Res* 1994; **54**: 6137–42.
12. Kurbacher CM, Bruckner HW, Cree IA, *et al.* Mitoxantrone combined with paclitaxel as salvage therapy for platinum-refractory ovarian cancer: laboratory study and clinical pilot trial. *Clin Cancer Res*: in press.
13. Bosari S, Viale G, Radaelli U, Bossi P, Bonoldi E, Coggi G. p53 accumulation in ovarian carcinoma and its prognostic implications. *Hum Pathol* 1993; **24**: 1175–9.
14. Niwa N, Itoh M, Murase T, Itoh N, Mori H, Tamaya T. Alteration of p53 gene in ovarian carcinoma: clinicopathological correlation and prognostic significance. *Br J Cancer* 1994; **70**: 1191–7.
15. Levesque MA, Katsaros D, Yu H, *et al.* Mutant p53 protein overexpression is associated with poor outcome in patients with well or moderately differentiated ovarian carcinoma. *Cancer* 1995; **75**: 1327–38.
16. van der Zee AG, Hollema H, Suurmeijer AJ, *et al.* Value of P-glycoprotein, glutathione S-transferase pi, c-*erbB*-2, and p53 as prognostic factors in ovarian carcinomas. *J Clin Oncol* 1995; **13**: 70–8.
17. Antman K, Ayash L, Elias A, *et al.* High dose cyclophosphamide, thiotepa, and carboplatin with autologous marrow support in women with measurable advanced breast cancer responding to standard dose therapy: analysis by age. *Monogr Natl Cancer Inst* 1994; **16**: 91–4.
18. Stewart THM, Retsky MW, Tsai SCJ, Verma S. Dose response in the treatment of breast cancer. *Lancet* 1994; **343**: 402–4.
19. Leonard RC. The importance of dose and schedule in cancer chemotherapy: breast cancer. *Anti-Cancer Drugs* 1995; **6** (suppl 5): 17–27.
20. Andreotti PE, Linder D, Hartmann DM, Cree IA, Pazzagli M, Bruckner HW. TCA-100 tumor chemosensitivity assay: differences in sensitivity between cultured tumor cell lines and clinical studies. *J Biolum Chemilum* 1994; **9**: 373–8.
21. Cree IA, Pazzagli M, Mini E, *et al.* Methotrexate chemosensitivity by ATP luminescence in human leukaemia cell lines and in breast cancer primary cultures: comparison of the TCA-100 assay with a clonogenic assay. *Anti-Cancer Drugs* 1995; **6**: 398–404.
22. Hunter EM, Sutherland LA, Cree IA, *et al.* The influence of storage on cytotoxic drug activity in an ATP-based chemosensitivity assay. *Anti-Cancer Drugs* 1994; **5**: 171–6.
23. Bonadonna G, Valagussa P. Combined modality approach for high-risk breast cancer. The Milan Cancer Institute experience. *Surg Oncol Clin N Am* 1995; **4**: 701–14.
24. Hudis C, Seidman A, Raptis G, *et al.* Sequential adjuvant therapy: the Memorial Sloan-Kettering Cancer Center experience. *Semin Oncol* 1996; **23**: 58–64.
25. Aas T, Borresen AL, Geisler S, Smith-Sorensen B, *et al.* Specific P53 mutations are associated with *de novo* resistance to doxorubicin in breast cancer patients. *Nat Med* 1996; **2**: 811–4.
26. Delia D, Mizutani S, Lamorte G, Goi K, Iwata S, Pierotti MA. p53 activity and chemotherapy. *Nat Med* 1996; **2**: 724–5.
27. Steel M. Cancer genes: complexes and complexities. *Lancet* 1993; **342**: 754–5.
28. Hamm JT, Allegra JC. Hormonal therapy. In: Moosa AR, Schimpff SC, Robson MC, eds. *Comprehensive textbook of oncology*, 2nd edn. Baltimore: Williams & Wilkins 1991: 597–600.
29. Zeigler LD, Connelly JH, Frye D, Smith TL, Hortobagyi GN. Lack of correlation between histologic findings and response to chemotherapy in metastatic breast cancer. *Cancer* 1991; **68**: 628–33.
30. Kernohan NM, Cox LS. Immunochemical challenges and therapeutic implications. *J Pathol* 1996; **179**: 1–3.
31. Bellamy WT. Prediction of response to drug therapy of cancer. A review of *in vitro* assays. *Drugs* 1992; **44**: 690–708.
32. Bosanquet AG. In vitro drug sensitivity testing for the individual testing: an adjunct to current methods of treatment choice. *Clin Oncol* 1993; **5**: 195–7.
33. Cree IA, Petty RD, Kurbacher CM, Untch M. Comments on tumor chemosensitivity and chemoresistance assays. *Cancer* 1996; **78**: 2031–2.
34. Von Hoff DD. He's not going to talk about *in vitro* predictive assays again, is he? *J Natl Cancer Inst* 1990; **82**: 96–101.
35. Welander CE, Homesley HD, Jobson VW. *In vitro* chemotherapy testing of gynecologic tumors: basis for planning therapy? *Am J Obstet Gynecol* 1983; **147**: 188–95.
36. Von Hoff DD, Kronmal R, Salmon SE, *et al.* A Southwest Oncology Group study on the use of a human tumor cloning assay for predicting response in patients with ovarian cancer. *Cancer* 1991; **67**: 20–7.
37. Fruehauf JP, Bosanquet AG. *In vitro* determination of drug response: a discussion of clinical applications. *Principles Practice Oncol* 1993; **7**: 1–16.
38. Cree IA, Petty RD, Sutherland LA, *et al.* Elucidation of molecular determinants of tumor chemosensitivity by ATP-based luminescence assay. In: Campbell AC, Stanley PE, Kricka LJ, eds. *Chemluminescence and bioluminescence*. Chichester: John Wiley 1994; 407–410.
39. Fidler IJ, Wilmanns C, Staroselsky A, Radinsky R, Dong

Z, Fan D. Modulation of tumor cell response to chemotherapy by the organ environment. *Cancer Metast Rev* 1994; **13**: 209–22.
40. Maehara Y, Anai H, Tamada R, Sugimachi K. The ATP assay is more sensitive than the succinate dehydrogenase inhibition test for predicting cell viability. *Eur J Cancer Clin Oncol* 1987; **23**: 273–6.
41. Petty RD, Sutherland LA, Hunter EM, Cree IA. Comparison of MTT and ATP-based assays for the measurement of viable cell number. *J Biolum Chemilum* 1995; **10**: 29–34.
42. Hanauske AR. *In vitro* assays for antitumor activity: more pitfalls to come? *Eur J Cancer* 1993; **29A**: 1502–3.
43. Pacliacci MC, Spinozzi F, Migliorati G, *et al.* Genistein inhibits tumor cell growth *in vitro* but enhances mitochondrial reduction of tetrazolium salts: a further pitfall in the use of the MTT assay for evaluating cell growth and survival. *Eur J Cancer* 1993; **29A**: 1573–7.
44. Kaspers GJL, Pieters R, Van Zantwijk CH, *et al. In vitro* drug sensitivity of normal peripheral blood lymphocytes and childhood leukaemic cells from bone marrow and peripheral blood. *Br J Cancer* 1991; **64**: 469–74.
45. Mitsuhashi Y, Inaba M, Sugiyama Y, Kobayashi T. *In vitro* measurement of chemosensitivity of human small cell lung and gastric cancer cell lines toard cell cytlce phae-nonspecific agents under the clnically equivalent area under the curve. *Cancer* 1992; **70**: 2540–6.
46. Furukawa T, Kubota T, Hoffman RM. Clinical applications of the histoculture drug response assay. *Clin Cancer Res* 1995; **1**: 305–11.
47. Hoffman RM. Three-dimensional histoculture: origins and applications in cancer research. *Cancer Cells* 1991; **3**: 86–92.
48. Bosanquet AG, Bell PB. Novel *ex vivo* analysis of nonclassical, pleiotropic drug resistance and collateral sensitivity induced by therapy provides a rationale for treatment strategies in chronic lymphocytic leukemia. *Blood* 1996; 87: 1962–71.
49. Shaw GL, Gazdar AF, Phelps R *et al.* Individualized chemotherapy for patients with non-small cell lung cancer determined by prospective identification of neuroendocrine markers and *in vitro* drug sensitivity testing. *Cancer Res* 1993; **53**: 5181–7.
50. Frankfurt OS. Detection of apoptosis in leukemic and breast cancer cells with monoclonal antibody to single-stranded DNA. *Anticancer Res* 1994; **14**: 1861–9.
51. Csoka K, Nygren P, Graf W, Pahlman L, Glimelius B, Larsson R. Selective sensitivity of solid tumors to suramin in primary cultures of tumor cells from patients. *Int J Cancer* 1995; **63**: 356–60.
52. Maurer HR. *In vitro* screening for potential anti-cancer drugs; new approaches including induction of differentiation. In: Crommelin D, Couvreur P, Duchene D, eds. *In vitro and ex vivo test systems to rationalize drug design and delivery.* Paris: Editions de Sante 1994; 99–107.
53. Andreotti PE, Thornthwaite JT, Morse IS. ATP Tumor chemosensitivity assay. In Stanley PE, Kricka LJ, eds. *Bioluminescence and chemiluminescence: current status.* Chichester: John Wiley 1991, 417–20.
54. Andreotti PE, Linder D, Hartmann DM, *et al.* ATP tumor chemosensitivity assay application for solid tumors and leukaemias. In: Szalay A, Kricka LJ, Stanley PE, eds. *Chemiluminescence and bioluminescence: current Status.* Chichester: John Wiley 1993: 271–5.
55. Andreotti PE, Cree IA, Kurbacher CM, *et al.* Chemosensitivity testing of human tumors using a microplate ATP luminescence assay: clinical correlation for cisplatin resistance of ovarian carcinoma. *Cancer Res* 1995; **55**: 5276–82.
56. Kurbacher CM, Nagel W, Mallman P, *et al. In vitro* activity of titanocenedichloride in human renal cell carcinoma compared to conventional antineoplastic agents. *Anticancer Res* 1994; **14**: 1529–34.
57. Kurbacher CM, Cree IA, Brenne U, *et al.* Heterogeneity of *in vitro* chemosensitivity in perioperative breast cancer cells to mitoxantrone versus doxorubicin evaluated by microplate ATP bioluminescence assay. *Breast Can Res Treat* 1996; **41**: 161–70.
58. Lundin A, Hasenson M, Persson J, Pousette A. Estimation of biomass in growing cell lines by adenosine triphosphate assay. *Methods Enzymol* 1986; **133**: 27–42.
59. Kangas L, Gronroos M, Nieminen AL. Bioluminescence of cellular ATP: a new method for evaluating cytotoxic agents *in vitro. Med Biol* 1984; **62**: 338–43.
60. Garewal HS, Ahmann F, Schifman RB, Celniker A. ATP assay: ability to distinguish cytostatic from cytocidal anticancer drug effects. *J Natl Cancer Inst* 1986; **77**: 1039–45.
61. Sevin BU, Perras JP, Averette HE, Donato DM, Penalver M. Chemosensitivity testing in ovarian cancer. *Cancer* 1993; **71**: 1613–20.
62. Hirai T, Kawano K, Hirabayashi N, *et al.* A novel *in vitro* chemosensitivity test using materials collected by endoscopic biopsy. *Anti-Cancer Drugs* 1991; **2**: 269–74.
63. Cree IA, Kurbacher CM, Untch M, *et al.* Correlation of the clinical response to chemotherapy in breast cancer with ex vivo chemosensitivity. *Anti-Cancer Drugs* 1996; **7**: 630–5.
64. Brown E, Markman M. Tumor chemosensitivity and chemoresistance assays. *Cancer* 1996; **77**:1020–5.
65. Alberts DS, Salmon SE, Moon TE. Human tumor cloning: drug sensitivity testing clinical correlations. *Prog Clin Cancer* 1982; **8**: 147–79.
66. Daniels JR, Daniels AM, Luck EE, Whitman B, Casagrande JT, Skinner DG. Chemosensitivity of human neoplasms with *in vitro* clone formation. Experience at the University of Southern California—Los Angeles County Medical Center. *Cancer Chemother Pharmacol* 1981; **6**: 245–51.
67. Federico M, Alberts DS, Garcia DJ, *et al. In vitro* drug testing of ovarian cancer using the human tumor colony-forming assay: comparison of *in vitro* response and clinical outcome. *Gynecol Oncol* 1994; **55**: S156–63.
68. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977; **197**: 461–3.
69. Salmon SE, Hamburger AW, Soehnlen B, Durie BG, Alberts DS, Moon TE. Quantitation of differential sensitivity of human-tumor stem cells to anticancer drugs. *N Engl J Med* 1978; **298**: 1321–7.

70. Von Hoff DD, Cowan J, Harris G, Reisdorf G. Human tumor cloning: feasibility and clinical correlation. *Cancer Chemother Pharmacol* 1981; **6**: 265–71.
71. Von Hoff DD, Sandbach JF, Clark GM, *et al.* Selection of cancer chemotherapy for a patient by an *in vitro* assay versus a clinician. *J Natl Cancer Inst* 1990; **82**: 110–6.
72. Kern DH, Drogemuller CR, Kennedy MC, Hildebrand-Zanki SU, Tanigawa N, Sondak VK. Development of a miniaturized, improved nucleic acid precursor incorporation assay for chemosensitivity testing of human solid tumors. *Cancer Res* 1985; **45**: 5436–41.
73. Kern DH, Weisenthal LM. Highly specific prediction of antineoplastic drug resistance with an *in vitro* assay using suprapharmacologic drug exposures. *J Natl Cancer Inst* 1990; **82**: 582–8.
74. Weisenthal LM, Kern DH. Prediction of drug resistance in cancer chemotherapy: the Kern and DiSC assays. *Oncology* 1991; **5**: 93–103.
75. Bosanquet AG, Bird MC, Price WJP, Gilby ED. An assessment of a short term tumor chemosensitivity assay in chronic lymphocytic leukaemia. *Br J Cancer* 1983; **47**: 781–9.
76. Bosanquet AG. Short-term *in vitro* drug sensitivity tests for cancer chemotherapy. A summary of correlations of test results with both patient response and survival. *Forum Trends Exp Clin Med* 1994; **4**: 179–95.
77. Meitner PA. The fluorescent cytoprint assay: a new approach to *in vitro* chemosensitivity testing. *Oncology* 1991; **5**: 75–81.
78. Klumper E, Pieters R, Kaspers GJ, *et al. In vitro* chemosensitivity assessed with the MTT assay in childhood acute non-lymphoblastic leukemia. *Leukemia* 1995; **9**: 1864–9.

(Received 12 May 1997; accepted 22 May 1997)