Anti-Cancer Drugs 1999, **10**, pp. 437–444

Report

Heterogeneity of chemosensitivity of metastatic cutaneous melanoma

Ian A Cree, Michael H Neale, Nyree E Myatt, Pippa G de Takats,[1] Per Hall,[1] John Grant,[1] Christian M Kurbacher,[2] Uwe Reinhold,[3] Karsten Neuber,[4] Rona M MacKie,[5] Jagdeep Chana,[6] Paul C Weaver,[7] Ghassan G Khoury,[7] Christian Sartori[8] and Peter E Andreotti[9]

Department of Pathology, Institute of Ophthalmology, University College London, London EC1V 9EL, UK. [1]Oncology Centre, Adenbrookes Hospital NHS Trust, Cambridge CB2 2AA, UK. [2]Labor fur Chemosensitivitatestungen, Universitats–Frauenklinik, University of Cologne Medical Center, 50931 Cologne, Germany. [3]Department of Dermatology, The Saarland University Hospital, 66421 Homburg/Saar, Germany. [4]Universitats-Krankenhaus-Eppendorf, Hautklinik, 20246 Hamburg, Germany. [5]Department of Dermatology, Western Infirmary, Glasgow G11 6NT, UK. [6]RAFT Institute of Plastic Surgery, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR, UK. [7]Portsmouth Oncology Center, St Mary's Hospital, Portsmouth PO3 6AD, UK. [8]DCS Innovative Diagnostik Systeme, 22397 Hamburg, Germany. [9]Atlantic Scientific Development Inc., Fort Lauderdale, FL 33308, USA.

Advanced melanoma has a poor prognosis and chemotherapy provides little benefit for most patients. This may be related to heterogeneity of chemosensitivity as well as frequent constitutive resistance to individual cytotoxic drugs. We have therefore examined the heterogeneity of chemosensitivity in metastatic cutaneous melanoma specimens using an *ex vivo* ATP-based chemosensitivity assay (ATP-TCA). Melanoma deposits (n=55) in skin or lymph node were tested using the ATP-TCA, performed in three separate laboratories. Analysis of the data collected (based on an arbitrary sensitivity index <300) shows considerable heterogeneity of chemosensitivity. The most active single cytotoxic agents in the assay were identified as cisplatin, treosulfan, paclitaxel, vinblastine, gemcitabine and mitoxantrone. There was also a limited direct inhibition of melanoma cell growth by interferon-α2b, although this agent is known to have a number of indirect biological antitumor effects. Exposure of tumor cells to combinations of drugs at the concentrations tested as single agents showed the most active combinations to be treosulfan+gemcitabine, cisplatin+paclitaxel and vinblastine+paclitaxel. There was considerable heterogeneity of chemosensitivity: some tumors responded well to one agent or combination, while others showed no response to this and instead responded to one of the alternatives tested. Occasional highly resistant tumors showed no response to any of the single agents or combinations tested. The degree of heterogeneity observed suggests that the ATP-TCA could be used to select patients who might benefit from specific chemotherapeutic agents alone or in combination. This provides the rationale for future randomized controlled trials of ATP-TCA-directed chemotherapy versus physician's choice to determine whether assay-directed chemotherapy can improve patient response and survival. [© 1999 Lippincott Williams & Wilkins.]

Key words: Chemosensitivity, luciferase, melanoma.

Introduction

Chemotherapy for advanced melanoma remains disappointing. Although it is now possible to achieve up to 50% response rates with combined chemotherapy, the regimens used are often highly toxic and responses tend to be short lived.[1] It is debatable whether immunotherapy fares any better, although rarely prolonged clinical remissions occur.[2] Adjuvant immunotherapy with high-dose interferon (IFN)-α2b may be useful in high-risk patients.[3]

It is clear from a large number of reported clinical trials that melanoma responds best to alkylating agents such as DTIC (dacarbazine), but that other drugs (e.g. vinca alkaloids) may be effective in certain patients.[1] This heterogeneity of chemosensitivity which appears to exist between different tumors is currently unpredictable and may be one explanation for the poor results of chemotherapy: in short, many patients are

This research was funded in part by a donation from Schering-Plough Ltd. IAC is a director of Can Tech Ltd.

Correspondence to IA Cree, Department of Pathology, Institute of Ophthalmology, University College London, Bath Street, London EC1V 9EL, UK.
Tel: (+44) 171 608 6808; Fax: (+44) 171 608 6862;
E-mail: i.cree@ucl.ac.uk

being treated with ineffective agents and being exposed to potential systemic toxicity without benefit.

Chemosensitivity assays have been developed over the last 20 years to try to predict responsiveness of primary human tumors and to overcome this problem of heterogeneity.[1-6] Unfortunately early assays proved more useful for cell lines than human tumors and the approach as a whole failed to meet initial expectations. The situation has changed recently as the result of technical developments: newer assays such as the ATP-based tumor chemosensitivity assay (ATP-TCA)[7] have high evaluability rates with solid tumors, producing interpretable results for greater than 90% of tumors tested.[8] A case-control intervention study in recurrent ovarian carcinoma has shown dramatic improvement in response rate and survival attributed to use of this assay.[9] The assay has also been applied to study the chemosensitivity of primary intra-ocular melanoma[10] allowing definition of a new chemotherapy regimen with potential for use in these patients.[11] A randomized phase III study is now in progress for platinum-refractory ovarian cancer[12] and it is clearly time to examine the applicability of this approach to other tumors in which clinical heterogeneity of chemotherapeutic responses has been observed.

This study was undertaken to determine the degree of heterogeneity of chemosensitivity in cutaneous melanoma. We also wished to identify any technical problems for the application of the ATP-TCA to this tumor and to provide data, which will permit interpretation of assay results for subsequent clinical intervention studies.

Materials and methods

Patients

A total of 54 patients with metastatic cutaneous melanoma were included in the study, with a median age of 59 years (range 27–84). There were 32 males and 22 females in the series. Eight patients had received DTIC-containing chemotherapy before assay. Each tumor deposit (completely replaced lymph node or skin deposit) was tested using a standardized assay protocol[7] in one of three laboratories by IAC (London), KN (Hamburg) or CMK (Cologne). All tumor samples were removed as part of patient treatment with local ethics committee approval for use of tissue for chemosensitivity testing. Tumor samples representing part of the biopsy not required for diagnosis following local examination of the specimen by a surgeon or pathologist were sent to the assay laboratory in clearly labeled specimen bottles containing culture medium with antibiotics (DMEM; Sigma, Poole, UK). The samples were taken under aseptic conditions and assayed within 24 h of surgery.

ATP-TCA

Tumor material was trimmed of fat/epidermis and minced prior to enzymatic tumor cell dissociation according to the assay protocol.[7,10] Cells were resuspended in complete assay medium (CAM; DCS Innovative Diagnostik Systeme, Hamburg, Germany), assessed for viability by Trypan blue exclusion and counted. If less than 75% viability or excessive tissue debris was present, cells were purified using Lymphoprep (Nycomed, Birmingham, UK) and washed in CAM before resuspension to 200 000 cells/ml. Test drugs were added to polypropylene 96-well plates (Costar, High Wycombe, UK) at six dilutions (6.25–200%) of the test drug concentrations (TDC) shown in Table 1. Dilutions were prepared in the plate (100 μl/well) from an 800% TDC solution made up freshly from frozen aliquots of each drug in CAM.[13] The TDC for each drug is determined by reference to known pharmacokinetic and response data as previously

Table 1. Drugs tested and their 100% TDC as used in the *ex vivo* assay

Drug/combination	100% TDC (μg/ml)
Paclitaxel	13.6
Cisplatin	3.8
BCNU	8
Paclitaxel/cisplatin	13.6+3.8
Temozolomide	20
Dacarbazine	10
Vincristine	0.4
Temozolomide+BCNU+cisplatin	20+8+3.8
Temozolomide+vincristine+cisplatin	20+0.4+3.8
Fotemustine	8
Ara-C	2.4
Treosulfan	20
Doxorubicin	0.5
Mitoxantrone	0.3
Gemcitabine	12.5
Vinblastine	0.5
IFN-α2b	1000 IU/ml
Vinblastine+paclitaxel	0.5+13.6
Treosulfan+gemcitabine	20+12.5
Temozolomide+cisplatin	20+3.8
Doxorubicin+paclitaxel	0.5+13.6
Mitoxantrone+paclitaxel	0.3+13.6
Cisplatin+fotemustine	3.8+8

Temozolomide was used as the active agent as the activity of DTIC *in vitro* depends upon the degree to which cells possess p450, but subsequent analysis showed that this concentration needs further adjustment

described.[7] Two rows of the plate were reserved for controls: a no drug 'MO' row containing 100 μl/well CAM and a 'MI' row containing 100 μl/well maximum inhibitor of cell survival (DCS). A volume of 100 μl cells was added to each well giving a final plating density of 20 000 cells/well in 200 μl total volume. Following incubation of the plates at 37°C at 100% humidity in 5% CO_2 for 6–7 days, the cells were lysed

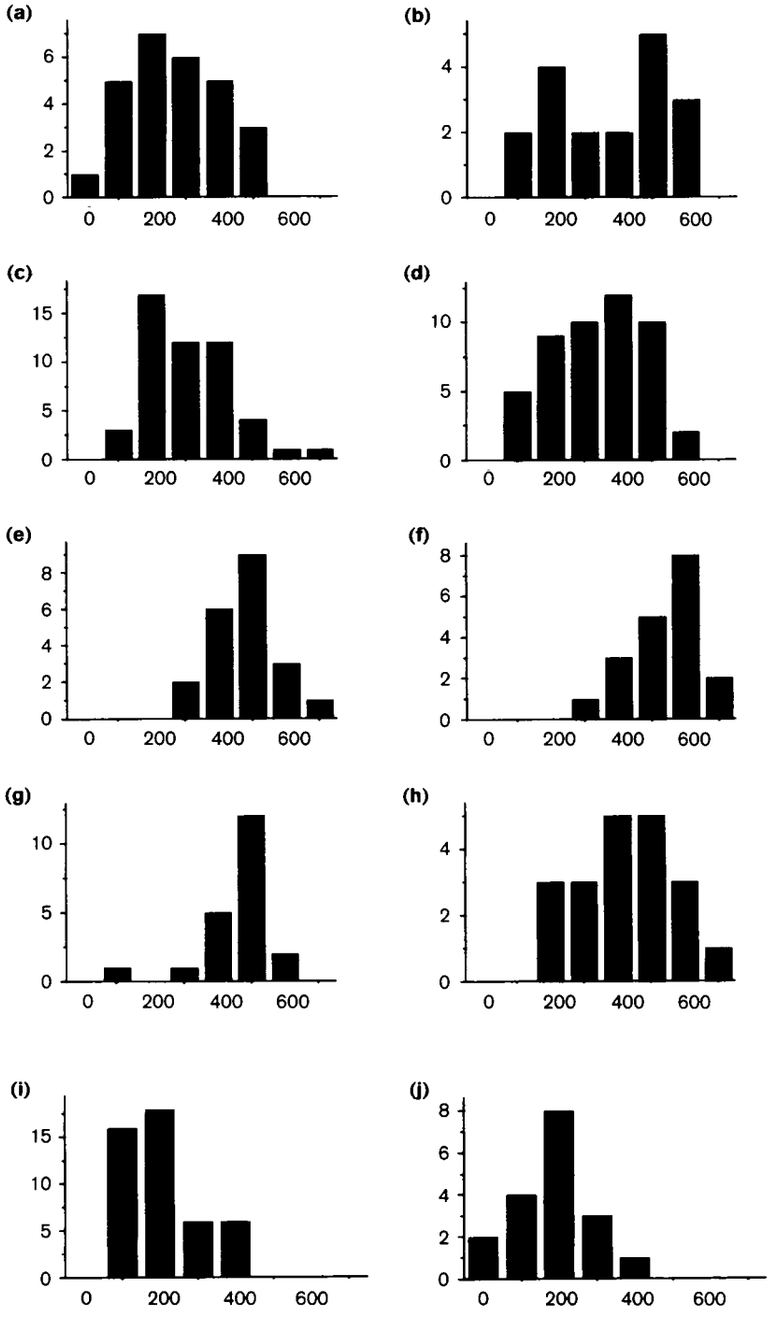

Figure 1. Frequency histograms showing heterogeneity of the sensitivity index (*y*-axis) for each single agent and combination. (A) Treosulfan (*n*=27). (b) Gemcitabine (*n*=18). (c) Paclitaxel (*n*=50). (d) Cisplatin (*n*=49). (e) DTIC (*n*=22). (f) Temozolomide (*n*=20). (g) Fotemustine (*n*=21). (h) Vinca (*n*=18 vinblastine and *n*=4 vincristine). (i) Cisplatin+paclitaxel (*n*=46). (j) Treosulfan+gemcitabine (*n*=18).

by addition of 50 μl Tumor Cell Extraction Reagent (DCS) to each well and the ATP content of each well measured using the luciferin-luciferase system according to the manufacturer's instructions (DCS). Luminescence measurements were made in a Dynatech ML1000, Berthold LB96P or LB953 luminometer. The results were entered into a spreadsheet (Excel, Microsoft) and calculations of the percent inhibition at each concentration were used directly to graph the results. A 'TCA Index' was calculated by summing the inhibition at each concentration tested to allow simple comparison of results between patients (Index=Sum [$Inhibition_{6.25200}$]), in addition to IC_{90} and IC_{50} parameters.

Data analysis

Thresholds for sensitivity and resistance were calculated using percentiles based on previous trials of melanoma therapy using the agents and combinations included in the study as previously described.[7,8]

Results

The evaluability rate (i.e. the number of tumors with interpretable results) in London was 23 of 24 (96%) in a consecutive series of tumors assayed over a period of 1 year. The failure was a tumor with extensive necrosis, which produced too few cells for assay. No tumors with sufficient cells for assay were lost. Evaluability rates in Germany were comparable. The amount of tumor submitted for testing varied but was generally 1-2 cm^3, allowing a median of seven drugs or drug combinations to be tested (range 2-14).

The results show considerable heterogeneity of chemosensitivity to single agents and drug combinations (Figures 1-4 and Table 2). Some patients responded well to one drug or combination, while others showed no response to this and instead responded to one or more of the alternative regimens tested (Figure 3). Occasional highly resistant tumors (five of 55, 9%) showed no response to any of the single agents or combinations tested.

The most active single agents were identified as cisplatin, treosulfan, paclitaxel, vinca alkaloids and mitoxantrone. Eight tumors were tested with vinorelbine and there was activity (index >300) in four tumors (50%). There was limited sensitivity to gemcitabine and IFN-α2b. Alkylating agents, particularly cisplatin and treosulfan, appeared to be the most active class of drugs against melanomas in the assay.

Since single-agent DTIC is currently the 'gold

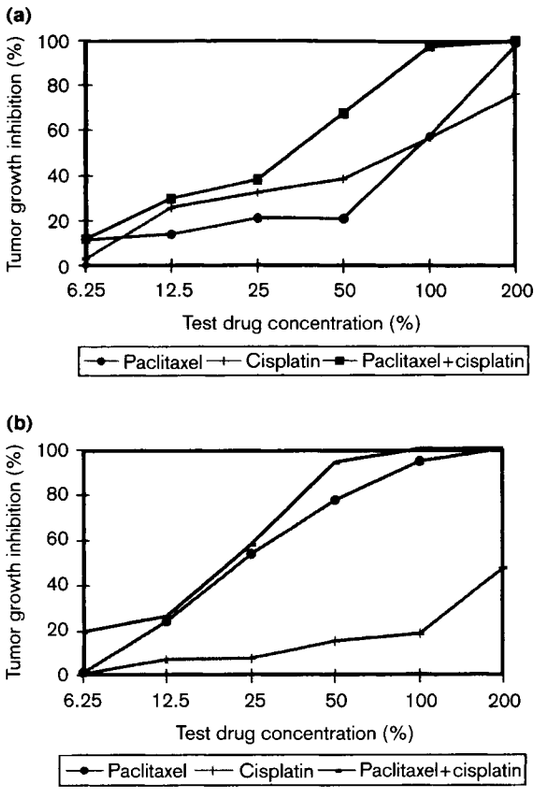

Figure 2. Typical TCA results for (a) a tumor sensitive to cisplatin+paclitaxel with sensitivity to both drugs tested, and (b) a tumor resistant to cisplatin with some sensitivity to paclitaxel and similar sensitivity to the combination of cisplatin+paclitaxel.

standard' for treatment of metastatic melanoma, it was considered important to include this agent in the assay. However, technical problems preclude full analysis of the data obtained with DTIC, as this agent is usually activated *in vivo* by liver microsomal p450 and activation of the drug by endogenous p450 within melanoma cells in the *ex vivo* assay may not have the same biological relevance. The related drug temozolomide, which does not require conversion, was also included in the study. The degree of sensitivity was lower than expected on the basis of 20% response rates to the single agent, with only one of 20 tumors sensitive to temozolomide using the arbitrary threshold of TCA Index <300 and two of 22 for DTIC.

Relatively few tumors were tested with multiple agents of the same class, so that it is difficult to determine cross-resistance/sensitivity patterns from this data. However, only one of six paclitaxel-sensitive tumors tested showed sensitivity to vinca alkaloids

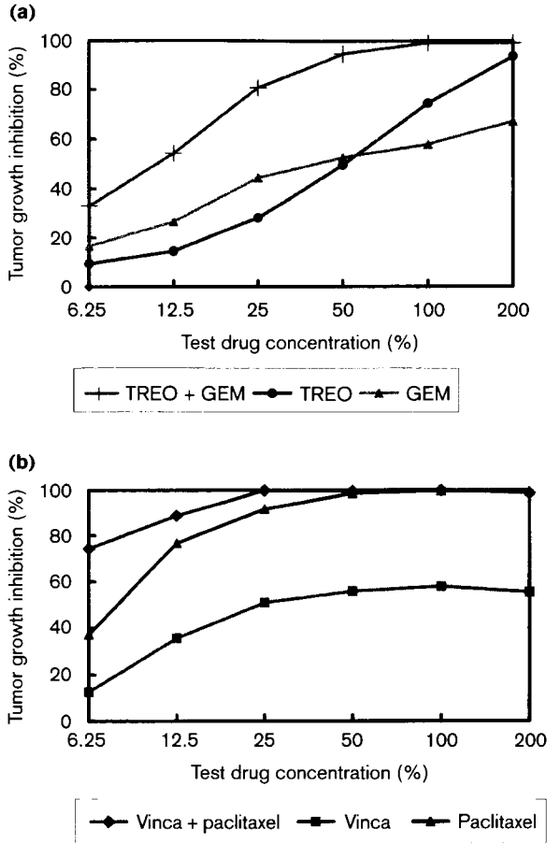

Figure 3. (a) Results for gemcitabine+treosulfan in one tumor, showing little activity of the gemcitabine, but a marked increase in activity of the combination compared with treosulfan alone. (b) Results for vinblastine+paclitaxel in one tumor, showing activity of both agents with an increase in activity of the combination.

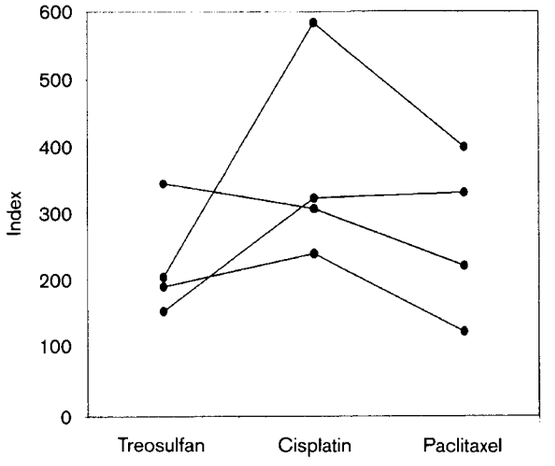

Figure 4. Heterogeneity data for three individual drugs expressed as TCA Indices for four tumors: a low index (<300) indicates probable sensitivity to the single agent. The degree of heterogeneity between tumors taken from different patients is evident with a low index for all three drugs in one case and resistance to all but treosulfan in another.

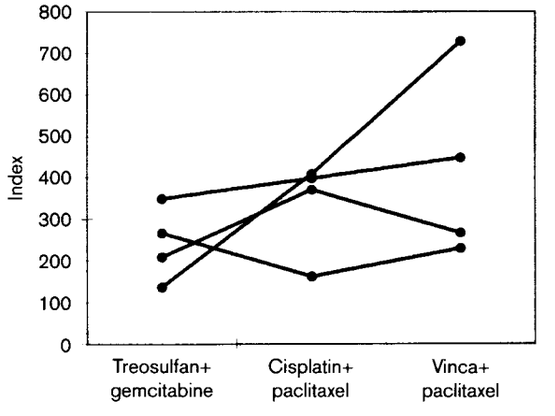

Figure 5. Heterogeneity data for four tumors from different patients with three combinations: a low index (<300) indicates probable sensitivity to the combination.

(vincristine or vinblastine). Of those sensitive to treosulfan, three of seven were also sensitive to cisplatin, consistent with some cross-sensitivity to alkylating agents in melanoma. Of six tumors resistant to temozolomide, four of six were resistant to BCNU and three of six were resistant to cisplatin. None of these six tumors showed resistance to treosulfan.

Simultaneous addition of drugs with different mechanisms of action using the same concentrations tested as single agents showed the best combinations to be treosulfan+gemcitabine, cisplatin+paclitaxel and vinblastine+paclitaxel. Most tumors were sensitive to the combinations of treosulfan+gemcitabine (16 of 18) and cisplatin+paclitaxel (38 of 46), with a lesser proportion sensitive to vinblastine+paclitaxel (six of nine) or vinorelbine+paclitaxel (five of eight). However, these figures hide considerable heterogeneity of sensitivity to combinations of drugs within individual tumors: in some the best combination was vinorelbine+paclitaxel (Figure 5), while in others it was one of the alkylating agent-containing regimens. Thus even for combinations, the ATP-TCA was able to distinguish a 'best' regimen for each tumor tested.

Discussion

Metastatic melanoma shows considerable heterogeneity of chemosensitivity to single cytotoxic agents and

Table 2. Summary of sensitivity data using an arbitary threshold of sensitivity defined as a TCA index <300 for six concentrations tested

Drug	No. sensitive in ATP-TCA	No. assessed	Sensitivity (%)
Treosulfan	11	27	41
Temozolomide	1	20	5
Cisplatin	18	49	37
Taxol	28	50	56
Doxorubicin	5	11	45
Mitoxantrone	3	10	30
Gemcitabine	6	18	33
Vinblastine	3	18	17
BCNU	2	15	13
IFN-α2b	0	16	0
Fotemustine	1	21	5
DTIC	2	22	9
Vincristine	1	4	25
Temozolomide+vincristine+cisplatin	6 (5/9 Vin B)	11	55
Cisplatin+taxol	38	46	83
Vinblastine+taxol	6	9	67
Temozolomide+BCNU+cisplatin	3	7	43
Treosulfan+gemcitabine	16	18	89
Temozolomide+cisplatin	0	6	0
Doxorubicin+taxol	4	5	80
Mitoxantrone+taxol	5	5	100
Cisplatin+fotemustine	11	16	69

combinations in keeping with clinical studies. Our data shows that the ATP-TCA is a robust method of investigating such heterogeneity with a near 100% evaluability record for specimens from which melanoma cells were extracted. Evaluability rates with other tumors are similar: over 90% in both breast and ovarian cancer.[8,9] Previous studies using a variety of different *ex vivo* chemosensitivity assays have also shown heterogeneity of chemosensitivity in melanoma, whether using cell lines[14,15] or tumor tissue.[16–19] In this study, we have used drugs at levels related to their peak plasma concentration (C_{max}), taking into account their degree of protein binding.[7] This approach has its problems, since C_{max} is not always a good indicator of clinically attainable intra-tumor concentrations. While we have considerable experience with many of the agents tested,[7,8,13] some are new to the assay and require refinement of the TDC to obtain good correlation with clinical outcome. However, our results provide useful comparative data between tumors and in general those agents that show activity clinically also show activity in the assay.

In the UK, chemotherapy with DTIC alone is regarded as standard therapy for metastatic melanoma. This agent has an overall response rate of 12–25%,[2] but does not significantly increase overall survival in treated patients. Unfortunately, DTIC causes difficulties in chemosensitivity studies since it is a prodrug requiring microsomal activation by p450. It is possible to use light to activate the drug,[20] but the metabolic pathway is then different and the effects may not be identical. The recent development of temozolomide, a DTIC derivative which is rapidly hydolysed to an active compound, circumvents this problem, but in practice, we have observed effects with DTIC alone and therefore include them in this paper. It is probable that melanoma cells contain enough p450 to activate the drug sufficiently for an effect to be observed during the assay period (MF Stevens, pers. commun.), but a full explanation is awaited. The sensitivity data obtained for DTIC and temozolomide are similar to those obtained by Von Hoff and co-workers[21] and to clinical experience.

DTIC is a frequent component of chemotherapy combination regimens used to treat melanoma patients, most of which have historically included other alkylating agents (nitrosoureas) or cisplatin with or without a vinca alkaloid. Nitrosoureas such as BCNU, CCNU or fotemustine have activity with response rates varying from 10 to 24%. Single-agent cisplatin or carboplatin have response rates of 15–16%.[1] In our hands, all of these DNA-damaging agents show activity, although the threshold used to calculate sensitivity for the purposes of comparison may not be appropriate

for interpretation of the assay results to direct therapy. However, the striking sensitivity of many tumours to treosulfan may be real: one of us (KN) has recently reported the results of a phase II study of this agent used alone in patients with metastatic melanoma with promising results.[22] Treosulfan induces O^7-methylation rather than O^6-methylation and is therefore potentially less susceptible to resistance mediated by O^6-methyl-guanyl transferase.[23]

Response rates to single-agent vinca alkaloids (vinblastine and vincristine) are similar at 12–14%[1] to those observed here based on the sensitivity index threshold. There is generally clear distinction between those tumors that are sensitive or resistant to these agents in the assay, although 100% kill is rarely observed. This differential sensitivity may be due to differences in apoptotic susceptibility induced by these agents.[24] Taxanes are potentially active single agents in melanoma with response rates around 24% as single agents.[1,25–29] In the assay, many tumors showed good responses consistent with this high sensitivity in comparison to other agents.

Anthracyclines are not generally thought to have much activity in melanoma and we were surprised to see how well they compared with other drugs in the assay. Such differences may reflect rapid development of resistance during chemotherapy that is not present at first treatment. We have previously shown that this occurs in some breast tumors re-tested after just three cycles of chemotherapy.[8] Melanoma cells can express and potentially up-regulate classical mechanisms of drug resistance including MDR1, LRP and MRP.[30,31]

Limited sensitivity was seen to gemcitabine, the only anti-metabolite tested. This agent rarely achieved high per cent inhibition, but was included in view of recent success in using it to modulate the *ex vivo* chemosensitivity of alkylating agents in both ovarian cancer[9] and uveal melanoma.[11]

Drug combinations tested included a number of experimental regimens not currently used in clinical practice, which generally compared well with standard regimens such as DTIC+cisplatin+vinblastine. In general, combination of an active single agent with an inactive single agent produced no enhancement of chemosensitivity (as expected), but combination of treosulfan (active) with gemcitabine (often relatively inactive) did produce synergism as previously described for uveal melanoma.[11] Combination of agents which both showed single-agent activity tended to produce additive effects, although this was less marked for combinations of alkylating agents or cisplatin than with alkylating agents+anthracyclines or paclitaxel.

Paclitaxel-containing regimens showed consistently high activity, whether combined with cisplatin, mitoxantrone or doxorubicin. Antagonism was not seen in this study, in contrast to one previous report for cisplatin+paclitaxel using a cell line.[32] Cell line data can be misleading, as has previously been shown in melanoma[33] and in other tumors.[34] We have performed some preliminary experiments combining paclitaxel with vinca alkaloids, including those which have previously been reported to have activity *in vitro* and *in vivo*.[35–37] The results are impressive in some patients, particularly those showing activity for both agents in the assay. The effects appear to be additive rather than synergistic, but we have not yet examined schedule dependence issues for this regimen and only have preliminary data for vinorelbine.

The degree of heterogeneity of chemosensitivity observed in this study suggests that the ATP-TCA may be an excellent technique for the selection of patients who will benefit from particular agents alone or in combination. These data further suggest that most patients are sensitive to something—the problem is knowing which drugs to give the individual patient. This study provides the rationale for undertaking randomized controlled trials of ATP-TCA-directed chemotherapy in patients with metastatic cutaneous melanoma.

Acknowledgments

We are grateful to all those who made this multinational study possible, especially members of the Preclinical Therapeutic Models Group of the EORTC and the International Society for Chemosensitivity Testing in Oncology.

References

1. Lee SM, Betticher DC, Thatcher N. Melanoma: chemotherapy. *Br Med Bull* 1995; **51**: 609–30.
2. Atkins MB. The treatment of metastatic melanoma with chemotherapy and biologics. *Curr Opin Oncol* 1997; **9**: 205–13.
3. Kirkwood JM, Strawderman MH, Ernstoff MS, Smith TJ, Borden EC, Blum RH. Interferon α-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. *J Clin Oncol* 1996; **14**: 7–17.
4. Bellamy WT. Prediction of response to drug therapy of cancer. A review of *in vitro* assays. *Drugs* 1992; **44**: 690–708.
5. Bosanquet AG. *In vitro* drug sensitivity testing for the individual patient: an ideal adjunct to current methods of treatment choice. *Clin Oncol* 1993; **5**: 195–7.
6. Cree IA, Kurbacher CM. Individualizing chemotherapy for solid tumors—is there any alternative? *Anti-Cancer Drugs* 1997; **8**: 541–8.

7. Andreotti PE, Cree IA, Kurbacher CM, *et al.* Chemosensitivity testing of human tumors using a microplate adenosine triphosphate luminescence assay: clinical correlation for cisplatin resistance of ovarian carcinoma. *Cancer Res* 1995; **55**: 5276–82.
8. 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.
9. Kurbacher CM, Cree IA, Bruckner HW, Mallmann P, Andreotti PE. Use of an *ex vivo* ATP luminescence assay to direct chemotherapy for recurrent ovarian cancer. *Anti-Cancer Drugs* 1998; **9**: 51–7.
10. Myatt N, Cree IA, Kurbacher CM, Foss AJE, Hungerford JL, Plowman PN. The *ex vivo* chemosensitivity profile of choroidal melanoma. *Anti-Cancer Drugs* 1997; **8**: 756–62.
11. Neale MH, Myatt N, Cree IA, *et al.* Combination chemotherapy for chroroidal melanoma: *ex vivo* sensitivity to treosulfan with gemcitabine or cytosine arabinoside. *Br J Cancer* 1999; **79**: 1487–93.
12. Kurbacher CM, Untch M, Cree IA. A randomised trial of chemotherapy directed by a tumour chemosensitivity assay versus physician's choice in patients with recurrent platinum-resistant ovarian adenocarcinoma. *Lancet Internet publication* 1997: http://www.thelancet.com/newlancet/sub/author//menu_NOD7.html
13. 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.
14. Schadendorf D, Jurgovsky K, Worm M, Czarnetzki BM. *In vitro* sensitivity of human melanoma cells to chemotherapeutic agents and interferons. *Melanoma Res* 1994; **4**: 243–9.
15. Tachihara R. The effect of dacarbazine and vincristine sulfate on human melanoma cell lines. *In vitro* analysis of interaction on DNA synthesis, RNA synthesis and protein synthesis. *Nippon Ika Daigaku Zasshi* 1997; **64**: 238–48 (abstr).
16. Marshall ES, Finlay GJ, Matthews JH, Shaw JH, Nixon J, Baguley BC. Microculture-based chemosensitivity testing: a feasibility study comparing freshly explanted human melanoma cells with human melanoma cell lines. *J Natl Cancer Inst* 1992; **84**: 340–5.
17. Schadendorf D, Worm M, Algermissen B, Kohlmus CM, Czarnetzki BM. Chemosensitivity testing of human malignant melanoma. A retrospective analysis of clinical response and *in vitro* drug sensitivity. *Cancer* 1994; **73**: 103–8.
18. Muhonen T, Pyrhönen S, Laasonen A, *et al.* Mouse subrenal capsule assay chemosensitivity and DNA flow cytometry parameters of human melanoma metastases. *Acta Oncol* 1994; **33**: 431–7.
19. Hahka-Kemppinen M, Muhonen T, Kangas L, Pyrhönen S. Chemosensitivity of human melanoma metastases in mouse subrenal capsule assay—can it predict tumour response to combined cytostatic plus interferon therapy in metastatic melanoma? *Melanoma Res* 1996; **6**: 215–21.
20. Metelmann HR, Von Hoff DD. *In vitro* activation of dacarbazine (DTIC) for a human tumor cloning system. *Int J Cell Cloning* 1983; **1**: 24–32.
21. Raymond E, Izbicka E, Soda H, Gerson S, Dungan M, Von Hoff DD. Effects of temozolomide against human tumor using the human tumor cloning assay. *Ann Oncol* 1998; **9** (suppl 2): 38.
22. Neuber K, tom Dieck A, Bloedorn-Schlicht N, Itschert G, Karnback C. Treosulfan is an effective alkylating cytostatic for malignant melanoma *in vitro* and *in vivo*. *Melanoma Res* 1999: **9**: 125–32.
23. Hartley JA, O'Hare CC, Baumgart J. DNA alkylation and interstrand cross-linking by treosulfan. *Br J Cancer* 1998; **79**: 264–6.
24. Li G, Tang L, Zhou X, Tron V, Ho V. Chemotherapy-induced apoptosis in melanoma cells is p53 dependent. *Melanoma Res* 1998; **8**: 17–23.
25. Wiernik PH, Schwartz EL, Einzig A, Strauman JJ, Lipton RB, Dutcher JP. Phase I trial of taxol given as a 24-hour infusion every 21 days: responses observed in metastatic melanoma. *J Clin Oncol* 1987; **5**: 1232–9.
26. Legha SS, Ring S, Papadopoulos N, Raber M, Benjamin RS. A phase II trial of taxol in metastatic melanoma. *Cancer* 1990; **65**: 2478–81.
27. Wiernik PH, Einzig AL. Taxol in malignant melanoma. *J Natl Cancer Inst Monogr* 1993; **15**: 185–7.
28. Bedikian AY, Weiss GR, Legha SS, *et al.* Phase II trial of docetaxel in patients with advanced cutaneous malignant melanoma previously untreated with chemotherapy. *J Clin Oncol* 1995; **13**: 2895–9.
29. Einzig AI, Schuchter LM, Recio A, Coatsworth S, Rodriquez R, Wiernik PH. Phase II trial of docetaxel (Taxotere) in patients with metastatic melanoma previously untreated with cytotoxic chemotherapy. *Med Oncol* 1996; **13**: 111–7.
30. Berger W, Hauptmann E, Elbling L, Vetterlein M, Kokoschka EM, Micksche M. Possible role of the multidrug resistance-associated protein (MRP) in chemoresistance of human melanoma cells. *Int J Cancer* 1997; **71**: 108–15.
31. Schadendorf D, Makki A, Stahr C, *et al.* Membrane transport proteins associated with drug resistance expressed in human melanoma. *Am J Pathol* 1995; **147**: 1545–52.
32. Mohith A, Photiou A, Retsas S. The combination of paclitaxel with cisplatin exhibits antagonism *in vitro* against human melanoma. *Anti-Cancer Drugs* 1996; **7**: 493–8.
33. Tveit KM, Fodstad O, Pihl A. The usefulness of human tumor cell lines in the study of chemosensitivity. A study of malignant melanomas. *Int J Cancer* 1981; **28**: 403–8.
34. 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 Biolumin Chemilumin* 1994; **9**: 373–8.
35. Aamdal S, Wolff I, Kaplan S, *et al.* Docetaxel (Taxotere) in advanced malignant melanoma: a phase II study of the EORTC Early Clinical Trials Group. *Eur J Cancer* 1994; **30A**: 1061–4.
36. Retsas S, Mohith A, Mackenzie H. Taxol and vinorelbine: a new active combination for disseminated malignant melanoma. *Anti-Cancer Drugs* 1996; **7**: 161–5.
37. Photiou A, Shah P, Leong LK, Moss J, Retsas S. *In vitro* synergy of paclitaxel (Taxol) and vinorelbine (navelbine) against human melanoma cell lines. *Eur J Cancer* 1997; **33**: 463–70.

(Received 23 February 1999; accepted 3 March 1999)