

***In vitro* Drug Sensitivity Predicts Response and Survival after Individualized Sensitivity-Directed Chemotherapy in Metastatic Melanoma: A Multicenter Phase II Trial of the Dermatologic Cooperative Oncology Group**

Selma Ugurel,¹ Dirk Schadendorf,¹ Claudia Pföhler,² Karsten Neuber,³ Adina Thoenke,¹ Jens Ulrich,⁴ Axel Hauschild,⁵ Konstanze Spieth,⁶ Martin Kaatz,⁷ Werner Rittgen,⁸ Stefan Delorme,⁹ Wolfgang Tilgen,² and Uwe Reinhold²

Abstract Purpose: *In vitro* sensitivity assays are promising tools to predict the individual outcome of different chemotherapy regimens. However, a direct association between *in vitro* and *in vivo* chemosensitivity has to be shown by clinical studies. This multicenter phase II trial was aimed to investigate the efficacy of a sensitivity-directed, first-line chemotherapy in metastasized melanoma patients, and to prove an association between *in vitro* sensitivity and therapy outcome.

Patients and Methods: The primary study end point was objective response; secondary end points were safety, overall survival, and progression-free survival. Viable tumor cells obtained from metastatic lesions were tested for chemosensitivity to seven single drugs and five drug combinations using an ATP-based luminescence viability assay.

Results: Out of 82 recruited patients (intention-to-treat), 57 received assay-directed chemotherapy and 53 were evaluable for all study end points (per protocol). The drug combinations used were gemcitabine + treosulfan, paclitaxel + cisplatin, paclitaxel + doxorubicin, and gemcitabine + cisplatin. The per protocol population could be divided into 22 (42%) chemosensitive and 31 (58%) chemoresistant patients by an arbitrary chemosensitivity index. Objective response was 36.4% in chemosensitive patients compared with 16.1% in chemoresistant patients ($P = 0.114$); progression arrest (complete response + partial response + stable disease) was 59.1% versus 22.6% ($P = 0.01$). Chemosensitive patients showed an increased overall survival of 14.6 months compared with 7.4 months in chemoresistant patients ($P = 0.041$).

Conclusion: *In vitro* chemosensitivity testing may be worthy of further exploration to see if it could be a useful tool to predict the outcome of melanoma patients treated with a sensitivity-directed chemotherapy. Therefore, these preliminary results will be evaluated by a planned phase III trial using a randomized, standard-regimen controlled setting.

Authors' Affiliations: ¹Skin Cancer Unit, German Cancer Research Center Heidelberg and Department of Dermatology, University Hospital of Mannheim, Mannheim, Germany; ²Department of Dermatology, The Saarland University Hospital, Homburg/Saar, Germany; ³Department of Dermatology, University Hospital Eppendorf, Hamburg, Germany; ⁴Department of Dermatology, Otto von Guericke University, Magdeburg, Germany; ⁵Department of Dermatology, Christian Albrechts University, Kiel, Germany; ⁶Department of Dermatology, Johann Wolfgang Goethe University, Frankfurt/Main, Germany; ⁷Department of Dermatology, Friedrich Schiller University, Jena, Germany; and ⁸Central Unit of Biostatistics and ⁹Department of Radiology, German Cancer Research Center, Heidelberg, Germany

Received 12/16/05; revised 4/21/06; accepted 5/3/06.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Selma Ugurel, Skin Cancer Unit, German Cancer Research Center Heidelberg, Department of Dermatology, University Hospital of Mannheim, Theodor-Kutzer-Ufer 1, 68167 Mannheim, Germany. Phone: 49-621-383-3905; Fax: 49-621-383-2163; E-mail: s.ugurel@dkfz.de.

©2006 American Association for Cancer Research.
doi:10.1158/1078-0432.CCR-05-2763

Melanoma is a cutaneous neoplasm known for its high aggressiveness, its early dissemination of metastases, and its poor prognosis once metastasized. Chemotherapy with dacarbazine (DTIC) does actually apply as the standard treatment regimen in metastasized melanoma, with reported response rates of only 10% to 18% (1). Even these might be overestimated, as recent studies using new standardized evaluation criteria (2) revealed much lower response rates of 6% to 7% (3, 4). This poor outcome does not rely on an impaired penetration of chemotherapeutics into the tumor, but has been proposed to be caused by chemoresistance mechanisms intrinsic to melanoma cells (5, 6). Moreover, biochemotherapy and immunotherapy regimens did not prove to be superior to DTIC (1, 7).

Due to this unfavorable situation, a number of nonstandard chemotherapeutics were tested in small pilot studies to prove a stronger efficacy in melanoma. Although complete remissions of metastatic lesions could only be observed in few patients

(8–14), these observations indicate a subgroup of patients exhibiting high sensitivity to certain anticancer drugs. Diagnostic tools are needed to identify this subgroup among the presumably high number of overall chemoresistant patients. The ideal assay would reveal reliable information about the individual drug sensitivity profile of a tumor, combined with a considerable association with treatment response and survival of the corresponding patient.

For these purposes, various *in vitro* chemosensitivity assays have been developed and tested in the preclinical and clinical setting, predominantly in ovarian, breast, and lung cancer (15, 16). Although the majority of older technologies like, e.g., tumor clonogenic assays and [³H]thymidine incorporation quantified drug sensitivity by growth inhibition of tumor cells, newer approaches like, e.g., the differential staining assay and the ATP bioluminescence assay (ATP-TCA) use the rate of tumor cell death as a readout (16). These latter assays take advantage of the quantification of cell viability as measures of anticancer drug effectiveness, whereas growth inhibition assays often promote single clones, thus failing to reflect the *in vivo* situation (15, 17). ATP-TCA was shown to comprise high sensitivity, high reproducibility, and a low failure rate (18). Prospective studies using this approach revealed an association between *in vitro* sensitivities and *in vivo* tumor responses in metastasized breast cancer (19) and recurrent ovarian cancer (20). First applications in melanoma showed heterogenous chemosensitivity profiles of tumor specimens, but yet allowed no association with the clinical response of the corresponding patients (21–24).

The present study was intended (a) to investigate the feasibility of pretherapeutic *in vitro* chemosensitivity testing using the ATP-TCA method in a multicenter setting, (b) to investigate the efficacy of an individualized assay-directed chemotherapy, and (c) to prove a putative association between *in vitro* chemosensitivities and *in vivo* therapy outcomes by means of tumor response and survival in melanoma patients. An exploratory data analysis from this phase II trial was planned to provide insights into patient subgroups and sensitivity thresholds, which will be implemented into the design of a currently planned randomized phase III trial comparing sensitivity-directed with nonsensitivity-directed chemotherapy.

Patients and Methods

Study design. The primary end point of this multicenter prospective phase II trial was objective response, secondary end points were safety, overall survival, and progression-free survival. All end points were evaluated on intention-to-treat and per protocol basis. Patient recruitment was outlined as a total 50 patients evaluable for all study end points. This sample size, on the one hand, was small enough to ensure feasibility, and, on the other hand, provided sufficient data for the calculation of a threshold index differentiating between chemosensitive and chemoresistant patients. However, it should be noticed that the study was underpowered to detect a doubling in objective response from 15% to 30%, a difference that would have required a total sample size of 190 patients with a power of 80% and a significance level of 5%.

Patient population. Patients with histologically confirmed metastatic melanoma were enrolled in accordance with the following eligibility criteria: stage IV disease following American Joint Committee on Cancer criteria (25), no prior systemic chemotherapy in stage IV, at least one

measurable target lesion following the response evaluation criteria in solid tumors (2), willing and physically able to receive polychemotherapy, age ≥ 18 years, adequate bone marrow function (leukocytes $\geq 3,000/\mu\text{L}$, platelets $\geq 100,000/\mu\text{L}$), and satisfactory hepatic and renal functions. All types of metastatic sites were considered eligible, including metastases to the brain. Primary cutaneous or mucosal melanomas, as well as melanomas of unknown primary, were eligible; primary ocular melanomas were excluded. The study protocol was approved by the Institutional Review Board, and a written informed consent was signed by all patients before enrollment.

Chemosensitivity assay. After enrollment, an excision biopsy of a metastatic lesion was done in every patient and shipped to the central test laboratories (Homburg/Saar and Mannheim) within 24 hours.

Table 1. Pretreatment patient characteristics

	ITT	PP
	82 (100.0%)	53 (100.0%)
Gender		
Male	45 (54.9%)	28 (52.8%)
Female	37 (45.1%)	25 (47.2%)
Median age/y (range)	61.7 (24.1-80.9)	60.4 (30.2-80.9)
Localization of primary		
Skin	58 (70.7%)	41 (77.4%)
Mucosa	10 (12.2%)	7 (13.2%)
Unknown	14 (17.1%)	5 (9.4%)
Previous chemotherapy*		
Yes	15 (18.3%)	9 (17.0%)
No	67 (81.7%)	44 (83.0%)
Previous IFN- α therapy*		
Yes	25 (30.5%)	17 (32.1%)
No	57 (69.5%)	36 (67.9%)
Serum LDH [†]		
\leq UNL	50 (61.0%)	35 (66.0%)
$>$ UNL	24 (29.3%)	16 (30.2%)
NA	8 (9.7%)	2 (3.8%)
ECOG performance status		
0	37 (45.1%)	31 (58.5%)
1	20 (24.4%)	14 (26.4%)
2	14 (17.1%)	6 (11.3%)
3	4 (4.9%)	2 (3.8%)
NA	7 (8.5%)	0 (0.0%)
Metastatic sites [‡]		
Skin/lymph nodes	67 (81.7%)	49 (92.5%)
Lung	45 (54.9%)	30 (56.6%)
Liver	30 (36.6%)	19 (35.8%)
Bone	14 (17.1%)	10 (18.9%)
Brain	15 (18.3%)	9 (17.0%)
Other	28 (34.1%)	19 (35.8%)
No. metastatic sites		
0	5 (6.1%)	0 (0.0%)
1	17 (20.8%)	12 (22.6%)
2	22 (26.8%)	17 (32.1%)
3	22 (26.8%)	13 (24.5%)
>3	16 (19.5%)	11 (20.8%)
AJCC M category		
M _{1a}	9 (11.0%)	8 (15.1%)
M _{1b}	13 (15.8%)	9 (17.0%)
M _{1c}	55 (67.1%)	36 (67.9%)
NA	5 (6.1%)	0 (0.0%)

Abbreviations: ITT, intention to treat; PP, per protocol; UNL, upper normal limit; NA, not assessed; ECOG, Eastern Cooperative Oncology Group; AJCC, American Joint Committee on Cancer.

*Treatment received in stage I to III.

[†]Serum LDH values at study entry, classified according to the normal laboratory ranges of each study center.

[‡]Multiple entries possible.

There, the tumor tissue was cleared from connective and fatty tissues, and ~1 cm³ was subjected to chemosensitivity testing. The remaining tissue material was used for routine histopathology and cryopreservation. Chemosensitivity testing was done using a nonclonogenic ATP-TCA assay (DCS Innovative Diagnostic Systems, Hamburg, Germany; ref. 18). Briefly, the tissue sample was minced and thereafter enzymatically dissociated. The obtained single-cell suspension was depleted of RBC and debris by Ficoll-Hypaque density gradient centrifugation and thereafter assessed for tumor cell count and viability by trypan blue dye exclusion. Minimum tumor cell viability was defined as 25%; otherwise, the assay was considered inevaluable. The cell suspension was given into polypropylene round-bottomed 96-well plates (2 × 10⁴ per well) with or without different chemotherapeutic agents at six different dilutions (6.25, 12.5, 25, 50, 100, and 200) of the individual test drug concentrations (TDC), each tested in triplicates. The drugs and test drug concentrations used were 20 µg/mL dacarbazine (DTIC), 3.8 µg/mL cisplatin, 0.5 µg/mL doxorubicin, 0.5 µg/mL vindesine, 13.6 µg/mL paclitaxel, 12.5 µg/mL gemcitabine, and 20 µg/mL treosulfan, as described before (18). After 7 days of incubation at 37°C, 5% CO₂, and 100% humidity, the cells were lysed and their ATP content was quantified by a luciferin-luciferase luminescence reaction using a microplate luminometer (Berthold Detection Systems, Pforzheim, Germany). Cell suspensions incubated without cytotoxic drugs were used as reference for 100% tumor cell viability.

Best sensitivity index. Individual sensitivity indices ranging from 0 to 600 for each test drug or drug combination were calculated by summing up the percentages of cell viability at the six drug concentrations tested (18). Thus, a sensitivity index of 600 indicates full cell viability/minimal drug sensitivity, whereas a sensitivity index of 0 reflects complete cell death/maximal drug sensitivity. The lowest individual sensitivity index resulting from *in vitro* drug testing, corresponding to the highest individual *in vitro* chemosensitivity, was defined as the best sensitivity index, and was calculated for each individual patient. This index was planned as a variable for patient stratification within the forthcoming phase III trial, which uses overall survival as primary end point. Thus, the best sensitivity index was analyzed for a cutoff value, at which the most pronounced differentiation between survival probabilities takes place. This was done using critlevel analysis (26), an exploratory procedure for the evaluation of quantitative prognostic factors, as well as arbitrary testing. Patients with a best sensitivity index below the determined cutoff value were considered "chemosensitive," whereas the others were considered "chemoresistant."

Sensitivity-directed chemotherapy. The patients received an assay-directed chemotherapy using the individual drug or drug combination showing the highest *in vitro* sensitivity. The therapy regimens used were gemcitabine + treosulfan: gemcitabine 1,000 mg/m² i.v. for 30 minutes, treosulfan 3.5 g/m² i.v. for 30 minutes, intermitted by a 3-hour interval

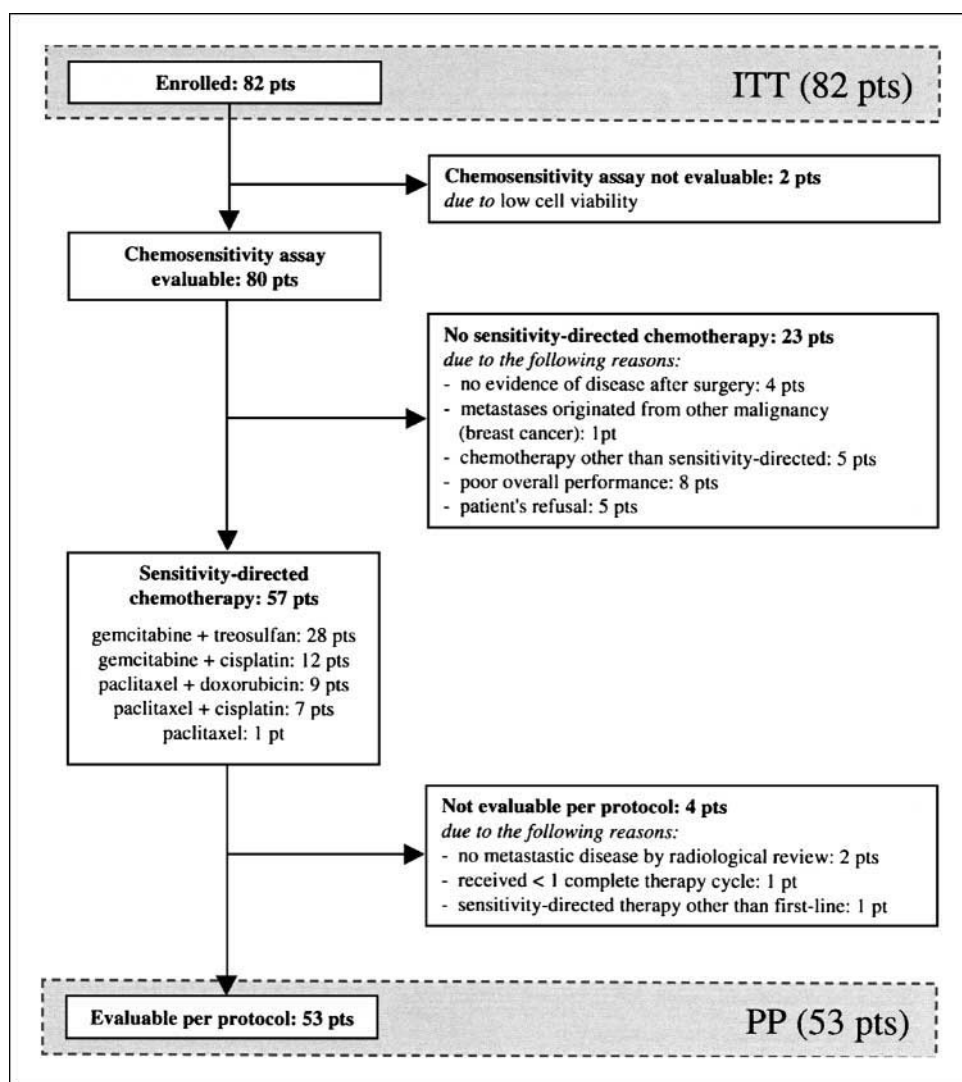


Fig. 1. Schematic presentation of the study flow. ITT, intention to treat; PP, per protocol.

Table 2. *In vitro* chemosensitivity assay results

	ITT	PP
Chemosensitivity assays done	82 (100.0%)	53 (100.0%)
Evaluable	80 (97.6%)	53 (100.0%)
Not evaluable	2 (2.4%)	0 (100.0%)
Tissue sample origin*		
Skin/s.c.	36 (43.9%)	27 (50.9%)
Lymph node	38 (46.3%)	25 (47.2%)
Organ	8 (9.8%)	1 (1.9%)
Cell viability [†] , mean (range)	84.4% (25-100%)	85.2% (25-100%)
Sensitivity index [‡] , mean (range), no. patients		
DTIC	510 (221-600), 79	512 (221-600), 53
Cisplatin	423 (145-600), 79	448 (150-600), 53
Doxorubicin	396 (124-600), 79	426 (176-600), 53
Vindesine	471 (163-600), 79	479 (163-600), 53
Paclitaxel	262 (58-600), 79	246 (58-600), 53
Gemcitabine	346 (45-600), 79	353 (45-600), 53
Treosulfan	345 (26-600), 79	351 (26-600), 53
Gemcitabine + treosulfan	188 (9-514), 79	190 (9-514), 53
Paclitaxel + cisplatin	225 (47-557), 79	224 (47-557), 53
Paclitaxel + doxorubicin	222 (53-600), 79	219 (53-567), 53
Gemcitabine + vindesine	337 (42-600), 79	336 (42-600), 53
Gemcitabine + cisplatin	213 (1-600), 79	226 (1-600), 53
Best sensitivity index [§] , mean (range), no. patients		
Paclitaxel	219 (—), 1	219 (—), 1
Treosulfan	233 (—), 1	—
Gemcitabine + treosulfan	124 (9-262), 35	122 (9-256), 27
Paclitaxel + cisplatin	153 (60-360), 8	155 (60-360), 7
Paclitaxel + doxorubicin	145 (78-225), 13	146 (78-225), 9
Gemcitabine + cisplatin	93 (1-214), 21	76 (1-157), 9

NOTE: Number and results of chemosensitivity assays done in all study patients (for details, see Fig. 1).

*Localization of metastatic lesion biopsied for chemosensitivity testing.

[†]Viability of the cells subjected to *in vitro* sensitivity testing was measured by trypan blue exclusion.

[‡]Individual sensitivity indices were calculated in all patients for each cytotoxic drug as described in Patients and Methods.

[§]Lowest individual sensitivity index of each patient, corresponding to the highest individual *in vitro* drug sensitivity.

(days 1 and 8, every 28 days); gemcitabine + cisplatin: gemcitabine 1,000 mg/m² i.v. for 30 minutes, cisplatin 40 mg/m² i.v. for 60 minutes, intermitted by a 2-hour interval (days 1 and 8, every 28 days); paclitaxel + doxorubicin: paclitaxel 175 mg/m² i.v. for 180 minutes, doxorubicin 30 mg/m² i.v. for 30 minutes, intermitted by a 1-hour interval (day 1 every 28 days); paclitaxel + cisplatin: paclitaxel 200 mg/m² i.v. for 180 minutes, cisplatin 40 mg/m² i.v. for 60 minutes, intermitted by a 1-hour interval (day 1), followed by only cisplatin 40 mg/m² i.v. for 60 minutes (day 8), every 28 days; and paclitaxel as a single agent: paclitaxel 200 mg/m² i.v. for 180 minutes, day 1 every 28 days. Treatment was continued at a tumor response of stable disease or better, and stopped due to disease progression or intolerable side effects. Recommended concomitant medications were serotonin antagonists in all regimens and corticosteroids (one single dose of dexamethasone 20 mg p.o.) in regimens containing paclitaxel. Toxicity was evaluated in all patients who received study treatment using Common Toxicity Criteria 2.0.¹⁰

Response and survival assessment. Patients who completed at least one cycle of sensitivity-directed chemotherapy were considered evaluable for response. Tumor response was assessed by computed tomography and/or magnetic resonance imaging in 8-week intervals and evaluated according to response evaluation criteria in solid tumor (2). Complete and partial responses were combined as objective response. All objective responses had to be confirmed by repeated computed tomography or magnetic resonance imaging scans after

4 weeks. Patients who died from melanoma rapidly after onset of study treatment, so that no assessment of tumor response could be done, were considered as progressive disease (2). Best overall response was defined as the best response recorded from the start of treatment until disease progression; best overall responses of stable disease or better (complete response + partial response + stable disease) were considered as progression arrest (2). All computed tomography and magnetic resonance imaging scans from these patients were retrospectively reviewed by an independent radiologist (stable disease), and tumor response was adjusted following his advice. Overall survival and progression-free survival were measured from the date of enrollment until the date of death or disease progression, respectively. If no such event occurred, the date of the last patient contact was used as end point of survival assessment (censored observation).

Statistical analysis. The database was frozen in March 2005. Fisher's exact test was used to compare tumor response rates and toxicities between groups. Survival curves and median survival times were calculated using the Kaplan-Meier method for censored failure time data. The log-rank test was used for comparison of survival probabilities between two groups, as well as for a global test of differences between more than two groups, respectively. Confidence intervals (95% CI) for median survival were calculated using the method of Brookmeyer (27). Multivariate analysis of factors influencing survival was done using the proportional hazard model of Cox (28). Statistical analyses were done using the statistical packages ADAM of the Biostatistics Unit and SAS 8.1 (SAS Institute, Cary, NC). *P* values <0.05 were considered statistically significant. All *P* values are two-tailed and unadjusted for potential multiple comparisons.

¹⁰ <http://ctep.cancer.gov/reporting/ctc.html>.

Table 3. Treatment efficacy and survival

	ITT	PP
	82 (100.0%)	53 (100.0%)
Best overall response		
CR	5 (6.1%)	5 (9.4%)
PR	8 (9.7%)	8 (15.1%)
SD	10 (12.2%)	7 (13.2%)
PD	34 (41.5%)	33 (62.3%)
Not evaluable*	25 (30.5%)	0 (0.0%)
Objective response (CR + PR)	13 (15.9%)	13 (24.5%)
Progression arrest (CR + PR + SD)	23 (28.0%)	20 (37.7%)
Median progression-free survival mo (95% CI)	3.6 (3.0-4.8)	3.6 (2.9-4.8)
Median overall survival mo (95% CI)	7.9 (5.7-13.0)	8.8 (5.8-14.2)
AJCC M category		
M _{1a/b}	20.8 (9.7-28.4)	20.8 (8.8-24.8)
M _{1c}	5.4 (4.1-7.4)	5.8 (4.2-11.6)
Serum LDH		
≤UNL	14.2 (9.7-20.8)	14.2 (8.8-20.8)
>UNL	4.0 (2.8-4.7)	4.2 (4.0-7.4)
ECOG performance state		
0	20.8 (9.7-24.8)	14.2 (8.8-21.2)
1-3	4.7 (3.6-5.7)	4.7 (4.0-7.9)
Therapy regimen		
Gemcitabine + treosulfan		14.2 (5.7-20.8)
Gemcitabine + cisplatin		4.4 (4.0-5.8)
Paclitaxel + doxorubicin		7.2 (4.8-14.2)
Paclitaxel + cisplatin		13.0 (7.9-28.1)

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

*For reasons for exclusion from evaluation, see study flow (Fig. 1). Best overall response was defined as the best tumor response recorded from the start of treatment until removal of the patient from the trial. Survival was measured from the date of enrollment until the date of death or disease progression, respectively; if no such event occurred, the date of the last patient contact was used as end point.

Results

Patient characteristics and study flow. Between January 2001 and May 2004, 82 patients (intention-to-treat) were enrolled into the study from 11 participating centers (see Acknowledge-

ments); detailed patient characteristics are presented in Table 1. Fifty-seven patients (69.5%) received an assay-directed chemotherapy within 1 month after enrollment, 25 patients (30.5%) received other than test-directed or no chemotherapy (see Fig. 1). Four of 57 patients treated per protocol had to be

Table 4. Characteristics of patients with objective response

Patient ID	Sex/ Age (y)	Stage (AJCC)	Sites of metastases	LDH (serum)	OPS (ECOG)	Best sensitivity index	Treatment regimen	Best overall response	PFS (mo)	OS (mo)
Ha060	F/73	M _{1c}	SQ, LN, gall bladder	<UNL	0	96	Paclitaxel + doxorubicin	CR	8.6	28.9+
Ho029	M/60	M _{1c}	LN, lung, liver	<UNL	0	60	Paclitaxel + cisplatin	PR	21.8	28.0
Ho014	M/54	M _{1b}	lung	<UNL	0	1	Gemcitabine + cisplatin	CR	10.8	21.2
Ma070	M/70	M _{1b}	SQ, LN, lung	<UNL	0	214	Gemcitabine + treosulfan	PR	12.2	19.1+
Ho063	M/68	M _{1c}	LN, lung, liver	<UNL	1	175	Gemcitabine + treosulfan	PR	14.4	19.0
Ma066	M/71	M _{1a}	LN	<UNL	1	256	Gemcitabine + treosulfan	PR	14.7	16.9+
Ma078	M/46	M _{1b}	LN, lung	<UNL	0	9	Gemcitabine + treosulfan	PR	11.3	15.9+
Ma076	M/69	M _{1a}	LN	<UNL	0	148	Gemcitabine + treosulfan	CR	15.8	15.8+
Ma083	F/72	M _{1b}	LN, lung	<UNL	0	221	Gemcitabine + treosulfan	PR	10.3	12.9+
Ha007	F/70	M _{1b}	LN, lung	<UNL	0	77	Gemcitabine + treosulfan	PR	10.5	10.5+
Ha088	F/50	M _{1c}	LN, liver	<UNL	0	46	Gemcitabine + treosulfan	CR	9.5	9.5+
Ha006	F/81	M _{1a}	LN	<UNL	0	91	Gemcitabine + treosulfan	CR	5.1	8.8
Ha027	F/45	M _{1c}	SQ, LN, lung, liver	NA	1	30	Gemcitabine + cisplatin	PR	3.6	5.7

NOTE: Patients are sorted by overall survival. Age, stage of disease, sites of metastases, serum LDH, and overall performance index refer to the time point of enrollment. Best sensitivity index was defined as the lowest individual drug sensitivity index; best overall response was defined as the best tumor response recorded from the start of treatment until removal of the patient from the trial. All responses presented here were reviewed and confirmed by an independent radiologist.

Abbreviations: OPS, overall performance index; PFS, progression-free survival; OS, overall survival.

excluded from analysis due to different reasons (see Fig. 1). Fifty-three of 82 patients (64.6%) were evaluable for all study end points (per protocol).

Heterogenous chemosensitivity in melanoma. Chemosensitivity testing was done from different metastatic sites (see Table 2). The test assay showed a high yield with only 2 of 82 (2.4%) assays revealing inevaluable results (see Fig. 1; Table 2). Routine histopathology was done in all 82 patients, leading to the diagnosis of metastases from melanoma in all but one case, which showed metastasis from breast cancer. This patient was excluded from study treatment (see Fig. 1). The remaining 79 evaluable chemosensitivity assays on melanoma samples revealed a heterogenous sensitivity to different chemotherapeutics and combinations (see Table 2). The drug combinations with the highest *in vitro* sensitivities were gemcitabine + treosulfan, paclitaxel + cisplatin, paclitaxel + doxorubicin, and gemcitabine + cisplatin. These four combinations also most often showed the highest individual *in vitro* chemosensitivity, represented by the best sensitivity index (see Table 2). The combination gemcitabine + vindesin as well as all single agents tested revealed rather low *in vitro* sensitivities.

Response to treatment. Treatment responses are presented in Table 3. The characteristics of patients showing objective responses are given in Table 4.

Survival analysis. The median follow-up time for all patients, whether alive or dead, was 19.3 months. Considering the per protocol population, a total number of 41 deaths had occurred, 12 patients were still alive. Two of 41 patients responded to chemotherapy but died from septic infections following surgical procedures and were therefore considered as censored observations. The remaining 39 of 41 patients died from melanoma progression. A detailed presentation of overall survival and progression-free survival is provided in Table 3. As expected, patients with an elevated serum lactate dehydrogenase (LDH) showed a poorer survival than patients with normal LDH levels ($P = 0.0005$; Fig. 2A), patients presenting an unimpaired overall performance state revealed a favorable survival compared with patients with a reduced performance ($P = 0.0003$; Fig. 2B), and patients at stage $M_{1a/b}$ revealed a significantly prolonged survival compared with patients at stage M_{1c} ($P = 0.021$; Fig. 2C). Different chemotherapy regimens revealed differences in overall survival ($P = 0.032$; Fig. 3A), with gemcitabine + treosulfan and paclitaxel + cisplatin appearing to be associated with a higher probability of survival than gemcitabine + cisplatin and paclitaxel + doxorubicin.

In vitro chemosensitivity is associated with tumor response and survival. Using critical analysis and arbitrary testing of different cutoff values, a threshold value of 100 could be determined for the best sensitivity index to differentiate between chemosensitive and chemoresistant patients. Patients whose tumors were considered chemosensitive (best sensitivity index ≤ 100) revealed a better response than patients who were tested chemoresistant (best sensitivity index > 100) with an objective response of 36.4% versus 16.1% ($P = 0.114$) and a progression arrest (complete response + partial response + stable disease) of 59.1% versus 22.6% ($P = 0.01$; Table 5). Chemosensitive and chemoresistant patients moreover differed in overall survival (median 14.6 versus 7.4 months, $P = 0.041$; Fig. 3C) and progression-free survival (median 5.1 versus 3.0 months, $P = 0.052$; Fig. 3D). Multivariate analysis revealed serum LDH \leq versus $>$ upper normal limit ($P = 0.03$, hazard ratio 2.38; 95% CI, 1.07-5.32) and overall performance

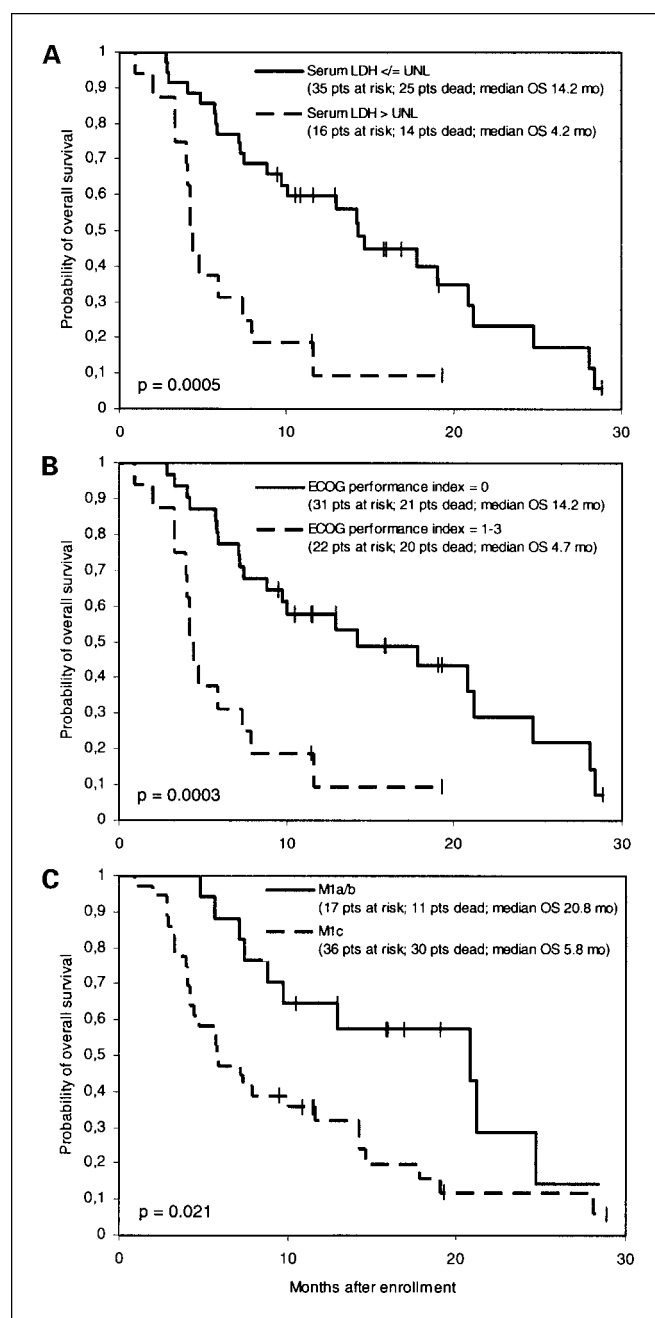


Fig. 2. Kaplan-Meier curves showing the probability of overall survival of the per protocol population by serum LDH (A), Eastern Cooperative Oncology Group (ECOG) performance status (B), and American Joint Committee on Cancer (AJCC) M category (C). In (A), two patients are not presented due to missing data (see Table 1). Differences between groups were calculated using the log-rank test. Vertical bars, censored observations.

state 0 versus 1 to 3 ($P = 0.03$, hazard ratio 2.21; 95% CI, 1.05-4.64) as the strongest independent predictors of overall survival, followed by best sensitivity index \leq versus $>$ 100 ($P = 0.18$, hazard ratio, 1.71; 95% CI, 0.77-3.75) and American Joint Committee on Cancer M category a/b versus c ($P = 0.82$, hazard ratio 0.90; 95% CI, 0.35-2.33). Comparing chemosensitive and chemoresistant patients, serum LDH, overall performance state, and American Joint Committee on Cancer M category showed balanced distributions (Table 5). Neither tumor response nor overall

survival revealed an association with previous chemotherapy and/or IFN- α treatment of the patients (data not shown).

Treatment-related toxicity. Common toxicity criteria grade 3/4 toxicities and the actions required by those are summarized in Table 6. These toxicities were experienced by 19 of 57 (33.3%) patients, with the majority presenting as myelosuppression. No differences could be observed in frequency or intensity of toxicities between different drugs or drug combinations. A treatment discontinuation was required in one patient only; no fatal outcome was observed. Patients who experienced grade 3/4 toxicities revealed a favorable overall survival compared with patients without (median 14.2 versus 5.9 months; $P = 0.036$; Fig. 3B). Grade 3/4 toxicities mainly occurred in patients who received multiple treatment cycles. Eight of 19 (42%) patients experiencing these toxicities received more than four cycles, compared with only 4 of 34 (12%) patients with less than four cycles.

Discussion

As the first major finding of the present study, pretherapeutic chemosensitivity testing of melanoma tissue samples using the

ATP-TCA proved as a feasible method and yielded interpretable results in 98% (80 of 82 samples), even if applied in a multicenter setting. Moreover, the test results were obtained within a time frame of 7 days in all cases, so that long latencies were no subject for a patient's refusal to be treated per protocol. Additional routine histopathology analysis of the tissue samples proved as a sensible procedure to exclude secondary malignancies, as detected in one patient. As described before (23, 24), melanoma tissues revealed heterogenous chemosensitivities, with drug combinations showing higher sensitivities than single agents. The most effective combinations were gemcitabine + treosulfan, paclitaxel + cisplatin, paclitaxel + doxorubicin, and gemcitabine + cisplatin. *In vitro* sensitivities to DTIC were extremely poor with nearly all tumor samples tested resistant to this drug. This observation might be explained by the prodrug status of DTIC. However, there is evidence for its activation by microsomal p450 in melanoma cells (23), and similar resistance profiles could be shown for temozolomide, which does not rely on activation by p450 (23, 24). Thus, it appears that DTIC and temozolomide are not suitable for chemosensitivity testing using the ATP-TCA, and therefore will be excluded from testing within the forthcoming phase III trial.

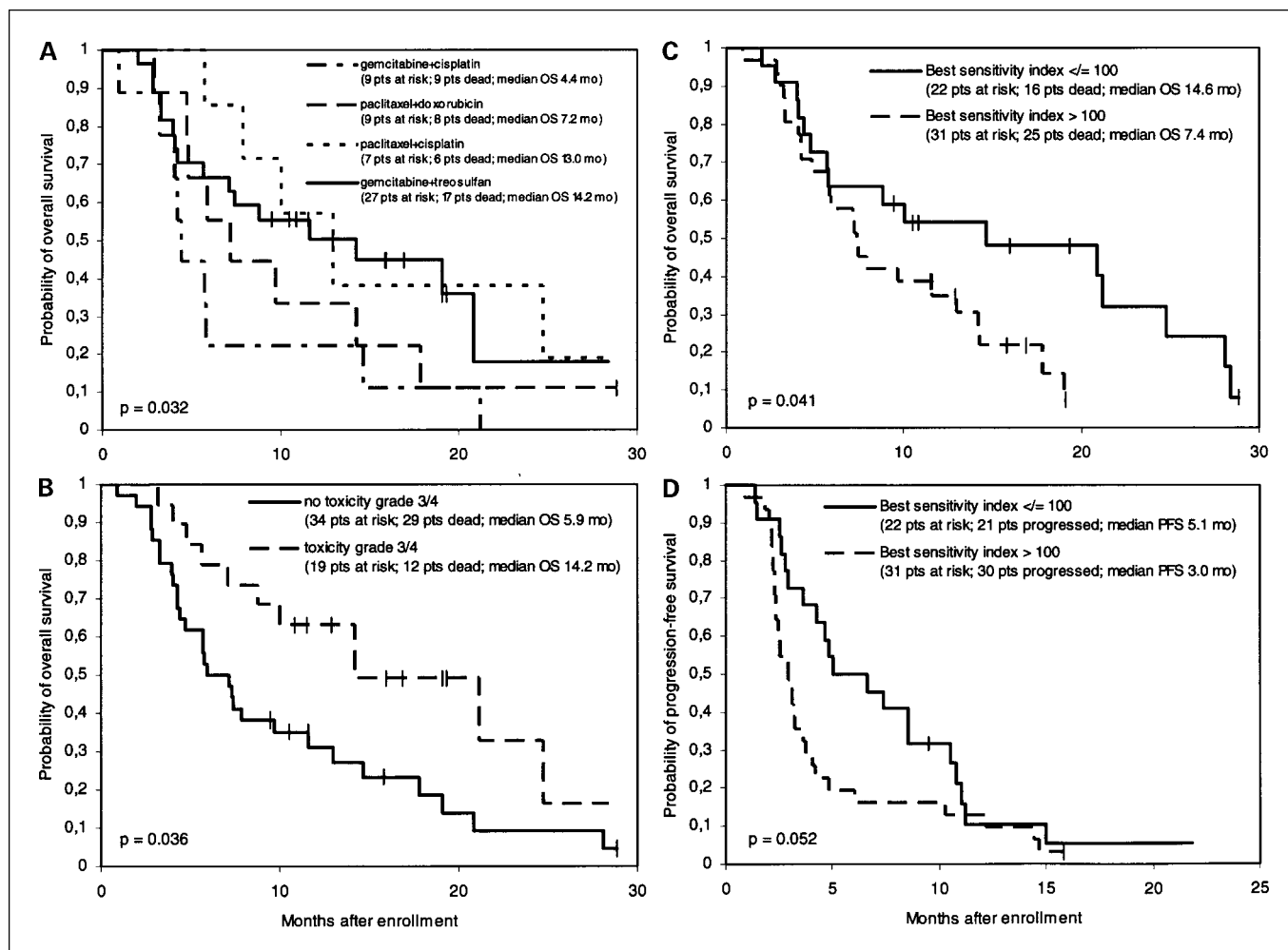


Fig. 3. Kaplan-Meier curves showing the probability of overall survival of the per protocol population by different treatment regimens (A), by the occurrence of grade 3 or 4 toxicities under assay-directed chemotherapy (B), and by best sensitivity index of *in vitro* chemosensitivity testing (C). D, probability of progression-free survival of the per protocol population by best sensitivity index. Differences between groups were calculated using the log-rank test. OS, overall survival; PFS, progression-free survival. Vertical bars, censored observations. Toxicity was graded according to Common Toxicity Criteria 2.0 (<http://ctep.cancer.gov/reporting/ctc.html>).

Table 5. Characteristics of chemosensitive versus chemoresistant patients

	Best sensitivity index ≤ 100	Best sensitivity index > 100	P
	22 (100.0%)	31 (100.0%)	
AJCC M category			
M _{1a/b}	8 (36.4%)	9 (29.0%)	>0.5*
M _{1c}	14 (63.6%)	22 (71.0%)	
Serum LDH			
\leq UNL	16 (72.7%)	19 (61.3%)	0.37*
$>$ UNL	5 (22.7%)	11 (35.5%)	
NA	1 (4.6%)	1 (3.2%)	
ECOG performance state			
0	13 (59.1%)	18 (58.1%)	>0.5
1-3	9 (40.9%)	13 (41.9%)	
Best overall response			
CR	4 (18.2%)	1 (3.2%)	0.03*
PR	4 (18.2%)	4 (12.9%)	
SD	5 (22.7%)	2 (6.5%)	
PD	9 (40.9%)	24 (77.4%)	
CR + PR	8 (36.4%)	5 (16.1%)	
CR + PR + SD	13 (59.1%)	7 (22.6%)	
Median overall survival, mo (95% CI)	14.6 (5.7-24.8)	7.4 (5.7-13.0)	0.036 [†]
Median time to progression, mo (95% CI)	5.1 (3.6-10.5)	3.0 (2.4-3.6)	0.041 [†]

NOTE: Per protocol patients were divided into two groups by *in vitro* best sensitivity index (≤ 100 , chemosensitive; > 100 , chemoresistant). Best sensitivity index was defined as sensitivity index of the test drug or drug combination with the highest individual *in vitro* chemosensitivity (see Patients and Methods).

*Differences between chemosensitive and chemoresistant patients analyzed with Fisher's exact test.

[†]Differences between chemosensitive and chemoresistant patients analyzed with log-rank test.

Concerning the design of this study, the rate of patients inevaluable per protocol was unexpectedly high (29 of 82; 35%). This was mainly caused by the inclusion of patients who were not able to receive combined chemotherapeutic regimens due to poor overall performance (13 of 29; 45%), and thus received either monotherapy with DTIC or temozolomide (five patients) or no chemotherapy at all (eight patients; see Fig. 1). We conclude that with regard to the design of future

studies, a threshold performance status should be defined for inclusion, rather than physical ability to receive polychemotherapy.

As a second major finding of this trial, we observed good efficacy of assay-directed individualized chemotherapy. Objective response was 24.5% (per protocol), and thus was superior to recent reports of an objective response of 6% to 7% under standard DTIC monotherapy (3, 4). Median overall survival was 8.8 months (per protocol), which is comparable with the results reported under DTIC (3, 4). However, it should be noticed that the prognostic indices of the present study cohort were extremely poor with brain metastases present in 17.0% of the patients, and 13.2% having primary mucosal melanomas (per protocol). Both of these conditions are well known to account for a poor prognosis and thus were not allowed for inclusion into the above-mentioned DTIC trials (3, 4). All objective responses of the present trial were observed in patients with normal serum LDH and an unimpaired overall performance state of 0 to 1 (see Table 4). No objective responses were observed in patients with brain metastases, prompting us to exclude this patient group from the planned phase III trial.

Our third and most important finding was that the *in vitro* chemosensitivity obtained by the ATP-TCA was associated with the *in vivo* therapy outcome of the investigated melanoma patients. Patients whose tumor tissue samples were tested sensitive to one of the investigated drugs or drug combinations revealed higher response rates and a prolonged overall survival than patients who were tested resistant. For the differentiation between "chemosensitive" and "chemoresistant," a threshold sensitivity index could be defined, which, after careful evaluation in subsequent studies, might be used as a predictor

Table 6. Treatment-related toxicities grade 3 and 4

	Patients treated per protocol, 57 (100.0%)
Grade 3 or 4 toxicity	19 (33.3%)
Laboratory changes	
Hemoglobin	3 (5.3%)
Leukocytes	14 (24.6%)
Platelets	6 (10.5%)
Gastrointestine	2 (3.5%)
Neurology	3 (5.3%)
Endocrine	1 (1.8%)
General/lethargy	2 (3.5%)
Action required	
Dose reduction	
25%	5 (8.8%)
50%	2 (3.5%)
Cycle delay	5 (8.8%)
Treatment discontinuation	1 (1.8%)

NOTE: Toxicity was classified and graded according to Common Toxicity Criteria 2.0 (ctep.cancer.gov/reporting/ctc.html). Data represent the worst Common Toxicity Criteria grade by patient.

of chemotherapy outcome in terms of tumor responsiveness and overall survival. Such a predictor would offer the possibility of future therapy decisions based on the results of *in vitro* chemosensitivity testing, thus enhancing treatment efficacy in sensitive patients while sparing toxicity in resistant patients, who might then be admitted to alternative treatment regimens. However, it should be noticed that the sensitivity index was not independent predictor of overall survival, as were serum LDH and overall performance.

Without an obvious association with chemosensitivities, distinct therapy regimens (gemcitabine + treosulfan, paclitaxel + cisplatin) showed higher response and survival rates than others (paclitaxel + doxorubicin, gemcitabine + cisplatin). This might be due to the well-known limitations inherent to drug sensitivity assays, mainly caused by the biology of the tumor, which cannot be completely imitated by *in vitro* test conditions. In addition, it should be mentioned that we had to define dosing regimens for each drug or drug combination, which were chosen based on empirical data gained by small phase I/II studies and possibly might not lead to full effectiveness of the drug at the tumor site. Subsequent studies will be necessary to optimize drug dosing and treatment schedules.

In conclusion, this cooperative group study contributes to the recognition of *in vitro* chemosensitivity testing as a reasonable tool for the selection of individualized chemotherapy regimens. As recently controversially discussed (17, 29–31), the American Society of Clinical Oncology Working Group on Chemotherapy Sensitivity and Resistance Assays stated that based on the current level of evidence, chemosensitivity assays should not be recommended for clinical use outside of study protocols (32). Moreover, the working group recommended a comparison of patients for whom chemotherapy was chosen based on the results of chemosensitivity testing with patients whose therapy was chosen empirically, to be the only effective study design. However, in the situation of melanoma, one should consider that, currently, no distinct alternative to empirical therapy, which is DTIC monotherapy, exists (1, 7). In fact, the

present study was not designed to compare two different therapy regimens, but rather to help identify the individually most effective drugs among multiple nonstandard options. Our results show that the assay used in this study is predictive of therapy outcome, and indicate that nonstandard chemotherapeutics are effective in melanoma if they are applied selectively based on individual chemosensitivity profiles. However, these encouraging results need further evaluation by prospectively randomized trials. A subsequent phase III study protocol comparing patients treated on the basis of chemosensitivity assay results with patients treated with DTIC standard chemotherapy is currently being developed by the Dermato-cooperative Oncology Group.

Appendix. Participating centers and investigators

Center	Investigators
Homburg/Saar	Claudia Pöhler, Alexandra Stark, Wolfgang Tilgen, Uwe Reinhold
Hamburg	Thomas Haalck, Jürgen Altenhoff, Karsten Neuber
Mannheim	Adina Thölke, Robert Figl, Antje Sucker, Dirk Schadendorf, Selma Ugurel
Magdeburg	Vassiliki Bekou, Jens Ulrich
Kiel	Katharina Kähler, Axel Hauschild
Frankfurt/Main	Konstanze Spieth
Jena	Martin Kaatz
Köln	Cornelia Mauch
Aachen	Faris Abuzahra
Erlangen	Gerold Schuler
Stuttgart/ Bad Cannstadt	Peter von den Driesch

Acknowledgments

We thank the investigators from all participating centers whose concerted efforts were of essential value for the successful implementation and completion of this study, and Jürgen C. Becker for critically reading the manuscript and providing helpful discussions.

References

- Crosby T, Fish R, Coles B, Mason MD. Systemic treatments for metastatic cutaneous melanoma (Cochrane Review). The Cochrane library. vol. 4. Oxford: Update Software; 2001.
- Therasse P, Arbuuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–16.
- Food and Drug Administration Center for Drug Evaluation and Research: Oncologic Drugs Advisory Committee, Briefing Material: May 3, 2004 AM Session-Genasense. <http://www.fda.gov/ohrms/dockets/ac/04/briefing/4037B1.02.FDA-Genasense.pdf>.
- Schadendorf D, Ugurel S, Schuler-Thurner B, et al. Dacarbazine (DTIC) versus vaccination with autologous peptide-pulsed dendritic cells (DC) in first-line treatment of patients with metastatic melanoma: a randomized phase III trial of the DC study group of the DeCOG. *Ann Oncol* 2006;17:563–70.
- Joukhadar C, Klein N, Mader RM, et al. Penetration of dacarbazine and its active metabolite 5-aminoimidazole-4-carboxamide into cutaneous metastases of human malignant melanoma. *Cancer* 2001;92:2190–6.
- Helmbach H, Rossmann E, Kern MA, Schadendorf D. Drug-resistance in human melanoma. *Int J Cancer* 2001;93:617–22.
- Eigentler TK, Caroli UM, Radny P, Garbe C. Palliative therapy of disseminated malignant melanoma: a systematic review of 41 randomised clinical trials. *Lancet Oncol* 2003;4:748–59.
- Kleeberg UR, Engel E, Israels P, et al. Palliative therapy of melanoma patients with fotemustine. Inverse relationship between tumour load and treatment effectiveness. A multicentre phase II trial of the EORTC-Melanoma Cooperative Group (MCG). *Melanoma Res* 1995;5:195–200.
- Petit T, Borel C, Rixe O, et al. Complete remission seven years after treatment for metastatic malignant melanoma. *Cancer* 1996;77:900–2.
- Neuber K, tom Dieck A, Blodorn-Schlicht N, Itschert G, Karnbach C. Treosulfan is an effective alkylating cytostatic for malignant melanoma *in vitro* and *in vivo*. *Melanoma Res* 1999;9:125–32.
- Iqbal M, Marshall E, Green JA. Ten-year survival in advanced malignant melanoma following treatment with interferon and vindesine. *Ann Oncol* 2000;11:483–5.
- Nieboer P, Mulder NH, Van Der Graaf WT, Willemse PH, Hospers GA. Dacarbazine DTIC and carboplatin as an outpatient treatment for disseminated malignant melanoma. *Anticancer Res* 2001;21:3115–6.
- Guven K, Kittler H, Wolff K, Pehamberger H. Cisplatin and carboplatin combination as second-line chemotherapy in dacarbazine-resistant melanoma patients. *Melanoma Res* 2001;11:411–5.
- Bafaloukos D, Aravantinos G, Fountzilas G, et al. Docetaxel in combination with dacarbazine in patients with advanced melanoma. *Oncology* 2002;63:333–7.
- Cortazar P, Johnson BE. Review of the efficacy of individualized chemotherapy selected by *in vitro* drug sensitivity testing for patients with cancer. *J Clin Oncol* 1999;17:1625–31.
- Samson DJ, Seidenfeld J, Ziegler K, Aronson N. Chemotherapy sensitivity and resistance assays: a systematic review. *J Clin Oncol* 2004;22:3618–30.
- Nagourney R. Chemosensitivity and resistance assays: a systematic review? *J Clin Oncol* 2005;23:3640–1.
- 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.
19. Cree IA, Kurbacher CM, Untch M, et al. Correlation of the clinical response to chemotherapy in breast cancer with *ex vivo* chemosensitivity. *Anticancer Drugs* 1996;7:630–5.
20. Kurbacher CM, Cree IA, Bruckner HW, et al. Use of an *ex vivo* ATP luminescence assay to direct chemotherapy for recurrent ovarian cancer. *Anticancer Drugs* 1998;9:51–7.
21. Neale MH, Myatt N, Cree IA, et al. Combination chemotherapy for choroidal melanoma: *ex vivo* sensitivity to treosulfan with gemcitabine or cytosine arabinoside. *Br J Cancer* 1999;79:1487–93.
22. Myatt N, Cree IA, Kurbacher CM, Foss AJ, Hungerford JL, Plowman PN. The *ex vivo* chemosensitivity profile of choroidal melanoma. *Anticancer Drugs* 1997;8:756–62.
23. Cree IA, Neale MH, Myatt NE, et al. Heterogeneity of chemosensitivity of metastatic cutaneous melanoma. *Anticancer Drugs* 1999;10:437–44.
24. Ugurel S, Tilgen W, Reinhold U. Chemosensitivity testing in malignant melanoma. *Recent Results Cancer Res* 2003;161:81–92.
25. Balch CM, Buzaid AC, Soong SJ, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *J Clin Oncol* 2001;19:3635–48.
26. Abel U, Berger J, Wiebelt H. Critlevel: An exploratory procedure for the evaluation of quantitative prognostic factors. *Meth Inform Med* 1984;23:154–6.
27. Brookmeyer R, Crowley J. A confidence interval for the median survival time. *Biometrics* 1982;38:29–41.
28. Kalbfleisch JD, Prentice RL. *The statistical analysis of failure time data*. New York: Wiley; 1980.
29. Fruehauf JP, Alberts DS. *In vitro* drug resistance versus chemosensitivity: two sides of different coins. *J Clin Oncol* 2005;23:3641–3.
30. Wieand HS. Chemotherapy sensitivity and response assays: are the ASCO guidelines for clinical trial design too restrictive? *J Clin Oncol* 2005;23:3643–4.
31. Castro M. Resisting a fundamentalist policy. *J Clin Oncol* 2005;23:3645–6.
32. Schrag D, Garewal HS, Burstein HJ, Samson DJ, Von Hoff DD, Somerfield MR. American Society of Clinical Oncology Technology Assessment: chemotherapy sensitivity and resistance assays. *J Clin Oncol* 2004;22:3631–8.