Eur J Med Res (2007) 12: 497-502

Does Chemosensitivity-assay-directed Therapy Have an Influence on the Prognosis of Patients with Malignant Melanoma Stage IV?

A Retrospective Study of 14 Patients with Malignant Melanoma Stage IV

M. Doerler[1], J. Hyun[2], I. Venten[1], A. Potthoff[1], U. Bartke[1], K. Serova[3], S. Hoextermann[1], P. Altmeyer[1], N. H. Brockmeyer[1]

[1]Department of Dermatology, Ruhr-University Bochum,
[2]Department of Dermatology, Ruprecht-Karls-University Heidelberg,
[3]Institute of Development Research and Development Policy, Ruhr-University Bochum, Germany

Abstract
Objective: To evaluate the efficacy of chemosensitivity-testing directed chemotherapy in comparison with empirically chosen therapy regimens in patients with malignant melanoma stage IV.
Patients and Methods: Retrospective study including 14 patients with histologically confirmed malignant melanoma and diagnosis of stage IV disease by routine diagnostic procedures. Patients in group A (n = 7) were treated according to their individual chemosensitivity testing results, whereas patients in group B (n = 7) received empirically chosen treatment regimens. Chemosensitivity testing was performed using a nonclonogenic ATP-TCA assay. For statistical analysis the Kaplan-Meier method was used to calculate survival curves. The log-rank test was performed to compare the overall survival according to treatment group, LDH level in serum and AJCC-category. To compare the distribution of sex, LDH level in serum and AJCC-category between the treatment groups, the Fisher exact test was used.
Results: The median overall survival of group A exceeded the median overall survival of group B by 8 versus 3 months, respectively with a median overall survival of 5 months for the whole study population. LDH level in serum at study entry showed a strong correlation with overall survival, with normal LDH levels leading to a statistically significant longer survival (p = 0.006 for the log-rank test, respectively). Moreover, stage AJCC M1a/b yielded to a better prognosis compared with stage AJCC M1c (log-rank test p = 0.066; not statistically significant).
Conclusion: Chemosensitivity-assay directed therapy might be a useful tool in determining the optimized chemotherapeutic drug or drug combination in the individual patient and might contribute to a better prognosis in patients with metastatic melanoma stage IV.

Introduction

Whereas early diagnosed melanoma can be cured by surgical excision, the prognosis of patients with malignant melanoma stage IV is still poor. According to Cummins et al. [1], patients with metastatic malignant melanoma show a median survival rate of 6 months and a 5-year-survival rate of less than 5%.

Many ongoing studies are investigating new therapeutic modalities including recombinant cytokines (IL-2, GM-CSF), vaccination and blockades of the neoplastic signal transduction [2]. One of the most promising and advanced substances is BAY 43-9006 (Sorafenib), which functions as a kinase inhibitor with the targets VEGFR, PDGFR as well as BRAF and CRAF [3]. As a single agent, sorafenib showed modest activity. However in combination with carboplatin and paclitaxel the results are more encouraging [4]. Indeed, within current research interest there is a huge variety of drugs for targeted melanoma therapy with most of them inhibiting kinases leading to reduced proliferation in cancer cells. However, a ground-breaking success in melanoma stage IV therapy could not yet been achieved.

Standard treatment with dacarbacine (DTIC) has recently shown low response rates of 6% to 7% [5, 6]. Many studies could not demonstrate a significant benefit of other chemotherapeutic drugs compared to dacarbacine.

However, a recently published phase II trial comparing the outcome of chemosensitive versus chemoresistant patients by Ugurel et al. [7] revealed markedly better response rates for chemosensitive patients (59.1% vs. 22.6% for progression arrest, defined as complete response + partial response + stable disease). Chemosensitivity was tested using a nonclonogenic ATP-TCA assay. For each patient a chemosensitivity index was calculated by summing up the cell viability in percent at the tested drug concentrations (0-600). Thus, a higher sensitivity index indicated higher cell viability and lower drug sensitivity. The authors defined a threshold sensitivity index of 100 to distinguish between chemosensitive (index ≤ 100) and chemoresistant (index ≥ 100) patients.

In their trial the overall survival of chemosensitive patients almost doubled the overall survival of chemoresistant patients (14.6 months vs. 7.4 months). All patients received chemosensitivity-directed therapy according to their individual testing results.

Whether chemosensitivity-assay-directed treatment improves the outcome of patients with malignant melanoma stage IV compared to empirically chosen reference treatment still remains to be investigated.

We performed a retrospective study to compare the prognosis (overall survival) of patients with malignant melanoma stage IV treated either according to their chemosensitivity assay results or with empirically and clinically chosen therapy.

Patients and Methods

Study design and patient population

This retrospective study included 14 patients with malignant melanoma, diagnosed stage IV disease between October 2001 and May 2005. At this time, chemosensitivity assays were performed in 25 malignant melanoma patients in our clinic (Department of Dermatology, Ruhr-University Bochum). All patients were informed about the tests and had given written consent. The main purpose of this study was to compare the overall survival of patients who had received chemotherapy according to their chemosensitivity assay results (group A) with patients who were treated with empirically and clinically chosen therapy regimens in accordance with their individual requests (group B). Time of study entrance and the beginning of therapy was defined as the month of diagnosis of stage IV disease.

The following criteria were necessary for inclusion into the study: patients with histologically confirmed malignant melanoma (1), stage IV disease according to the American Joint Committee on Cancer criteria (2), known medical history including overall survival and known regularly performed staging results (at least twice yearly) (3).

We excluded patients tested for chemosensitivity because of clinically diagnosed satellite and/or intransit metastases without other metastases leading to stage IV disease (1) and patients who did not survive for at least 2 months after diagnosis of stage IV disease because of advanced metastatic melanoma treated palliatively without chemotherapy (2) (n = 11).

Chemosensitivity assay

Tumor tissue was gained from metastatic lesions by surgical excision and immediately sent to our laboratory where it was cleared from fatty and connective tissue. Then, chemosensitivity testing was performed using a nonclonogenic ATP-TCA assay as described before (DCS Innovative Diagnostic Systems, Hamburg, Germany) [8, 9]. Drugs tested were: dacarbacine, gemcitabine, treosulfan, doxorubicin and the combinations gemcitabine/treosulfan with or without doxorubicin, gemcitabine/vindesine, gemcitabine/cisplatin, paclitaxel/cisplatin and paclitaxel/doxorubicin. The drug or drug combination leading to the lowest cell viability was considered the most effective.

Staging

All patients received regular physical examinations by dermatologists (at least 4 times a year) and routine diagnostic procedures at least twice yearly including chest x-ray, abdominal/lymph node ultrasound and computed tomography of the skull, thorax and abdomen to evaluate the progress of the disease.

Therapy regimens

Group A (7 patients) received chemotherapy according to the individual chemosensitivity assay results. In this group the following therapy regimens were applied: gemcitabine (1000 mg/m^2) in combination with treosulfan (35mg/kg) days 1 and 8 every 28 days and liposomal doxorubicin (20mg/m^2) day 2 i.v. (4 patients); gemcitabine (1000mg/m^2) in combination with treosulfan (35mg/kg) days 1 and 8 every 28 days (2 patients) and doxorubicin (30mg/m^2) day 1 in combination with paclitaxel (100mg/m^2) day 2 every 28 days i.v. (1 patient).

The patients in group B were treated according to the following regimens: temozolomide (200mg/m^2) days 1-5 every 28 days p.o. (3 patients); temozolomide (200mg/m^2) p.o. days 1-5 in combination with vindesine (3mg/m^2) and cisplatin (100mg/m^2) day 1 i.v. every 28 days (1 patient); interferon-alpha-2b (20 Mio I.E./m^2) 5x/week for 4 weeks i.v. with a maintenance dose of 10 Mio I.E./m^2 3x/week for 48 weeks s.c. (1 patient), interferon alpha-2a 3 Mio I.E. 3x/weeks s.c. for 18 months (1 patient) and Iscador® P 1mg (Viscum alb. ssp. austriac) 3x/week 1 ml s.c. (1 patient).

Statistical analysis

To calculate survival curves (Fig. 2-4) we used the Kaplan-Meier method. The log-rank test was performed to compare survival probabilities between the two treatment groups (group A and B) and to compare the overall survival according to LDH level in serum and AJCC-category. The level of significance was set at alpha = 0.05. To determine whether the LDH levels, the AJCC categories and the sex of the patients were distributed equally among the two treatment groups we performed the Fisher exact test (alpha = 0.05).

Results

In this study, a total of 14 patients could be included. Detailed patient characteristics are shown in Table 1. Seven (50%) patients received assay-directed chemotherapy (group A), whereas the remaining seven (50%) patients were treated according to empirically chosen therapy regimens (group B). One patient (group B) was treated with Iscador® because he refused other therapeutic options.

Patient age at study entry ranged from 39-76 years (median 64.5), respectively, with a median age of 61.0 years for group A and 65.0 years for group B. Group A included 71.4% women, whereas in group B 42.9% were female. Before study entry, 50% of patients had received interferon-based treatment regimens or chemotherapy. LDH in serum was elevated in 35.7% of the study population (42.9% in group A and 28.6% in group B, respectively). Detailed data about age, sex, previous therapy regimens, LDH levels in serum, AJCC category and metastases are shown in Table 1. To determine, whether sex, LDH level in serum and AJCC category were distributed equally among the two treatment groups, we performed the Fisher exact test, not leading to significant results, which suggests

Table 1. Characteristics of study population.

	Group A (assay-directed therapy) **n = 7 (100%)**	**Group B** (empirically chosen therapy) **n = 7 (100%)**	**Overall n = 14 (100%)**
Sex			
Male	2 (28.6%)	4 (57.1%)	6 (42.9%)
Female	5 (71.4%)	3 (42.9%)	8 (57.1%)
Median age at study entry (range)	61.0 years (39 - 73 years)	65 years (58 – 76 years)	64.5 years (39 – 76 years)
Median age at primary diagnosis (range)	60.5 years (35 – 72 years)	64 years (56 – 73 years)	63.0 years (35 – 73 years)
Previous therapy			
Yes	2 (28.6%)	5 (71.4%)	7 (50.0%)
No	5 (71.4%)	2 (28.6%)	7 (50.0%)
Previous therapy in detail			
Interferon-alpha-2b	1 (14.3%)	1 (14.3%)	2 (14.3%)
Interferon-alpha-2a	1 (14.3%)	3 (42.9%)	4 (28.6%)
Localized chemotherapy	0 (0.0%)	1 (14.3%)	1 (7.1%)
None	5 (71.4%)	2 (28.6%)	7 (50.0%)
Serum LDH			
In range	4 (57.1%)	5 (71.4%)	9 (64.3%)
Elevated	3 (42.9%)	2 (28.6%)	5 (35.7%)
Metastases			
Skin and/or lymph nodes	6 (25.0%)	7 (35.0%)	13 (29.5%)
Lung	6 (25.0%)	2 (10.0%)	8 (18.2%)
Liver	3 (12.5%)	4 (20.0%)	7 (15.9%)
Bone	2 (8.3%)	1 (5.0%)	3 (6.8%)
Brain	4 (16.7%)	4 (20.0%)	8 (18.2%)
Others	3 (12.5%)	2 (10.0%)	5 (11.4%)
Total	24 (100.0%)	20 (100.0%)	44 (100.0%)
AJCC-category			
M1 a/b	4 (57.1%)	3 (42.9%)	7 (50.0%)
M1c	3 (42.9%)	4 (57.1%)	7 (50.0%)

comparable distribution of these parameters (p = 1.000 2-sided for LDH level and AJCC category; p = 0.592 two-sided for sex). The differences in median age at study entry were not statistically significant (p = 1.000).

Our study revealed a median overall survival (calculated from the time of study entry to death) of 5 months for the study population. All patients had died by the time of evaluation of the results due to the progress of melanoma. In group A (assay-directed therapy) the median overall survival exceeded the overall survival of group B (empirically chosen therapy) with 8 versus 3 months, respectively (compare Fig. 1 and Fig. 2).

All patients in group A had died 18 months after study entry, whereas the longest overall survival in group B was 11 months, respectively (data shown in Fig. 2). To compare the survival probabilities between the two treatment groups, we performed the log-rank test, which yielded to a clearly longer overall survival of group A in comparison to group B with a p-value of 0.055, respectively (data not significant for alpha = 0.05). To evaluate overall survival subject to LDH in

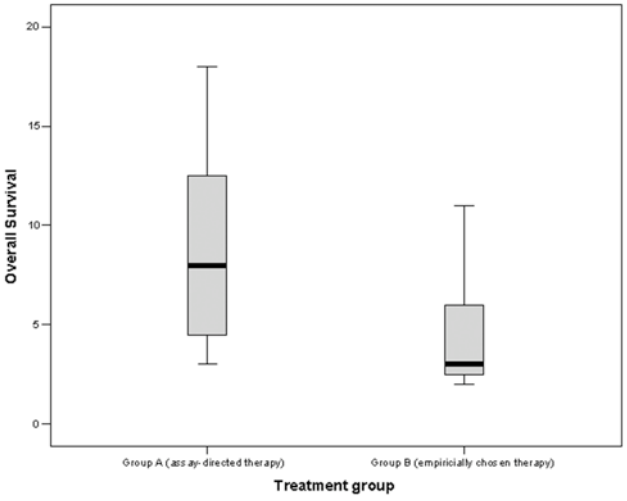

Fig. 1. Boxplot showing the overall survival subject to treatment group. The median overall survival of group A exceeded the median overall survival of group B with 8 vs. 3 months.

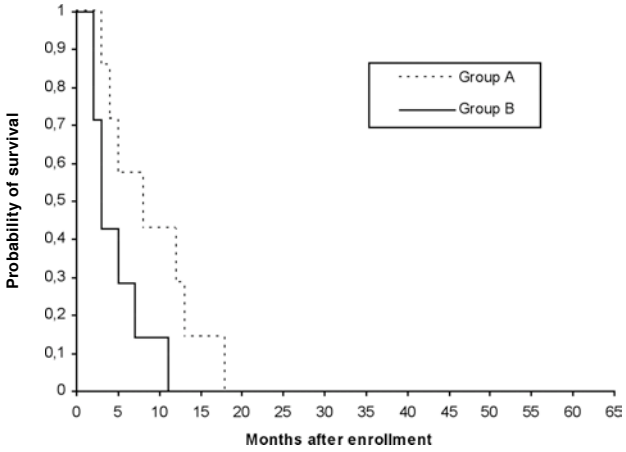

Fig. 2. Kaplan-Meier curves illustrating the overall survival of group A (assay-directed chemotherapy; dotted line) compared to group B (empirically chosen therapy; continous line).

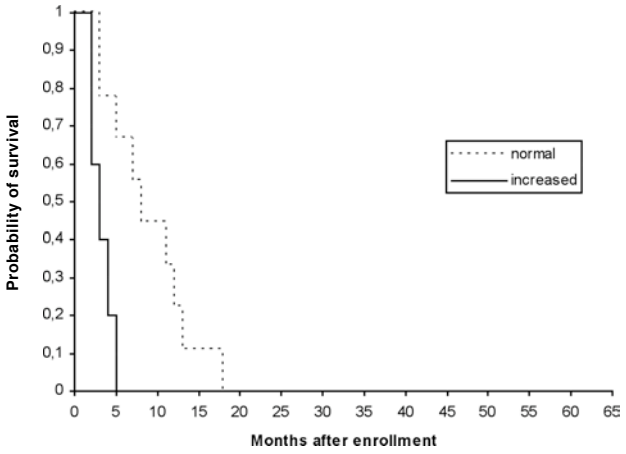

Fig. 3. Kaplan-Meier curves illustrating the overall survival of patients with normal serum LDH at study entry (dotted line) in comparison to patients with elevated serum LDH at study entry (continous line).

serum (normal vs. elevated at study entry) and AJCC category (M1a/b vs. M1c at study entry) we performed further statistical analysis using the Kaplan-Meier method. Patients with normal LDH levels in serum showed a significant longer overall survival with $p = 0.006$ for the log-rank test. Moreover, overall survival of patients at stage M1a/b exceeded overall survival of patients at stage M1c ($p = 0.066$ for the log-rank test; data not significant). Overall survival curves subject to LDH level in serum and AJCC category are shown in Figures 3 and 4.

Discussion

The main purpose of our study was to evaluate, whether chemosensitivity-directed chemotherapy improves the overall survival of patients with malignant melanoma stage IV in comparison with empirically chosen treatment regimens. It is known that metastatic malignant melanoma is extremely refractory to existing therapies with a median survival rate of 6 months and 5-year survival rate of less than 5% according to Cummins et al. [1]. Up to today, monochemotherapy with DTIC remains the standard treatment for stage IV melanoma since multiple studies could not show a significant benefit of other cytostatic drugs. A recently published review by Garbe et al. [10] lists similar response rates for interferon-alpha and several cytostatic drugs including dacarbacine and temozolomide in the treatment of metastatic melanoma [11, 12]. Moreover, combined treatment regimens including multiple cytostatic drugs and/or interferons could not demonstrate a prolonged survival of patients with metastatic malignant melanoma [13]. Middleton et al. [14] performed a phase III study to evaluate the effectiveness of temozolomide (TMZ) vs. dacarbacine (DTIC) in patients with metastatic malignant melanoma. Their trial included 305 patients and yielded to a median survival of 7.7 months for the TMZ group compared to 6.4 months for the DTIC group. Therefore we claim that temozolomide and interferon-alpha administered in the majoritiy of patients in group B (6/7) are possible reference treatments.

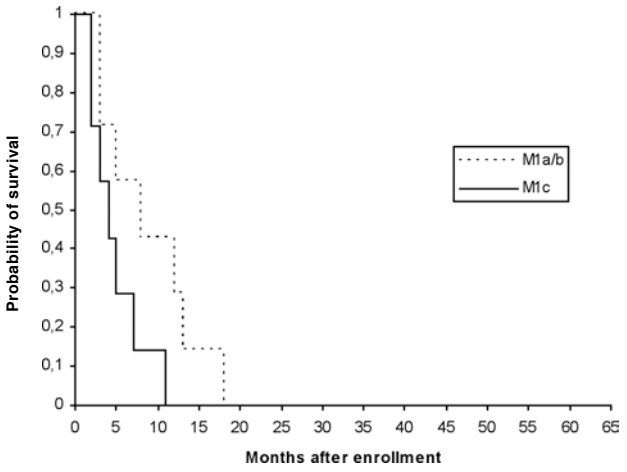

Fig. 4. Kaplan-Meier curves illustrating the overall survival subject to AJCC-category. The continous line shows the overall survival of patients at stage M1c at study entry. Overall survival of patients at stage M1a/b at study entry is shown by the dotted line.

Our results show that patients treated with the most effective drug or drug combination according to chemosensitivity testing had a much longer overall survival in comparison with patients, whose treatment was chosen empirically. In detail, patients in group A had a median overall survival of 8 months compared to 3 months in group B (compare Fig. 1 and Fig. 2). Ugurel et al. [7] showed that the objective response of assay-directed chemotherapy exceeded the objective response to DTIC monochemotherapy by 24.5% vs. 6-7% as determined in previous studies [5, 6]. A recently published review about chemotherapy for metastatic melanoma [15] lists response rates of less than 12% for single-agent DTIC. In our opinion, the longer overall survival of patients treated according to their chemosensitivity assay results (group A) and the finding that assay-directed chemotherapy shows higher

rates of objective response indicate, that chemosensitivity testing is a useful tool to determine which cytostatic drug or drug combinations should be used in the given patient.

The difference of overall survival between group A (assay-directed therapy) and B (empirically chosen therapy) was only slightly above the level of statistical significance with a p-value of 0.055 and possibly this was due to the small patient number.

In our study, group A included 71.4% women, whereas in group B only 42.9% were female. One could argue that this contributed to the prolonged overall survival in group A since many studies showed a better prognosis for women in comparison to men with malignant melanoma. However, the better course of the disease in women is usually confounded by differences in thickness, ulceration, and localization of the melanoma [16] since women in general show thinner melanomas and less frequently ulcerations [16, 17]. Our patients were diagnosed stage IV disease at study entry. In this stage of malignant melanoma, the outstanding prognostic factor seems to be the site of metastatic involvement. A study comparing the 1-year survival rates of 1158 patients with distant metastases yielded to 1-year survival rate of 59% for patients with lymph node or subcutaneous metastases and 57% for patients with lung involvement compared to only 41% for patients with other visceral organ involvement [18, 19]. In other words, patients at stage M1a/b showed a better prognosis than patients at stage M1c. Since there was no statistically significant difference in AJCC-category between the two treatment groups in our study, we claim that the sites of metastases were not crucial for the difference in overall survival between group A and group B.

Patients with elevated serum LDH showed a significantly poorer overall survival compared to patients with normal serum LDH at study entry. Moreover, the prognosis for patients at stage M1a/b was more promising than for patients at stage M1c (compare Fig. 3 and Fig. 4). These results correspond to the results of the prospective phase II trial by Ugurel et al. [7].

To our knowledge this is the first study evaluating the effectiveness of chemosensitivity-directed chemotherapy in comparison with empirically chosen treatment regimens in patients with malignant melanoma stage IV. Due to the small patient number, our results need to be interpreted with caution.

Contemplating other solid tumors, e.g. pretreated ovarian cancer, two prospective clinical trials showed triple the response rates and nearly double the survival for patients treated with assay-directed regimens compared to empirically chosen regimens [20].

Thus, we think that our results together with the findings of Ugurel et al. are encouraging for prospective randomized clinical trials comparing assay-directed treatment regimens with empirically chosen regimens in patients with malignant melanoma.

REFERENCES

1. Cummins DL, Cummins JM, Pantle H, Silverman MA, Leonard AL, Chanmugam A. Cutaneous malignant melanoma. Mayo Clin Proc 2006; 81: 500-507.
2. Hauschild A, Kleeberg UR. Adjuvant therapy of melanoma. From non-specific immune stimulants into the future. Hautarzt 2006; 57(9): 764-72.
3. Adnane L, Trail PA, Taylor I, Wilhelm SM. Sorafenib (BAY 43-9006, Nexavar), a dual action inhibitor that targets RAF/MEK/ERK pathway in tumor cells and tyrosine kinases VEGFR/PDGFR in tumor vasculature. Methods Enzymol 2005; 407: 597-612.
4. Flaherty KT. Chemotherapy and targeted therapy combinations in advanced melanoma. Clin Cancer Res 2006; 12: 2366s–2370s.
5. 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
6. Schadendorf D, Ugurel S, Schuler-Thurner B, Nestle FO, Enk A, Bröcker EB, Grabbe S, Rittgen W, Edler L, Sucker A, Zimpfer-Rechner C, Berger T, Kamarashev J, Burg G, Jonuleit H, Tüttenberg A Becker JC, Keikavoussi P, Kämpgen E, Schuler G; DC study group of the DeCOG. 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.
7. Ugurel S, Schadendorf D, Pfohler C, Neuber K, Thoelke A, Ulrich J, Hauschild A, Spieth K, Kaatz M, Rittgen W, Delorme S, Tilgen W, Reinhold U; Dermatologic Cooperative Oncology Group. 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. Clin Cancer Res 2006; 12(18): 5454-63.
8. Andreotti PE, Cree IA, Kurbacher CM, Hartmann DM, Linder D, Harel G, Gleiberman I, Caruso PA, Ricks SH, Untch M 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.
9. Kurbacher CM, Cree IA. Chemosensitivity testing using microplate adenosine triphosphate-based luminescence measurements. Methods Mol Med 2005; 110: 101-20.
10. Garbe C, Eigentler TK. Diagnosis and treatment of cutaneous melanoma: state of the art 2006. Melanoma Research 2007; 17/2: 117-127.
11. Robinson WA, Mughal TI, Thomas MR, Johnson M, Spiegel RJ. Treatment of metastatic malignant melanoma with recombinant interferon alpha 2. Immunobiology 1986; 172: 275-282.
12. Miller RL, Steis RG, Clark JW, Smith JW 2nd, Crum E, Mc Knight JE, Hawkins MJ, Jones MJ, Longo DL, Urba WJ. Randomized trial of recombinant alpha 2b-interferon with or without indomethacin in patients with metastatic malignant melanoma. Cancer Res 1989; 49: 1871-1876.
13. Eigentler TK, Caroli UM, Radny P, Garbe C. Palliative therapy of disseminated malignant melanoma: a systematic review of 41 randomized clinical trials. Lancet Oncol 2003; 4: 748-759.
14. Middleton M, Grob JJ, Aaronson N, Fierlbeck G, Tilgen W, Seiter S, Gore M, Aamdal S, Cebon J, Coates A, Creno A, Henz M, Schadendorf D, Kapp A, Weiss J, Fraass U, Statkevich P, Muller M, Thatcher N. Randomized Phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. J Clin Oncol 2000; 18: 158-166.
15. Gogas HJ, Kirkwood JM, Sondak VK. Chemotherapy for metastatic melanoma: time for a change? Cancer 2007; 109 (3): 455-64.

16. Homsi J, Kashani-Sabet M, Messina JL, Daud A. Cutaneous melanoma: prognostic factors. Cancer Control 2005; 12(4): 223-9.
17. Vossaert KA, Silverman MK, Kopf AW, Bart RS, Rigel DS, Friedman RJ, Levenstein M. Influence of gender on survival in patients with stage I malignant melanoma. J Am Acad Dermatol 1992; 26 (3 pt 2): 429-440.
18. Balch CM. Cutaneous melanoma: prognosis and treatment results worldwide. Semin Surg Oncol 1992; 8: 400-414.
19. Balch CM, Buzaid AC, Soong SJ, Atkins MB, Cascinelli N, Coit DG, Fleming ID, Gershenwald JE, Houghton A Jr, Kirkwood JM, McMasters KM, Mihm MF, Morton DL, Reintgen DS, Ross MI, Sober A, Thompson JA, Thompson JF. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol 2001; 19: 3635-3648.
20. Kurbacher CM, Grecu OM, Stier U, Gilster TJ, Janat MM, Untch M, Konecny G, Bruckner HW, Cree IA. ATP chemosensitivity testing in ovarian and breast cancer: early clinical trials. Recent Results Cancer Res 2003; 161: 221-30.

Received: December 8, 2006 / Accepted: July 11, 2007

Address for correspondence:
Professor Dr. Norbert Brockmeyer
Department of Dermatology
Ruhr-University Bochum
St. Josef-Hospital
Gudrunstr. 56
44791 Bochum
Germany
Phone: ++49-234/5093470
Fax: ++49-234/5093472
e-mail: n.brockmeyer@derma.de