Microphthalmia-Associated Transcription Factor Gene Amplification in Metastatic Melanoma Is a Prognostic Marker for Patient Survival, But Not a Predictive Marker for Chemosensitivity and Chemotherapy Response

Selma Ugurel, Roland Houben, David Schrama, Heike Voigt, Marc Zapatka, Dirk Schadendorf, Eva B. Bröcker, and Jürgen C. Becker

Abstract

Purpose: The microphthalmia-associated transcription factor (MITF) is regarded as a key oncogene of the melanocytic lineage since it was detected by a genome-wide analysis to be strongly amplified in 15% to 20% of metastatic melanomas. MITF gene amplification was shown to be associated with a reduced survival in metastatic melanoma patients, and reduction of MITF activity was shown to sensitize melanoma cell lines to chemotherapeutics, suggesting the intratumoral MITF gene copy number as a predictive biomarker of response and survival after chemotherapy.

Patients and Methods: To validate this hypothesis, we investigated MITF gene amplification in tumor tissues obtained from 116 metastatic melanoma patients before an individualized sensitivity-directed chemotherapy using quantitative real-time PCR. MITF amplification rates were correlated with tumor chemosensitivity quantified by an ATP-based luminescence assay and with chemotherapy outcome in terms of response and survival.

Results: Of 116 tumor tissues, 104 were evaluable for MITF gene amplification. Strong amplification (≥4 copies per cell) was detected in 24 of 104 tissues (23%), whereas 62 of 104 tissues (60%) harbored >3 copies per cell. Strong MITF gene amplification was associated with a reduced disease-specific survival (P = 0.031). However, no correlation was found between MITF copy number and in vitro chemosensitivity or in vivo chemotherapy response.

Conclusion: Our findings suggest that strong amplifications of the melanoma oncogene MITF affects patient survival but does not influence tumor chemosensitivity and chemotherapy response. Thus, the MITF gene copy number seems a useful prognostic marker in metastatic melanoma but could not be confirmed as a predictive marker of chemosensitivity and chemotherapy response.

The microphthalmia-associated transcription factor (MITF) is a basic helix-loop-helix leucine zipper protein, which is critical for melanocyte cell–fate determination during commitment from pluripotent neural crest stem cells. MITF is the major transcriptional regulator of pigmentation enzymes, like tyrosinase, Tyrp1, and Dct (1). Moreover, MITF was reported to regulate cell cycle exit associated with differentiation via induction of the tumor suppressor proteins p16 and p21 (2, 3). Mitogen-activated protein kinase pathway activation leads to a phosphorylation of MITF, which, on one hand, is an activation signal but, on the other hand, marks MITF for ubiquitination and degradation (4). Upon B-Raf transformation of murine melanocytes, MITF is down-regulated and MITF reexpression inhibits proliferation of the transformed cells (5). Therefore, two of the most frequent events associated with the pathogenesis of melanoma, the activation of B-Raf and the loss of p16INK4a, seem both to oppose an anticancerous action of MITF. However, despite of the fact that the expression of many molecules specific for the melanocytic lineage are lost during melanomagenesis, the expression of MITF seems to be maintained in the majority of cases and seems to be essential for melanoma cell survival (1, 6).

Recently, Garraway et al. reported a substantial number of melanomas characterized by amplified MITF genes (7). Using single-nucleotide polymorphism array analysis, the authors screened a panel of cell lines from different cancer entities (NCI60) for chromosomal alterations. Thus, 3p13-3p14 was identified as a region of copy gain in six of eight melanoma cell lines, and the MITF gene was identified to be located in the amplified region. Subsequent analysis of tissue samples from primary cutaneous melanomas and melanoma metastases by

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real-time PCR showed MITF amplification of at least four copies in 3 of 30 (10%) and 7 of 32 (21%) cases, respectively. Using fluorescence in situ hybridization, 4 to 13 MITF gene copies were detected in 2 of 19 primary tumors (10.5%) and 27 of 160 metastases (15.2%). Notably, a 3p copy gain was associated with chemoresistance of melanoma cell lines, and an inhibition of MITF function by dominant negative MITF or small interfering RNA led to an increased sensitivity to cytotoxic agents, like docetaxel and cisplatin (7). Recently, Koyanagi et al. showed that the MITF expression, as measured in circulating tumor cells of the peripheral blood, correlates with the disease stage of melanoma patients (8). Interestingly, in this study, the rate of MITF detection was significantly higher in patients relapsing after biochemotherapy compared with patients without a relapse.

To test if the MITF gene amplification rate can be used as a predictive marker of chemosensitivity and chemotherapy outcome, we analyzed genomic DNA from cryopreserved tumor tissues obtained from 116 metastatic melanoma patients before an individualized sensitivity-directed chemotherapy. This approach offers the possibility to correlate the MITF amplification rate with the in vitro chemosensitivity as measured in patients’ fresh tumor tissue, as well as the in vivo therapy outcome in terms of tumor response and disease-specific survival.

Patients and Methods

Patients and tissues. Frozen tissue samples obtained from metastatic lesions of 116 melanoma patients were selected from an in-house tissue biobank (Skin Cancer Unit, German Cancer Research Center) in accordance with the following criteria: histologically confirmed stage IV disease following American Joint Committee on Cancer criteria (9) at the time of tissue sampling; in vitro chemosensitivity testing done on fresh tissue derived from the same metastatic lesion; and complete documentation of the patient’s treatment and long-term follow-up. Of the 116 patients selected for analysis, 65 patients participated in a phase II study of the Dermatologic Cooperative Oncology Group, investigating an individualized sensitivity-directed chemotherapy in metastatic melanoma (10). The remaining 51 patients received chemosensitivity testing and subsequent therapy analogous to this study protocol. Detailed patient characteristics are provided in Table 1. Tissue sampling and analysis were approved by the Institutional Review Board, and a written informed consent was signed by all patients before the surgical procedure.

In vitro chemosensitivity assay. After informed consent, an excision biopsy of a metastatic lesion was done in every patient and 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-based luminescence assay (ATP-TCA, DCS Innovative Diagnostic Systems) as described before (10, 11). Briefly, the tissue samples were enzymatically dissociated and depleted from RBC and debris by Ficoll-Hypaque density gradient centrifugation. Thereafter, the cell suspensions were given into polypropylene round-bottom 96-well plates with or without chemotherapeutic agents (dacarbazine, cisplatin, doxorubicin, vindesine, paclitaxel, gemcitabine, treosulfan). After 7 days of incubation, the cells were lysed and the lysate’s ATP content as a measure of cell viability was quantified by a luciferin-luciferase luminescence reaction. Cell suspensions incubated without cytotoxic drugs were used as reference for 100% cell viability. Summing up the cell viabilities at the six drug concentrations tested, individual chemosensitivity indices ranging from 0 to 600 were determined for each test drug or drug combination. The lowest individual chemosensitivity index resulting from in vitro drug testing on each tissue sample was defined as the best

**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N = 116 (100.0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male 62 (53.4%)</td>
</tr>
<tr>
<td></td>
<td>Female 54 (46.6%)</td>
</tr>
<tr>
<td>Median age</td>
<td>y [range] 60.5 [15.5, 89.0]</td>
</tr>
<tr>
<td>Localization of primary</td>
<td>Skin 85 (73.3%)</td>
</tr>
<tr>
<td></td>
<td>Mucosa 10 (8.6%)</td>
</tr>
<tr>
<td></td>
<td>Uvea 4 (3.4%)</td>
</tr>
<tr>
<td></td>
<td>Unknown 17 (14.7%)</td>
</tr>
<tr>
<td>Prior chemotherapy in stage IV</td>
<td>Yes 43 (37.1%)</td>
</tr>
<tr>
<td>LDH (serum)</td>
<td>≤UNL 40 (34.5%)</td>
</tr>
<tr>
<td></td>
<td>&gt;UNL 39 (33.6%)</td>
</tr>
<tr>
<td></td>
<td>n.a. 37 (31.9%)</td>
</tr>
<tr>
<td>S100B (serum)</td>
<td>≤UNL 21 (18.1%)</td>
</tr>
<tr>
<td></td>
<td>&gt;UNL 58 (50.0%)</td>
</tr>
<tr>
<td></td>
<td>n.a. 37 (31.9%)</td>
</tr>
<tr>
<td>ECOG performance state</td>
<td>0 35 (30.2%)</td>
</tr>
<tr>
<td></td>
<td>1 26 (22.4%)</td>
</tr>
<tr>
<td></td>
<td>2 18 (15.5%)</td>
</tr>
<tr>
<td></td>
<td>3 7 (6.0%)</td>
</tr>
<tr>
<td></td>
<td>n.a. 30 (25.9%)</td>
</tr>
<tr>
<td>Tumor tissue biopsy origin</td>
<td>Skin/subcutis 56 (48.3%)</td>
</tr>
<tr>
<td></td>
<td>Lymph node 46 (39.7%)</td>
</tr>
<tr>
<td></td>
<td>Inner organ 14 (12.0%)</td>
</tr>
</tbody>
</table>

NOTE: Patient characteristics at the time point of tumor tissue biopsy. Abbreviations: n.a., not assessed; ECOG, Eastern Cooperative Oncology Group.
individual chemosensitivity index (BICSI) of the corresponding patient. The BICSI was shown to be a predictor of chemotherapy outcome in terms of therapy response and overall survival using a cutoff value of 100 (10). Thus, patients with a BICSI of ≤100 were considered "chemosensitive", whereas patients with a BICSI of >100 were considered "chemoresistant".

### Sensitivity-directed chemotherapy and outcome assessment.

Of 116 patients tested for chemosensitivity, 63 patients received an assay-directed chemotherapy using the individual drug or drug combination showing the highest in vitro sensitivity, and 53 patients were treated with other substances or did not receive disease-specific treatment. The regimens used for sensitivity-directed therapy were gemcitabine + treosulfan, gemcitabine + cisplatin, paclitaxel + doxorubicin, paclitaxel + cisplatin, and paclitaxel as a single agent (for detailed dosing schedules, see ref. 10). Treatment was continued at a tumor response of stable disease or better (complete response or partial response) and stopped due to disease progression or intolerable side effects. 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 Tumors (12). 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, and the tumor assessment scans of these patients were retrospectively reviewed by an independent radiologist. Overall survival was measured from tumor tissue biopsy until death from melanoma (disease-specific overall survival). For alive patients, the date of the last patient contact was used as the end point of survival assessment (censored observations).

### Quantitative real-time PCR.

DNA was extracted from tissue sections of cryopreserved samples with the QIAamp DNA mini kit (Qiagen) as described by the manufacturer. For quantitative real-time PCR, each tumor DNA was quantified by comparing the MITF locus to the reference locus line 1, a repetitive element whose copy numbers per haploid genome are similar among all human normal and neoplastic cells. The relative MITF copy number in the tumor DNAs was normalized to normal human genomic DNA as calibrator. The MITF gene amplification rate in tumor DNA (AR_Tu-DNA) was determined by using the formula AR_Tu-DNA = 2^ΔΔC_T with ΔΔC_T being [C_T (MITF;tumor) - C_T (LINE1;tumor)] - [C_T (MITF;calibr.) - C_T (LINE1;calibr.)], where C_T is the 2 negative log of the threshold cycle for each primer set. The primers for the LINE1 and MITF genes were designed to span exons to avoid PCR artifacts induced by alternative splicing. The amplification efficiencies were calculated using SYBRGreen real-time PCR assay (PCR1) as described by Bustin et al. (11) and for the DNA was amplified by 40 cycles at 94°C for 20 s, 56°C for 20 s, and 70°C for 20 s. Amplicons were subjected to a melting analysis to confirm specificity of PCR amplification. PCR2 was done with standard conditions, i.e., after activation of the Taq polymerase by incubation at 95°C for 10 min, the DNA was amplified by 40 cycles at 95°C for 15 s and 60°C for 60 s. The primers used were ACATGGGAACGGTCAAGAG for MITF R, AACCCCAACAGTACACTACT for MITF F, CCGGAC for Taqman probe MITF, AGATTCCGTGGGCGTAGGA for LINE1 R, CGGTCTTCCAGAGGCCTTGAA for LINE1 F, and CGGCAC-CACGAGACTATATCCACACGC for Taqman probe Line1. PCRs were done in triplicates on an ABI 5700 (Applied Biosystem) for PCR1 and on an ABI 7500 for PCR2 using qPCR Mastermix for SybrGreen Mastermix or qPCR Mastermix plus low rox (both Eurogentec).

### Statistical analysis.

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 groups. The optimal cutoff point for the MITF amplification rate with regard to differentiation between prolonged and reduced disease-specific survival was calculated using the method of exact distribution of maximally selected ranks (13). The Wilcoxon rank sum test was used to test for differences between therapy response groups. P values below 0.05 were considered statistically significant. Statistical analyses were done using the statistical software package R.4

### Results

**MITF gene amplification in metastatic melanoma.** To measure the MITF gene copy number, we applied either the SybrGreen real-time PCR assay (PCR1) as described by Garraway et al. (7) or a probe-based real-time PCR assay using different sets of primers (PCR2; Fig. 1). Both assays yielded comparable results. For 61 samples, which were tested thrice (twice PCR1 and once PCR2), the mean stable disease of the

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Table 2. Chemosensitivity assay results and MITF amplification rate

<table>
<thead>
<tr>
<th>Sensitivity-directed treatment regimen</th>
<th>No. patients, best test result/ received assay-directed treatment</th>
<th>Best treatment response</th>
<th>BICSI, mean (range)</th>
<th>MITF amplification rate</th>
<th>CR + PR + SD/PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemcitabine + cisplatin</td>
<td>20/10</td>
<td>2/8</td>
<td>91 (1-237)</td>
<td>1.60 (0.95-2.88)</td>
<td>6</td>
</tr>
<tr>
<td>Gemcitabine + treosulfan</td>
<td>49/30</td>
<td>12/18</td>
<td>126 (1-312)</td>
<td>1.61 (0.94-4.14)</td>
<td>16</td>
</tr>
<tr>
<td>Paclitaxel + cisplatin</td>
<td>25/14</td>
<td>6/8</td>
<td>151 (52-360)</td>
<td>1.78 (0.44-4.88)</td>
<td>10</td>
</tr>
<tr>
<td>Paclitaxel + doxorubicin</td>
<td>22/9</td>
<td>2/7</td>
<td>180 (67-303)</td>
<td>1.55 (0.24-3.25)</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>116/63</td>
<td>22/41</td>
<td>135 (1-360)</td>
<td>1.64 (0.24-4.88)</td>
<td>42</td>
</tr>
</tbody>
</table>

NOTE: Sensitivity-directed treatment regimens are given with the number of patients showing the highest in vitro chemosensitivity to these drugs or drug combinations, as well as the number of patients who were subsequently treated according to their in vitro chemosensitivity profile. The BICSI was defined as the lowest individual drug sensitivity index; best treatment response was defined as the best tumor response recorded from the start of treatment until treatment cessation. MITF amplification rate was assessed by real-time PCR (PCR2) as described in Patients and Methods.

Abbreviations: n.e., not evaluable; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

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[^4]: http://www.r-project.org
MITF amplification rate in tumor tissue ($AR_{Tu-Tissue}$) was 0.20. An $AR_{Tu-Tissue}$ of $>0.5$, which would indicate a gain of one gene copy, was observed in two cases only. However, in contrast to the SybrGreen assay (PCR1), the probe-based assay (PCR2) uses intron-targeted primers for MITF detection and does not, therefore, bear the risk to be disturbed by contaminating RNA. Moreover, PCR2 yielded a relatively constant amplification rate with a stable disease of 0.14 when applied on 20 different control DNAs from healthy donors, with none of these control samples classified as amplified. Thus, finding PCR2 being superior to PCR1, we consequently used PCR2 to analyze the complete set of 116 melanoma tissue samples, testing each sample at least twice. Of 116 tumor tissues, 104 were evaluable for MITF amplification. In the 12 samples considered as nonevaluable, 10 samples revealed $<50\%$ tumor cells determined by routine histopathology, and one failed to DNA extraction. An amplification rate ($AR_{Tu-Tissue}$) of $\geq 2$ (four gene copies or more), which was used as a cutoff by Garraway et al. (7), was detected in 24 of 104 tissues (23.0%; Table 2). When applying a less stringent cutoff ($AR_{Tu-Tissue} > 1.4$), which would correspond to a gain of at least one gene copy, we classified 62 of 104 melanoma tissues (59.6%) as MITF amplified.

**Correlation of MITF gene amplification with in vitro chemosensitivity.** Chemosensitivity testing of 116 metastatic melanoma tissues revealed a heterogeneous sensitivity to different chemotherapeutics and combinations (Table 2). The drug combinations with the highest in vitro sensitivities were paclitaxel + cisplatin and gemcitabine + treosulfan, reflected by the lowest BICSI. Routine histopathology was done from all 116 lesions, confirming the diagnosis of melanoma metastasis, as well as providing tumor cell percentages for the calculation of the tumor-specific MITF amplification rate ($AR_{Tu-Tissue}$). The MITF amplification rate detected in the metastatic tissue lesions ($AR_{Tu-Tissue}$) did not correlate with the in vitro chemosensitivity of these tissues, as reflected by the BICSI ($P = 0.6$; Fig. 2A).

**Correlation of MITF gene amplification with therapy response and survival.** Of 104 patients, whose metastatic tumor tissues were tested for chemosensitivity and for conclusive MITF amplification rates, 59 received an individualized sensitivity-directed chemotherapy. The patients' distribution on the different therapy regimens is given in Table 2. Of these 59 patients, 22 patients (37%) showed a best overall response of stable disease or better (progression arrest), whereas 37 patients (63%) experienced a disease progression. Correlating therapy response with MITF amplification rate ($AR_{Tu-Tissue}$), no significant differences could be detected between patients showing a disease progression and those showing a progression arrest ($P = 0.25$, Wilcoxon rank sum test; Fig. 2B). Analyzing all 104 patients for overall survival, we found no significant differences between patients whose tumor tissue showed an MITF amplification rate of $>1.4$, corresponding to a MITF gene copy number of 3 or more, and patients without such an amplification (amplification rate, $\leq 1.4$; $P = 0.39$; Fig. 3A). However, regarding patients with a MITF gene copy number of 4 or more, corresponding to an amplification rate of $>2.0$ (in our data set, the optimal cutoff was calculated as 2.01), we found a significantly decreased disease-specific survival in patients harboring the amplification compared with patients without ($P = 0.031$; Fig. 3B). The established prognostic serum markers lactate dehydrogenase (LDH; ref. 9) and S100B, measured in 79 patients at the time of tissue biopsy, correlated strongly with the disease-specific survival. Patients with serum levels equal or below the institution's upper normal limit (UNL) revealed a favorable survival compared with patients with elevated serum concentrations (LDH, $P < 0.0001$; S100B, $P = 0.0018$; Fig. 3B and C).

**Discussion**

To validate the MITF gene copy number as a predictive marker of chemosensitivity and chemotherapy outcome, we measured this variable by two independent methods, i.e., the SybrGreen real-time PCR assay described by Garraway et al.
and a probe-based real-time PCR assay in TaqMan technology. Notably, both assays yielded comparable results. A strong MITF gene amplification (≥4 copies per cell; amplification rate, >2) was found in 24 of 104 tissues (23%). This result is comparable with that reported by Garraway and coworkers (7), who found a strong MITF gene amplification in 21% of metastatic melanoma tissues by PCR analysis. In our present study, we further classified a subgroup of 62 of 104 tissues (60%), which were characterized by the acquisition of one or more additional gene copies (≥3 copies per cell; amplification rate, >1.4).

We thereafter questioned, which cutoff point might be the most meaningful to discriminate between patients with a prolonged and a reduced disease-specific survival. The method of exact distribution of maximally selected ranks (13) predicted an MITF amplification rate of 2.01 to be the optimal cutoff point for our patient cohort. Indeed, we found a significantly different disease-specific survival in patients showing an MITF amplification rate above this threshold compared with patients with an amplification rate of 2.01 or below. The optimal means to differentiate between “MITF-amplified” and “not MITF-amplified” using genomic quantitative PCR is difficult to
ascertain, because issues such as stromal contamination and technical factors can influence the signal. In future, fluorescence in situ hybridization studies may provide a more robust measurement. Interestingly, using a threshold of 1.4, corresponding to a MITF gene copy number of 3 or more, we found no significant survival differences between patients showing MITF amplification rates above and below this cutoff. These findings indicate that an MITF gene amplification becomes clinically relevant only at high amplification levels of two or more additional gene copies.

However, in the largest patient cohort reported to date correlating MITF amplification and functional and clinical consequences, we could not show an association between MITF copy number and in vitro chemosensitivity or in vivo chemotherapy response. This observation was unexpected, because apart from the evidence that MITF may be regarded as an oncogene (7), several reports directly implicated MITF in supporting melanocyte and melanoma survival and cell cycle progression (14). MITF was shown to increase the transcription of Bcl-2 (6) and CDK2 (15). Furthermore, MITF resides downstream of two key antiapoptotic pathways, the extracellular signal-regulated kinase and the phosphatidylinositol 3-kinase pathways, suggesting that MITF could integrate extracellular prosurvival signals (14). On the other hand, MITF can also up-regulate the expression of cell cycle inhibitors like INK4A (3) and p21 (2) and was shown to be degraded upon expression of activated B-Raf in murine melanocytes, whereas MITF reexpression inhibited proliferation of these cells (5). Moreover, in a very recent report, Larriber et al. showed a decrease in MITF expression during TRAIL-induced apoptosis which was due to MITF cleavage by caspases yielding a large N-terminal part and a small C-terminal fragment (16). Notably, the authors presented evidence that the physiologic role of this process is to amplify the apoptotic signal: (a) a caspase-resistant form of MITF impairs melanoma cell apoptosis, (b) down-regulation of MITF expression after caspase cleavage is not sufficient to explain the amplification of the apoptotic signal because MITF silencing does not promote melanoma cell death, and (c) forced expression of the MITF COOH terminus morphs morphologic changes characteristic of cell death and caspase-3 activation. Hence, the authors propose a functional duality of MITF that could operate in both prosurvival pathways through Bcl2 up-regulation and proapoptotic processes through the generation of death-inducing fragments upon its processing by caspases. Indeed, clinical observations support this Janus-faced role of MITF on the clinical course of melanoma. For example, Salti et al. showed in a multivariate analysis that the expression of MITF in intermediate-thickness cutaneous melanomas is inversely correlated with overall survival (17). Similarly, overexpression of MITF in human melanoma cells in a SCID mouse model led to a significantly reduced tumor growth (18). Garraway et al. reported that all six melanoma cell lines for which MITF amplification was detected were additionally characterized by an activating B-RAF mutation and p16 inactivation, leading to the speculation that MITF amplification might promote tumor formation especially in the setting of cell cycle deregulation and excess mitogen-activated protein kinase pathway activation (7).

Moreover, down-regulation of MITF expression in melanocytes by constitutively active, oncogenic B-RAF has been reported (5). However, in 63 of 104 tissue samples of the present study, the B-RAF mutation status was known, showing no correlation with the MITF amplification rate (data not shown).

It should be noted that, in 12 patients, we additionally tested tumor tissue from other metastases obtained within a time range of 8 weeks before or after the excision of the first one. In all but one patient, both tumor tissues showed similar MITF gene amplification rates. One patient showed discordant results with strong amplification within a lymph node metastasis and no amplification within a subcutaneous metastasis, indicating the possibility of heterogenous MITF amplification rates within different localizations of metastases of the same patient. Even if this phenomenon seems to be a rare event, it should be taken into account for the design and analysis of future studies.

The history of oncology has repeatedly shown that successful therapeutic approaches help to identify underlying biological features of particular cancer entities that have been ignored but subsequently lead to a useful prognostic classification. Nevertheless, the presented findings suggest that the effect of an amplification of the melanoma oncogene MITF on the clinical course of the disease is even more complex than previously anticipated (19, 20). Our data could not confirm the previously suggested use of the MITF gene copy number as a predictive marker of chemosensitivity and response to chemotherapy. Nevertheless, we could show that the MITF gene amplification rate is a useful prognostic marker in metastatic melanoma, with high amplification rates associated with poor disease-specific survival. However, previously established serologic markers, like LDH and S100B, showed a much stronger prognostic effect, at least in our patient cohort. Eventually, further genetic and molecular characterization of the responders and nonresponders is needed to deepen our knowledge of chemosensitivity and chemotherapy outcome in melanoma.

References
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