Circulating Tumor Cells as Prognostic Factor for Distant Metastases and Survival in Patients with Primary Uveal Melanoma

Ronny Schuster,[1] Nikolaos E. Bechrakis,[2] Andrea Stroux,[3] Antonia Busse,[1] Alexander Schmittel,[1] Carmen Scheibenbogen,[1] Eckhard Thiel,[1] Michael H. Foerster,[2] and Ulrich Keilholz[1]

Abstract **Purpose:** The aim of this study was to determine in patients with high-risk primary uveal melanoma whether the detection of circulating tumor cells by quantitative reverse transcription-PCR (RT-PCR) is of prognostic relevance.

Experimental Design: Blood samples from 110 patients with high-risk nonmetastatic uveal melanoma were collected on the occasion of primary treatment or follow-up visit. mRNA expression of *tyrosinase* and *MelanA/MART1* were analyzed by real-time RT-PCR and compared with clinical data at presentation and follow-up by univariate and multivariate analyses.

Results: The RT-PCR assay yielded a positive result in 11 of 110 patients, with five positive findings for *tyrosinase* and five for *MelanA/MART1*, and one sample positive for both markers. At a median follow-up of 22 months, 25% of patients had developed metastases and 15% had died. Univariate statistical analysis revealed RT-PCR and the largest tumor diameter as important prognostic factors for the development of metastases and for survival. In a Cox proportional hazard model, RT-PCR result and largest tumor diameter predicted metastases (hazard ratios 7.3 and 2.6, respectively), whereas PCR result, largest tumor diameter, and Karnofsky performance status were significant variables for disease-specific survival (hazard ratios 22.6, 4.7, and 6.0, respectively). Analysis of individual RT-PCR results revealed both *tyrosinase* and *MelanA/MART1* transcripts as independent prognostic factors.

Conclusion: The presence of *tyrosinase* or *MelanA/MART1* transcripts is an independent prognostic factor in patients with high-risk primary uveal melanoma for subsequent development of metastases and for survival and can be used to select patients for adjuvant treatment studies.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/13/4/1171/1923974/1171.pdf by guest on 04 February 2023

A major problem with uveal melanoma is the development of systemic metastases, which occur in up to 35% of cases even after successful treatment of the primary tumor (1 – 6). The survival rate of patients with metastatic uveal melanoma remains poor with a median of between 2 and 9 months in spite of a variety of systemic therapeutic approaches (5, 7 – 9). There is an urgent need to define risk factors for systemic metastasis to evaluate novel adjuvant treatments for high-risk patients.

Several clinical, histologic, and genetic factors (e.g., tumor size and monosomy 3) have been identified as important risk factors for local recurrence, development of metastases, and survival (5, 6, 10 – 15). However, histologic and genetic predictive factors are not usually identified because most patients with uveal melanoma are treated with radiotherapy (6, 16, 17). It is therefore necessary to rely on clinical features, such as largest basal tumor diameter, ciliary body involvement, and extraocular growth, which have firmly been established as important clinical prognostic factors by the Collaborative Ocular Melanoma Study (COMS; ref. 6).

The detection of disseminated tumor cells in the blood by reverse transcription-PCR (RT-PCR) is particularly relevant to uveal melanoma, which metastasizes early and exclusively via the hematogenous route (17). Transcripts of *tyrosinase* and *MelanA/MART1* can be detected in peripheral blood of a subset of patients with uveal melanoma (18, 19). In patients with cutaneous melanoma, prospective studies have confirmed the prognostic relevance of the RT-PCR assay results in most studies (20 – 34). Also, for sequential monitoring the prognostic significance of *tyrosinase* transcript, detection by RT-PCR was clearly shown in two studies (29, 34).

Here, we analyzed a cohort of 110 patients with primary uveal melanoma showing unfavorable clinical prognostic factors. Our results suggest that the detection of circulating tumor cells by quantitative RT-PCR for *tyrosinase* and *MelanA/MART1* reliably indicates a poor prognosis for survival.

Authors' Affiliations: Departments of [1]Medicine III (Hematology, Oncology, and Transfusion Medicine) and [2]Ophthalmology, and [3]Institute of Medical Informatics, Biometry, and Clinical Epidemiology, Charité, Campus Benjamin Franklin, Berlin, Germany

Received 9/20/06; revised 11/19/06; accepted 11/30/06.

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: Ulrich Keilholz, Department of Medicine III, Charité-Campus Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany. Phone: 49-30-8445-3906; Fax: 49-30-8445-4021; E-mail: ulrich.keilholz@charite.de.

© 2007 American Association for Cancer Research.

doi:10.1158/1078-0432.CCR-06-2329

Patients and Methods

Patients who underwent primary therapy for uveal melanoma in the department of Ophthalmology of the Charité-Campus Benjamin

Table 1. Primer and hybridization probe sequences, conditions, and annealing temperature of real-time RT-PCR

Marker	Primer/hybridization probe sequence	Amplicon (bp)	Annealing temperature (°C)	$MgCl_2$ concentration (mmol/L)
Porphobilinogen deaminase				
Forward	5′-TGCAGGCTACCATCCATGTCCCTGC-3′	187	65	4
Reverse	5′-AGCTGCCGTGCAACATCCAGGATGT-3′			
Probe	5′-CGTGGAATG TTACGAGCAGTGATGCCTACC-Fluo-3′			
Probe	5′-LCRed-640-TGTGGGTCATCCTCAGGGCCATCTTC-Pho-3′			
Tyrosinase				
Forward	5′-GTCTTTATGCAATGGAACGC-3′	207	60	3
Reverse	5′-GCTATCCCAGTAAGTGGACT-3′			
Probe	5′-GCGTAATCCTGGAAACCATGACAAA-Fluo-3′			
Probe	5′-LCRed-640-CACAACCCCAAGGCTCCCCTCTTC-Pho-3′			
MelanA/MART1				
Forward	5′-CACTCTTACACCACGGCTGA-3′	300	65	4
Reverse	5′-AGGTGAATAAGGTGGTGGTGA-3′			
Probe	5′-GCTGTCCCGATGATCAAACCCTTC-Fluo-3′			
Probe	5′-LCRed-640-TGTGGGCATCTTCTTGTTAAGGCACA-Pho-3′			

Franklin between 1998 and 2004 were invited to participate in this study if they had unfavorable clinical prognostic factor. Clinical features indicating poor prognosis included mid-size to large tumors with a largest tumor diameter exceeding 8 mm, ciliary body involvement, or extraocular tumor growth. Peripheral blood samples were obtained at the time of primary therapy or at a subsequent outpatient visit. All patients gave informed consent for the analysis. The investigation was done after approval by the institutional ethic committee. Absence of metastatic melanoma at the time of blood sampling was confirmed by clinical evaluation, routine biochemistry (i.e., liver enzymes and lactate dehydrogenase levels), and liver ultrasonography. Follow-up information was obtained by contacting each patient's ophthalmologist or general physician. Only two patients were excluded from final analysis because of incomplete follow-up.

RT-PCR assay. The method of analysis is described in detail elsewhere (18). In brief, 10 mL blood samples were collected in EDTA containers. Total RNA was isolated by acid guanidinium thiocyanate/phenol chloroform extraction (35) and further purified by the High Pure RNA Isolation kit (Roche Diagnostics, Mannheim, Germany). For reverse transcription, we used the Omniscript Reverse Transcriptase kit (Qiagen, Hilden, Germany). Quantitative RT-PCR was done to detect transcripts of the melanoma markers *tyrosinase* and *MelanA/MART1*, and of the housekeeping gene *porphobilinogen deaminase* (*PBGD*). PCR conditions for the LightCycler (Roche Diagnostics) are summarized in Table 1. Two microliters of each cDNA sample were diluted to a volume of 20 μL PCR mix (LightCycler Faststart DNA Master Hybridization Probes, Roche Diagnostics), containing the final $MgCl_2$ concentration listed in Table 1, 0.5 pmol of each primer (Metabion, Martinsried, Germany), and 0.2 pmol of each probe (TIB Molbiol Berlin, Germany, or Metabion). For amplification, an initial denaturation at 95°C for 10 min, followed by 55 cycles (0 s at 95°C, 12 s at the temperature provided in Table 1, and 10 s at 72°C), and a final extension of 2 min at 72°C was used. The expected size of the PCR products was confirmed by agarose gel electrophoresis.

Table 2. Clinical data

	n (%)
No. patients	110 (100)
Age	
Median (range), y	61 (16-93)
Sex	
Female/male	52/58 (47/53)
Karnofsky performance status <90%	7 (6.3)
Properties of uveal melanoma	
Largest tumor diameter (>14 mm)*	62 (56)
Ciliary body infiltration	40 (36)
Extraocular growth	10 (9)
Positive results of RT-PCR	
Tyrosinase	6 (55)
MelanA/MART1 †	6 (5.5)
PCR (*tyrosinase* or *MelanA/MART1*)	11 (10)
Follow-up interval from time of blood sampling	
Median (range), mo	22 (4-84)

*n = 105.
†n = 98.

Table 3. Clinical data in relation to PCR results (*tyrosinase or MelanA/MART1*)

Variable	PCR positive (n = 11)	PCR negative (n = 99)	P*
Age			
Median (y)	64	61	0.4
Range	36-85	16-93	
Karnofsky performance status			
≥90%	10 (91%)	93 (94%)	0.5
<90%	1 (9%)	6 (6%)	
Sex			
Female	5 (45%)	47 (47%)	1
Male	6 (55%)	52 (53%)	
Largest tumor diameter			
≤14 mm	4 (36%)	39 (39%)	1
>14 mm	6 (55%)	56 (57%)	
Ciliary body involvement			
No	7 (64%)	63 (64%)	1
Yes	4 (36%)	36 (36%)	
Extraocular growth			
No	9 (82%)	91 (92%)	0.3
Yes	2 (18%)	8 (8%)	

*Fisher's exact test or, in case of age, Mann-Whitney U test.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/13/4/1171/1923974/1171.pdf by guest on 04 February 2023

Table 4. Univariate Cox regression analysis of potential prognostic factors for time to metastases and DSS

Variable	Time to metastases, hazard ratio (95% CI)	*P*	DSS, hazard ratio (95% CI)	*P*
Karnofsky performance status				
≥90% versus <90%	0.6 (0.1-2.6)	0.50	0.4 (0.1-1.7)	0.19
Sex				
Female vs. male	0.9 (0.4-1.9)	0.82	0.7 (0.3-1.8)	0.49
Largest tumor diameter				
≤14 mm vs. >14 mm	2.3 (0.9-5.8)	0.07	3.2 (0.9-11.2)	0.06
Ciliary body involvement	1 (0.5-2.2)	0.96	0.6 (0.2-1.8)	0.40
Extraocular growth	0.8 (0.2-3.5)	0.81	1.2 (0.3-5.3)	0.79
*PCR**	6.3 (2.6-15.5)	<0.001	10.9 (4-29)	<0.001
Tyrosinase	6.8 (2.3-20.5)	0.001	8.9 (2.8-28)	<0.001
MelanA/MART1	5.2 (1.7-15.4)	0.003	10.3 (3.1-34)	<0.001

Abbreviation: 95% CI, 95% confidence interval.
**Tyrosinase* or *MelanA/MART1*.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/13/4/1171/1923974/1171.pdf by guest on 04 February 2023

All samples were analyzed in duplicate, and the average value of the two measurements was used as the quantitative value. If only one of the duplicates gave a positive signal, the positive result was taken. When results from the same sample showed a discrepancy, we checked whether the positive value was near the detection limit (i.e., non-detection was a sporadic event) and confirmed that the value of the housekeeping gene was in the reference range.

To reduce risk of contamination, thermocycling and post-PCR steps were done in a laboratory different from the one used for RNA extraction, cDNA synthesis, and PCR mixture preparation. PCR mixtures were set up in a template tamer (Oncor Appligene, Heidelberg, Germany). All reagents for cDNA synthesis were prepared with RNase-free water. For every PCR run, we did checks using negative controls consisting of a reverse transcriptase negative sample control for

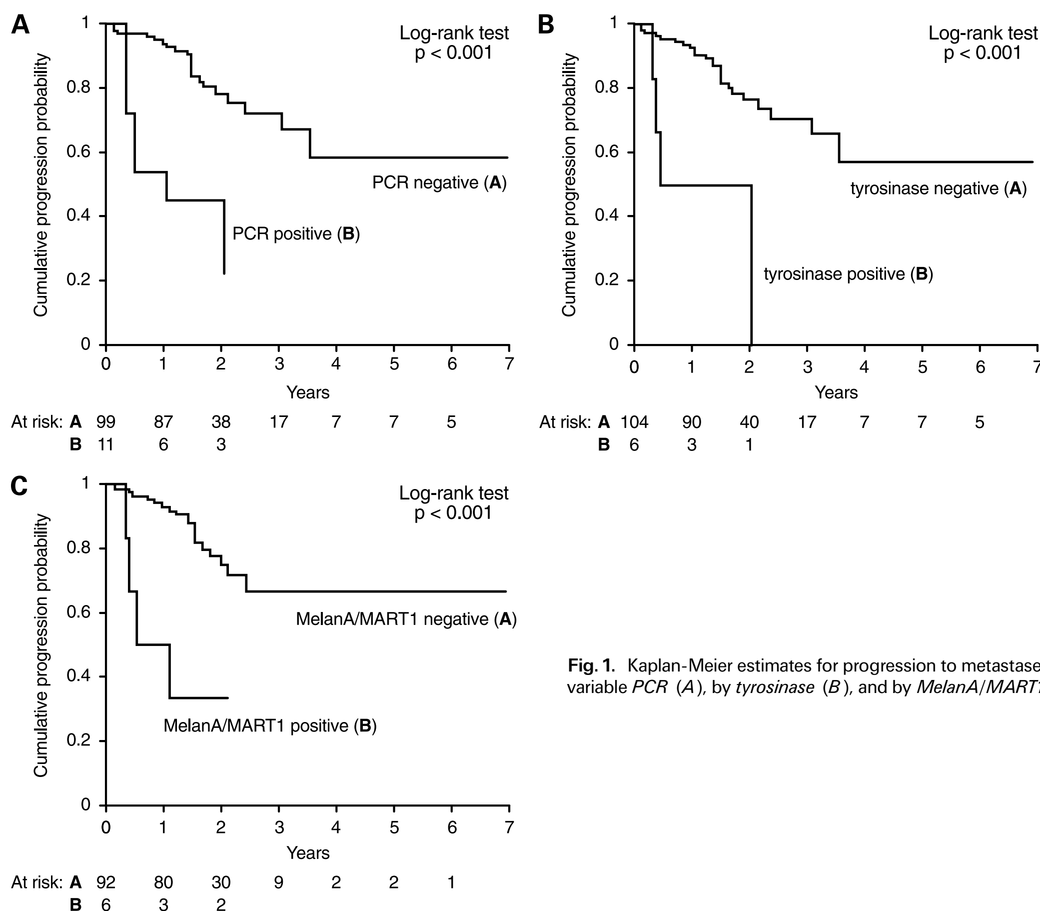

Fig. 1. Kaplan-Meier estimates for progression to metastases by combined variable *PCR* (*A*), by *tyrosinase* (*B*), and by *MelanA/MART1* (*C*).

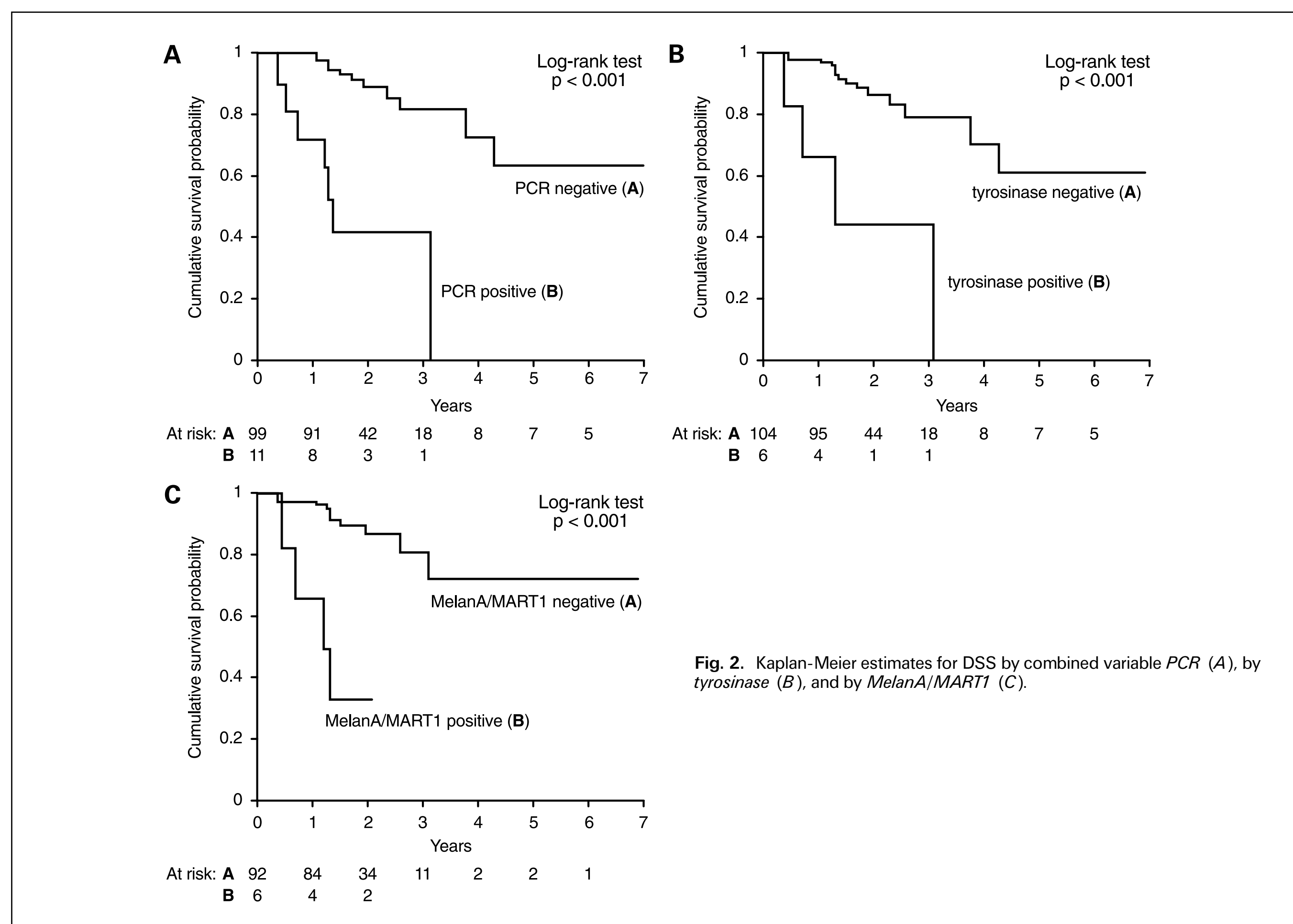

Fig. 2. Kaplan-Meier estimates for DSS by combined variable *PCR* (*A*), by *tyrosinase* (*B*), and by *MelanA/MART1* (*C*).

every sample and a water control, as well as plasmids for positive controls.

With the LightCycler software (version 3), crossing points (beginning of the PCR exponential phase) were assessed by the Second Derivative Maximum algorithm and plotted against the standard concentrations. Sample concentration was calculated using the plasmid standard curve, resulting in marker concentrations expressed as copy number of corresponding standard molecules per microliter. For comparing different blood samples, the marker concentration was normalized by concentration of the housekeeping gene. The relative sample amount was expressed as ratio marker (*tyrosinase or MelanA/MART1*)/(*PBGD*).

Because *tyrosinase* transcripts are not detected in blood samples from healthy persons (18), any detection of *tyrosinase* was considered positive. The transcripts of *MelanA/MART1* show a low background expression in hematopoietic cells. The cutoff for *MelanA/MART1/PBGD* ratio was defined as twice the background expression, 4.4×10^{-5} (18). Thus, only blood samples with *MelanA/MART1*/PBGD ratios above this cutoff were considered positive.

Statistical analysis. Descriptive statistics include mean, SD, median, and range for numerical variables, and absolute and relative frequencies for categorical factors. Associations between RT-PCR results and potential prognostic factors were analyzed with Fisher's exact test or, in the case of patient age at blood sampling, with the Mann-Whitney *U* test. RT-PCR results and clinical variables were assessed for their prognostic significance for time to metastases (TTP, time to progression) and disease-specific survival (DSS) by means of univariate Cox regression analysis, Kaplan-Meier analysis and log-rank test, and by multivariate Cox proportional hazard analysis with forward and backward selection. TTP, DSS, and age were related to the time of blood sampling. Lead-time bias was controlled by including the time between diagnosis and first blood sampling as a covariate into the Cox analysis and, additionally, by performing a subgroup analyses for that cohort of 75 patients with blood sampling at time of primary therapy. To verify the assumption of proportional hazards for explanatory variables in the selected models, model diagnostics was done by adding the corresponding time-dependent covariate (36). The analysis was done with the statistical package SPSS 12.0 (SPSS, Inc., Chicago, IL).

Results

Patient cohort characteristics. Over a 6-year period, blood samples from 110 patients with complete follow-up were obtained. For 75 patients, blood sampling was done at the time of primary therapy (i.e., enucleation or radiotherapy). Thirty-five patients were seen at a routine follow-up visit with a median interval between primary tumor diagnosis and blood sampling of 1 month (range 1-120 months). The clinical data, which are summarized in Table 2 for all patients, are comparable in both subgroups. The patient cohort selected for this study comprised a high-risk group with mid-size to large tumors and more than one third of patients had ciliary body involvement. In all patients, there were no signs of metastatic melanoma at the time of blood sampling. During the median follow-up interval

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/13/4/1171/1923974/1171.pdf by guest on 04 February 2023

of 22 months, 27 patients (25%) developed systemic metastases and 18 patients (15%) died from metastatic melanoma. Four patients had died of other causes.

RT-PCR results and relation to clinical variables. A total of 11 patients (10%) had a positive RT-PCR result, with blood samples from five patients positive only for *tyrosinase*, five patients positive only for *MelanA/MART1*, and one patient positive for both these markers. Correlation between RT-PCR results and age, sex, Karnofsky performance status, largest tumor diameter, extraocular growth, and ciliary body infiltration are shown in Table 3. The two mRNA markers *tyrosinase* and *MelanA/MART1* were pooled into one variable *PCR*, which was defined as positive if at least one of the single markers was positive. The PCR assay result showed no correlation with any clinical features, as assessed by Fisher's exact test, and no association with age, according to the Mann-Whitney *U* test.

Univariate analysis of potential prognostic factors. Univariate Cox regression analysis revealed highly significant results for *tyrosinase*, *MelanA/MART1*, and *PCR* (all *P* values ≤0.003) with respect to TTP and DSS (Table 4). The RT-PCR results were significant for both overall survival and DSS. The Kaplan-Meier estimates for TTP and DSS by RT-PCR results are shown in Figs. 1 and 2. Five of 11 PCR-positive patients (45%) relapsed and three died during the first year, in comparison with five relapses (5%) and no deaths in 99 PCR-negative patients. Altogether, there were seven relapses and deaths in PCR-positive patients and 20 (11) relapses (deaths) in PCR-negative patients. With univariate analysis, a positive RT-PCR result indicated an increased risk of metastasis and disease-specific mortality with a hazard ratio of 6.3 (95% confidence interval, 2.5-15.5) and 10.9 (95% confidence interval, 4.0-29.6), respectively. The univariate analysis did not yield any significant result for the clinical variables. Largest tumor diameter was of borderline significance, with a hazard ratio of 2.3 for TTP (P = 0.067) and of 3.2 for DSS (P = 0.064).

Multivariate analysis: all patients. In a multivariate Cox regression analysis, all clinical factors and RT-PCR results (*PCR* as combined variable or *tyrosinase* and *MelanA/MART1*) were considered for variable selection (Table 5). No violation of the proportional hazards assumption was detected, and the time from diagnosis to blood sampling was included as covariate in the model. Forward and backward selection gave basically identical results. For TTP, RT-PCR results and largest tumor diameter were selected as model variables. *PCR* was shown to be a relevant prognostic factor for the development of metastases with a hazard ratio of 7.3 (P < 0.001). When considered separately, both *tyrosinase* and *MelanA/MART1* were independent prognostic factors in the model with hazard ratios of 5.3 (P = 0.005) and 9.9 (P < 0.001), respectively. For DSS, the Karnofsky performance status was also included in the model. The prognostic relevance of the RT-PCR results for DSS was even more pronounced with a hazard ratio of 22.6 (P < 0.001) for *PCR*, 5.1 (P = 0.024) for *tyrosinase*, and 35.9 (P < 0.001) for *MelanA/MART1*. When overall survival was considered instead of DSS, the results of the model were essentially unchanged (not shown). If the PCR result was omitted from the analysis, only the largest tumor diameter was included in the model with a hazard ratio of 2.3 (P = 0.067) for TTP and a hazard ratio of 3.2 (P = 0.064) for DSS.

Subgroup analysis of patients with blood specimens taken at the time of the diagnosis. To exclude lead-time bias, the subgroup of 75 patients with blood sampling at the time of primary

Table 5. Prognostic factors selected for time to metastases and DSS by multivariate Cox regression

Factor	Time to metastases		DSS	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Model A with *PCR* as combined variable (*n* = 105)				
*PCR**	7.3 (2.9-18)	<0.001	22.6 (6.9-74)	<0.001
Largest tumor diameter	2.6 (1.04-6.5)	0.041	4.7 (1.3-17)	0.020
Karnofsky performance status			6.0 (1.2-30)	0.029
Age†			1.03 (0.9-1.1)	0.081
Model B with *tyrosinase* and *MelanA/MART1* as separate variables (*n* = 95)				
Tyrosinase	5.3 (1.7-17)	0.005	5.1 (1.2-21)	0.024
MelanA/MART1	9.9 (2.9-34)	<0.001	35.9 (5.5-198)	<0.001
Largest tumor diameter	3.8 (1.2-12)	0.019	10.0 (1.6-63)	0.014
Karnofsky performance status			6.7 (1.3-33)	0.021
Model C with *PCR* as combined variable (*n* = 75)				
*PCR**	14.6 (3-70)	0.001	134.0 (9-1,999)	<0.001
Largest tumor diameter	6.2 (1.2-34)	0.033	8.1 (0.8-79)	0.072
Karnofsky performance status			25.0 (1.6-333)	0.022
Sex‡			6.7 (0.7-50)	0.082

NOTE: Covariates included into the analysis are age, sex, Karnofsky performance status, largest tumor diameter, extraocular growth, ciliary body infiltration, *PCR*, *tyrosinase*, *MelanA/MART1*, and time from diagnosis to blood sampling. (A) Model with PCR as combined variable, all patients. (B) Model with tyrosinase and MelanA/MART1 as separate variables, all patients. (C) Model with PCR as combined variable, subgroup of patients with blood sampling at primary local therapy. Forward and backward elimination gave identical results for time to metastases in all models and for DSS in model (B).

**Tyrosinase* or *MelanA/MART1*.

†For disease-specific survival results of backward elimination are shown, in forward elimination age was not in the model.

‡For disease-specific survival, results of backward elimination are shown, in forward elimination sex was not in the model.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/13/4/1171/1923974/1171.pdf by guest on 04 February 2023

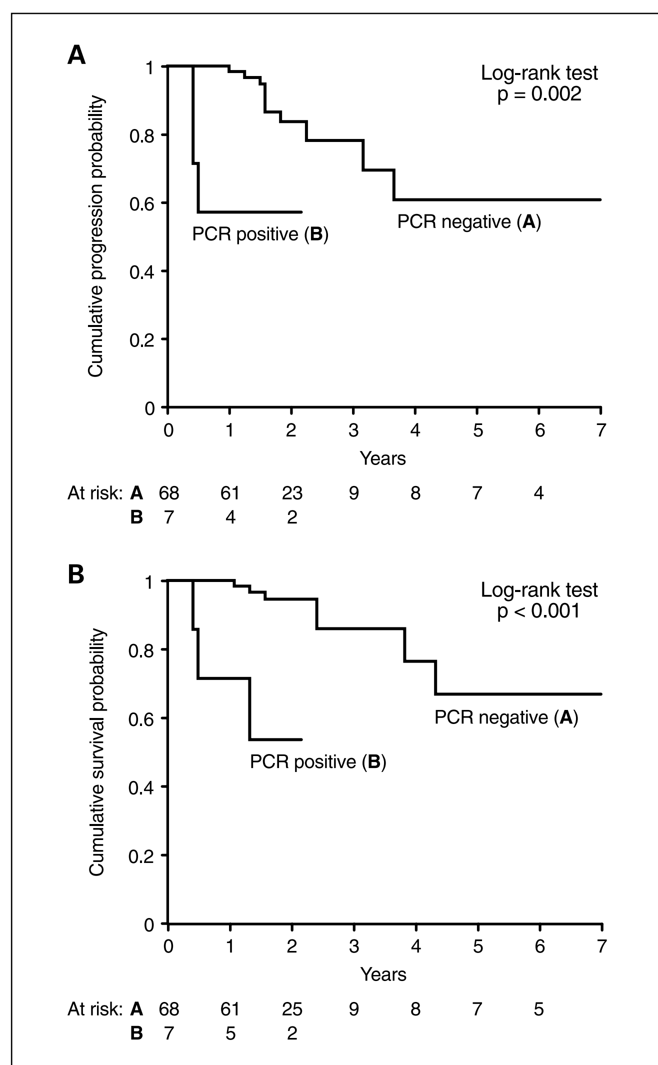

Fig. 3. Kaplan-Meier estimates for progression to metastases (*A*) and DSS (*B*) by combined variable *PCR* in the subgroup of patients with blood sampling at primary local therapy.

therapy was investigated, and comparable results were obtained (Table 5). Again, the detection of circulating tumor cells was a strong prognostic factor in the univariate analysis (Fig. 3). For the development of metastases, the largest tumor diameter and *PCR* were both significant and included in the model. For survival, the Karnofsky performance status, *PCR*, largest tumor diameter, and sex were included in the model (by backward elimination), whereas only the Karnofsky performance status and *PCR* produced *P* values <0.05. Because of the small number of RT-PCR–positive results (n = 7), the subanalysis for *tyrosinase* and *MelanA/MART1* was omitted within this subgroup.

Discussion

The study presented here shows a prominent relevance of RT-PCR for *tyrosinase* and *MelanA/MART1* in a cohort of 110 patients with uveal melanoma and unfavorable tumor characteristics. The presence of *tyrosinase* transcripts and expression of *MelanA/MART1* transcripts above the healthy volunteer cutoff value were both independently, and also as combined variable, associated with a very high risk for development of hematogenous metastases occurring within 2 years of follow-up. Both transcripts strongly predicted DSS and overall survival, whether assessed separately or together.

We are aware of six previous small studies on *tyrosinase* transcripts in peripheral blood of patients with uveal melanoma reported in the literature (37–41). Tobal et al. (39) detected *tyrosinase* mRNA in blood samples from six patients with uveal melanoma. Two of these patients had metastatic disease, and in one nonmetastatic patient the detection of *tyrosinase* mRNA was followed by occurrence of liver metastases 9 months later. Two years later, the same group reported absence of *tyrosinase* mRNA in 51 blood samples from 36 primary uveal melanoma patients (40). El Shabrawi (38) reported presence of *tyrosinase* transcripts in 2 of 12 from nonmetastatic uveal melanoma patients. In one of the two patients with positive PCR, histology of the primary tumor revealed tumor invasion of a vortex vein and the other patient developed liver metastases within 12 months. In a preceding report on quality assurance of diagnostic RT-PCR from our group (18), we had noted that the detection of *tyrosinase* transcripts preceded the development of liver metastases in two of three patients. Callejo et al. (41) showed that the detection rate of *tyrosinase* and *Melan A* transcripts in peripheral blood increased by multiple sampling in a study with 30 patients. Due to the short follow-up, prognostic importance of PCR results was not evaluated. In a recent study including 41 uveal melanoma patients, 16 were found positive for *tyrosinase* mRNA in peripheral blood samples and the prognostic role of the detection of *tyrosinase* transcripts was shown by univariate analysis (37).

Three of these studies concluded with the hypothesis that PCR detection of *tyrosinase* may be a relevant prognostic factor in uveal melanoma patients, which now has been verified by the study reported here.

In addition to *tyrosinase* as a marker for circulating melanoma cells, we included *MelanA/MART1* in our study as a second marker. Multimarker RT-PCR has been shown to be more sensitive for detection of circulating melanoma cells in patients with cutaneous melanoma (20). In a preceding report from our institution, several markers had been investigated for their applicability using quantitative RT-PCR, and *tyrosinase* and *MelanA/MART1* and have been found suitable in our hands (18). The univariate and multivariate analysis of the study presented here revealed detection of both transcripts as independent strong prognostic factors for the development of metastases and DSS. This result shows the advantage of using several melanoma-associated markers to increase the sensitivity of the method.

The patient cohort of 110 patients in our study is still rather small for achieving generalizable results; however, several features of our analysis support the robustness of the Cox models. These features most importantly include the absence of discrepancies when comparing forward and backward selection of variables, concordant results when comparing disease-specific with overall survival and also when omitting the 35 patients in whom blood samples were not obtained at the time of primary treatment but at a later outpatient clinic visit.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/13/4/1171/1923974/1171.pdf by guest on 04 February 2023

The results in our patient cohort are in general agreement with the results observed in the COMS series, the largest prospective series of uveal melanoma patients reported in the literature. The proportion of patients with tumors exceeding 14 mm in longest basal diameter was larger in our patient cohort compared with the patients in the COMS studies (56% versus 16%; ref. 42). The 22-month metastases rate in our study was therefore higher compared with the overall 24-month metastases rate reported for the COMS series (23% versus 10%; ref. 6). The rate of metastases of 24% at 2 years in the subgroup of patients with tumors exceeding 16 mm in largest basal diameter of the COMS series is similar to the 23% metastasis rate at 22 months in our high-risk patient cohort. Despite the overriding importance of the RT-PCR results in our study, the largest tumor diameter remained a significant predictor of metastasis and survival in our patient cohort as in the COMS reports. The positive and negative predictive values of the results of the PCR analysis with respect to the development of systemic metastases are 64% and 80%, respectively. Due to the small number of PCR-positive patients, the positive predictive value has to be considered with caution. By combining the variables PCR and largest tumor diameter in our study, the negative predictive value increases to 91%. Thus, patients with a negative result of the PCR analysis and a largest tumor diameter ≤14 mm should be candidates for observation only.

In summary, RT-PCR–based detection of the two melanoma markers *tyrosinase* and *MelanA/MART1* offers high prognostic value for patients with primary uveal melanoma and should be useful for patient counseling, development of follow-up recommendations, and selection of patients for investigation of novel adjuvant treatment approaches.

Acknowledgments

We thank Karin Heufelder for excellent technical assistance and Franziska Wellnitz for careful data collection and validation.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/13/4/1171/1923974/1171.pdf by guest on 04 February 2023

References

1. Augsburger JJ, Correa ZM, Freire J, Brady LW. Long-term survival in choroidal and ciliary body melanoma after enucleation versus plaque radiation therapy. Ophthalmology 1998;105:1670–8.

2. Egger E, Schalenbourg A, Zografos L, et al. Maximizing local tumor control and survival after proton beam radiotherapy of uveal melanoma. Int J Radiat Oncol Biol Phys 2001;51:138–47.

3. Lommatzsch PK, Werschnik C, Schuster E. Long-term follow-up of Ru-106/Rh-106 brachytherapy for posterior uveal melanoma. Graefes Arch Clin Exp Ophthalmol 2000;238:129–37.

4. Diener-West M, Earle JD, Fine SL, et al. The COMS randomized trial of iodine 125 brachytherapy for choroidal melanoma, III: initial mortality findings. COMS Report No. 18. Arch Ophthalmol 2001;119:969–82.

5. Schmittel A, Bechrakis NE, Martus P, et al. Independent prognostic factors for distant metastases and survival in patients with primary uveal melanoma. Eur J Cancer 2004;40:2389–95.

6. Diener-West M, Reynolds SM, Agugliaro DJ, et al. Development of metastatic disease after enrollment in the COMS trials for treatment of choroidal melanoma: Collaborative Ocular Melanoma Study Group report no. 26. Arch Ophthalmol 2005;123:1639–43.

7. Kath R, Hayungs J, Bornfeld N, Sauerwein W, Hoffken K, Seeber S. Prognosis and treatment of disseminated uveal melanoma. Cancer 1993;72:2219–23.

8. Eskelin S, Pyrhonen S, Hahka-Kemppinen M, Tuomaala S, Kivela T. A prognostic model and staging for metastatic uveal melanoma. Cancer 2003;97:465–75.

9. Peters S, Voelter V, Zografos L, et al. Intra-arterial hepatic fotemustine for the treatment of liver metastases from uveal melanoma: experience in 101 patients. Ann Oncol 2006;17:578–83.

10. Isager P, Ehlers N, Overgaard J. Prognostic factors for survival after enucleation for choroidal and ciliary body melanomas. Acta Ophthalmol Scand 2004;82:517–25.

11. Singh AD, Shields CL, Shields JA. Prognostic factors in uveal melanoma. Melanoma Res 2001;11:255–63.

12. Coupland SE, Anastassiou G, Stang A, et al. The prognostic value of cyclin D1, p53, and MDM2 protein expression in uveal melanoma. J Pathol 2000;191:120–6.

13. Hurks HM, Metzelaar-Blok JA, Barthen ER, et al. Expression of epidermal growth factor receptor: risk factor in uveal melanoma. Invest Ophthalmol Vis Sci 2000;41:2023–7.

14. Baggetto LG, Gambrelle J, Dayan G, et al. Major cytogenetic aberrations and typical multidrug resistance phenotype of uveal melanoma: current views and new therapeutic prospects. Cancer Treat Rev 2005;31:361–79.

15. Kivela T, Makitie T, Al-Jamal RT, Toivonen P. Microvascular loops and networks in uveal melanoma. Can J Ophthalmol 2004;39:409–21.

16. Kodjikian L, Roy P, Rouberol F, et al. Survival after proton-beam irradiation of uveal melanomas. Am J Ophthalmol 2004;137:1002–10.

17. Singh AD, Rennie IG, Kivela T, Seregard S, Grossniklaus H. The Zimmerman-McLean-Foster hypothesis: 25 years later. Br J Ophthalmol 2004;88:962–7.

18. Keilholz U, Goldin-Lang P, Bechrakis NE, et al. Quantitative detection of circulating tumor cells in cutaneous and ocular melanoma and quality assessment by real-time reverse transcriptase-polymerase chain reaction. Clin Cancer Res 2004;10:1605–12.

19. van Dinten LC, Pul N, van Nieuwpoort AF, Out CJ, Jager MJ, van den Elsen PJ. Uveal and cutaneous melanoma: shared expression characteristics of melanoma-associated antigens. Invest Ophthalmol Vis Sci 2005;46:24–30.

20. Hoon DS, Wang Y, Dale PS, et al. Detection of occult melanoma cells in blood with a multiple-marker polymerase chain reaction assay. J Clin Oncol 1995;13:2109–16.

21. Mellado B, Colomer D, Castel T, et al. Detection of circulating neoplastic cells by reverse-transcriptase polymerase chain reaction in malignant melanoma: association with clinical stage and prognosis. J Clin Oncol 1996;14:2091–7.

22. Kunter U, Buer J, Probst M, et al. Peripheral blood tyrosinase messenger RNA detection and survival in malignant melanoma. J Natl Cancer Inst 1996;88:590–4.

23. Ghossein RA, Coit D, Brennan M, et al. Prognostic significance of peripheral blood and bone marrow tyrosinase messenger RNA in malignant melanoma. Clin Cancer Res 1998;4:419–28.

24. Schittek B, Bodingbauer Y, Ellwanger U, Blaheta HJ, Garbe C. Amplification of MelanA messenger RNA in addition to tyrosinase increases sensitivity of melanoma cell detection in peripheral blood and is associated with the clinical stage and prognosis of malignant melanoma. Br J Dermatol 1999;141:30–6.

25. Hanekom GS, Stubbings HM, Johnson CA, Kidson SH. The detection of circulating melanoma cells correlates with tumour thickness and ulceration but is not predictive of metastasis for patients with primary melanoma. Melanoma Res 1999;9:465–73.

26. Palmieri G, Strazzullo M, Ascierto PA, Satriano SM, Daponte A, Castello G. Polymerase chain reaction-based detection of circulating melanoma cells as an effective marker of tumor progression. Melanoma Cooperative Group. J Clin Oncol 1999;17:304–11.

27. Proebstle TM, Jiang W, Hogel J, Keilholz U, Weber L, Voit C. Correlation of positive RT-PCR for tyrosinase in peripheral blood of malignant melanoma patients with clinical stage, survival and other risk factors. Br J Cancer 2000;82:118–23.

28. Hoon DS, Bostick P, Kuo C, et al. Molecular markers in blood as surrogate prognostic indicators of melanoma recurrence. Cancer Res 2000;60:2253–7.

29. Mellado B, Del Carmen Vela M, Colomer D, et al. Tyrosinase mRNA in blood of patients with melanoma treated with adjuvant interferon. J Clin Oncol 2002;20:4032–9.

30. Brownbridge GG, Gold J, Edward M, MacKie RM. Evaluation of the use of tyrosinase-specific and melanA/MART-1-specific reverse transcriptase-coupled-polymerase chain reaction to detect melanoma cells in peripheral blood samples from 299 patients with malignant melanoma. Br J Dermatol 2001;144:279–87.

31. Szenajch J, Jasinski B, Synowiec A, et al. Prognostic value of multiple reverse transcription-PCR tyrosinase testing for circulating neoplastic cells in malignant melanoma. Clin Chem 2003;49:1450–7.

32. Osella-Abate S, Savoia P, Quaglino P, et al. Tyrosinase expression in the peripheral blood of stage III melanoma patients is associated with a poor prognosis: a clinical follow-up study of 110 patients. Br J Cancer 2003;89:1457–62.

33. Quaglino P, Savoia P, Fierro MT, Osella-Abate S, Bernengo MG. Clinical significance of sequential tyrosinase expression in the peripheral blood of disease-free melanoma patients: a review of literature data. Melanoma Res 2004;14:S17–9.

34. Voit C, Kron M, Rademaker J, et al. Molecular staging in stage II and III melanoma patients and its effect on long-term survival. J Clin Oncol 2005;23:1218–27.

35. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–9.

36. Collett D. Modelling survival data in medical research. In: Chatfield C, Tanner M, Zidek J, editors.

Texts in statistical science series. London: Chapman & Hall/CRC; 2003. p. 146 – 9.

37. Boldin I, Langmann G, Richtig E, et al. Five-year results of prognostic value of tyrosinase in peripheral blood of uveal melanoma patients. Melanoma Res 2005;15:503 – 7.

38. El-Shabrawi Y, Langmann G, Hutter H, Kenner L, Hoefler G. Comparison of current methods and PCR for the diagnosis of metastatic disease in uveal malignant melanoma. Ophthalmologica 1998;212:80.

39. Tobal K, Sherman LS, Foss AJ, Lightman SL. Detection of melanocytes from uveal melanoma in peripheral blood using the polymerase chain reaction. Invest Ophthalmol Vis Sci 1993;34:2622 – 5.

40. Foss AJ, Guille MJ, Occleston NL, Hykin PG, Hungerford JL, Lightman S. The detection of melanoma cells in peripheral blood by reverse transcription-polymerase chain reaction. Br J Cancer 1995;72: 155 – 9.

41. Callejo SA, Antecka E, Blanco PL, Edelstein C, Burnier MN. Identification of circulating malignant cells and its correlation with prognostic factors and treatment in uveal melanoma. A prospective longitudinal study. Eye 2006; Epub 2006 Mar 31. (doi: 10.1038/sj.eye.6702322).

42. Diener-West M, Earle JD, Fine SL, et al. The COMS randomized trial of iodine 125 brachytherapy for choroidal melanoma, II: characteristics of patients enrolled and not enrolled. COMS Report No. 17. Arch Ophthalmol 2001;119:951 – 65.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/13/4/1171/1923974/1171.pdf by guest on 04 February 2023