original article

doi:10.1093/annonc/mdj138
Published online 16 January 2006

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

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Received 6 October 2005; revised 5 December 2005; accepted 14 December 2005

Background: This randomized phase III trial was designed to demonstrate the superiority of autologous peptide-loaded dendritic cell (DC) vaccination over standard dacarbazine (DTIC) chemotherapy in stage IV melanoma patients.
Patients and methods: DTIC 850 mg/m^2 intravenously was applied in 4-week intervals. DC vaccines loaded with MHC class I and II-restricted peptides were applied subcutaneously at 2-week intervals for the first five vaccinations and every 4 weeks thereafter. The primary study end point was objective response (OR); secondary end points were toxicity, overall (OS) and progression-free survival (PFS).
Results: At the time of the first interim analysis 55 patients had been enrolled into the DTIC and 53 into the DC-arm (ITT). OR was low (DTIC: 5.5%, DC: 3.8%), but not significantly different in the two arms. The Data Safety & Monitoring Board recommended closure of the study. Unscheduled subset analyses revealed that patients with normal serum LDH and/or stage M1a/b survived longer in both arms than those with elevated serum LDH and/or stage M1c. Only in the DC-arm did those patients with (i) an initial unimpaired general health status (Karnofsky = 100) or (ii) an HLA-A2+/HLA-B44− haplotype survive significantly longer than patients with a Karnofsky index <100 (P = 0.007 versus P = 0.057 in the DTIC-arm) or other HLA haplotypes (P = 0.04 versus P = 0.57 in DTIC-treated patients).
Conclusions: DC vaccination could not be demonstrated to be more effective than DTIC chemotherapy in stage IV melanoma patients. The observed association of overall performance status and HLA haplotype with overall survival for patients treated by DC vaccination should be tested in future trials employing DC vaccines.
Key words: advanced melanoma, dentric cells, DTIC, first-line therapy, melanoma

original article

introduction

Metastatic melanoma has a grave prognosis as it is largely resistant to all treatment modalities [1]. Dacarbazine (DTIC) is still considered the standard first-line treatment, despite the lack of any evidence of improving overall survival (OS) [2]. No other single agent or combination has demonstrated superiority to DTIC in terms of prolongation of survival, even if associated with higher response rates (RR) [2]. In conclusion, there is no evidence to date that any available treatment improves survival of patients with metastatic melanoma.

In small initial trials, notably using peptide vaccines, it has been reported that vaccines can induce regression of primarily locoregional metastases [3, 4]. Complete regressions, however, in particular in stage IV disease, were rarely observed [5]. In 1998 Nestle et al. [6] reported a 30% RR in 16 stage IV melanoma patients in a pilot trial upon repetitive intranodal injection of DC loaded with several MHC class I-restricted tumor peptides or tumor-lysate plus KLH as unspecific helper and control antigen. Thurner et al. [7] then proved the immunogenicity of mature DC loaded with a single Mage-3A1 peptide, and observed the regression of individual metastases.

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Others had also reported on an encouraging efficacy of DC vaccination in other settings, which was in congruence with data from animal experiments [8, 9]. As DC vaccination appeared to present a promising strategy for metastatic melanoma, we continued exploring this approach, in two ways. First, we wanted to address several of the obvious variables that required optimization, such as DC maturation and vaccination schedule, in order to collect data for an optimized, next generation DC vaccine [10]. Secondly, we decided in late 1999 to perform a randomized phase III trial to demonstrate superiority of DC vaccination compared with DTIC monochemotherapy, carefully weighing arguments for and against this endeavor. We were confident that no harm would be inflicted on surgically incurable stage IV melanoma patients given that standard therapy had been ineffective to date and that DC vaccination appeared non-toxic [11]. Furthermore, an early phase III trial would allow proof to be gained of clinical efficacy of a first generation DC vaccine, and would in addition represent a solid basis for any further step by step optimization. We therefore designed this randomized trial for the investigation of superiority of DC vaccination versus standard DTIC treatment in stage IV melanoma. The only other randomized phase III cancer vaccination trial to date compared the efficacy of a cellular vaccine versus placebo in prostate cancer patients [12]. Thus, we present here the first randomized trial that prospectively tested a DC-based vaccination in comparison to standard treatment in advanced cancer patients based on objective clinical end points as response and survival.

patients and methods

patient population

Patients with histologically confirmed metastatic melanoma were enrolled with the following eligibility criteria: at least one measurable target lesion following RECIST guidelines [13], HLA-A1, -A2, -A3, -A24 and/or -B44 positivity, no brain or bone metastases, no prior systemic chemotherapy, Karnofsky index ≥70%, age ≥18 years, life expectancy >3 months, no active infection or autoimmune disease, adequate bone marrow, hepatic and renal functions. The study protocol was approved by the institutional review committees of all participating centers and informed consent was obtained from all patients prior to randomization. Only centers with previous experience in the generation and administration of autologous DC were allowed to participate in this trial. Patients were stratified according to centers and randomization was performed centrally by the Central Unit for Biostatistics, DKFZ, Heidelberg.

treatment plan

Patients in arm A received DTIC, 850 mg/m^2 i.v. on day 1 in 4-week intervals, according to a previous DeCOG study protocol [14]. Patients randomized to arm B received autologous peptide-pulsed monocyte-derived DC administered s.c. at the ventromedial aspect of one extremity every 2 weeks for the first five vaccinations, followed by vaccinations in 4-week intervals. Recommended concomitant medication was 5HT3 antagonists in the DTIC arm.

DC vaccine

DC vaccines were generated at each center according to joint standard operating procedures. All reagents were centrally ordered and validated, and thereafter sent in aliquots to the individual centers. PBMCs ($\sim 10^{10}$) were isolated from leukapheresis products on Lymphoprep (Nycomed Pharma, Unterschleiβheim, Germany) and frozen in aliquots. To generate DC, thawed PBMCs were plated, and 1h-adherent monocyte-rich fractions were further cultured in RPMI 1640 (GMP quality; Bio-Whittaker, Walkersville, MD), 1% heat-inactivated autologous plasma, 800 U/ml GM-CSF (Leukomax; Schering-Plough, Kenilworth, NJ) and 1000 U/ml IL-4 (GMP quality; Schering-Plough) for 6 days [15], and then matured by adding 10 ng/ml TNF-α (Beromun; Boehringer-Ingelheim, Vienna, Austria), 2 ng/ml IL-1β (GMP quality; R&D, Wiesbaden, Germany), 1000 U/ml IL-6 (GMP quality; Novartis, Nürnberg, Germany), and 1 μg/ml prostaglandin E2 (Minprostin; Pfizer, Karlsruhe, Germany). Mature DCs were harvested on day 7 and characterized (viability; morphology; FACS analysis: forward/side scatter, staining for HLA-DR and CD83/CD80/CD86/CD1a/CD14/CD2/CD19/CD56; microbial tests) as described previously [16]. DC were then loaded with several MHC class I- and II-restricted peptides, all of pharmaceutical quality (Clinalfa, Läufelfingen, Switzerland). (For references regarding the peptides chosen and listed below see www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm.) Each of the individual four HLA-A1 (Mage-1, EADPTGHSY; Mage-3, EVDPIGHLY; Tyrosinase analogue, KSDICTDEY; and as a control FluNP, CTELKLSDY), six HLA-A2 (GnTV, VLPDVFIRCV; Tyrosinase, YMDGTMSQV; gp100 analogue, IMDQVPFSV; Melan-A analogue, ELAGIGILTV; Mage-3A2.1, FLWGPRALV; and as a control FluMP, GILGFVFTL), three HLA-A3 (Mage-1, SLFRAVITK; gp100, LIYRRRLMK; and FluNP, ILRGSVAHK), four HLA-A24 (Tyrosinase, AFLPWHRLF; Mage-1, NYKHCFPEI; gp100, VYFFLPDHL; Mage-3, IMPKAGLLI), and 2 HLA-B44 (Tyrosinase, SEIWRDIDF; Mage-3, MEVDPIGHLY) restricted peptides were pulsed at 30 μg/ml on separate batches of 4 million DCs to avoid uncontrollable competition at the MHC molecules. In the case of MHC class II–restricted peptides all DCs in all patients were pulsed with 18 μM Mage-3. DR13 (LLKYRAREPVTKAE) peptides, while the two DR4-restricted MHC class II peptides Tyrosinase (SYLQDSVPDSFQD; anchor-modified for high-affinity binding to DR4) and gp100 (WNRQLYPEWTEAQRLD) were loaded only onto half of the DCs in order to avoid competition for the DR4 molecules. Peptide-loaded DCs were then collected, washed and administered at a concentration of 4×10^6 DC in 1 ml PBS and 1% HSA by s.c. injection at four to 10 sites close to inguinal lymph nodes at the ventromedial aspect of one extremity.

response and survival assessment

Tumor response was assessed by CT and/or MRI imaging in 12-week intervals and evaluated according to RECIST guidelines. Patients who died from melanoma rapidly after onset of study treatment could not be assessed for tumor response and were classified as progressive disease (PD) [17]. Complete response (CR) and partial response (PR) were combined to evaluate OR, the primary study end point. All ORs had to be confirmed by repeated CT or MRI scans after 4 weeks. Best overall response was defined as the best response recorded from the start of treatment until disease progression [17]. OS and PFS were measured from the date of randomization until the date of death or disease progression, respectively. If no such event occurred, as well as in patients with unknown follow-up status, the date of last patient contact was used as censored observation. Patients who had received at least one cycle of DTIC or two DC vaccinations, respectively, were considered evaluable for per-protocol analysis of all study end points. Toxicity was evaluated using standard CTC-criteria (http://ctep.cancer.gov/reporting/ctc.html) and assessed prior to each treatment cycle. The study was externally monitored (Antaris, Lampertheim, Germany) in accordance to GCP guidelines.

statistical design

This study was designed as a multicenter, prospective-randomized, open-label, phase III trial to demonstrate the superiority of DC

vaccination over DTIC monochemotherapy in a one-sided statistical test. The primary study end point, OR, as well as the secondary end points, OS and PFS, were evaluated on an intention-to-treat (ITT) and on a per-protocol (PP) basis. Toxicity was analyzed in all patients that once received study treatment.

Patient recruitment was outlined as a total of 190 patients (95 per arm) evaluable for OR. Using this sample size, the study was planned to detect in a one-sided comparison a difference of the primary end point OR from 15% in the DTIC arm to 30% in the DC arm with a power of 80% and a significance level of 5%, corresponding to an odds ratio of 2.41. At the time of the protocol-based interim data analysis (December 2003), 108 patients were enrolled. With 93 evaluable cases at this time point, the power to detect the expected difference in OR at a significance level of 5% is 54% only. The odds ratio that would be detectable with this sample size using the originally planned power of 80% has increased to 3.41 instead of 2.41.

With regard to the secondary study end points, OS and PFS, conditional power calculations were based on survival data obtained from a clinical trial using DTIC in a comparable population of stage IV melanoma patients [18], revealing a median PFS of 1.5 months and a median OS of 6.4 months. With the originally planned sample size of 190 evaluable patients, the study would have had a good power of 90% to detect differences of 20%, both for PFS and OS.

statistical analysis

The database was frozen in October 2003 except for survival data, which were updated in August 2004. For the analysis of OS and PFS, survival curves and median survival times were calculated and graphically presented using the Kaplan–Meier method for censored failure time data. The log rank test was used for comparing the two treatment arms. In all graphs, censored observations are indicated by vertical bars. Confidence intervals for median survival at the 95% level were calculated using the method of Brookmeyer. The univariate Cox proportional hazards model was used to assess the impact of prognostic factors on survival. Multivariate Cox proportional hazards regression was used both to adjust the treatment comparison for the influence of prognostic factors as well as for the determination of influential prognostic factors on survival. Statistical analyses were performed using the statistical packages ADAM of the Biostatistics Unit, SAS 8.1 for Windows (SAS Institute, Cary, NC), R 1.6.2 (http://www.r-project.org) and StatXact 5.0.3 (Cytel Software, Cambridge, MA).

results

Between March 2000 and July 2003, 108 patients (ITT population) with a median age of 58 years (range 19–79) were accrued and randomized from six centers (Figure 1, Table 1). Detailed patient characteristics are provided in Table 1 and demonstrate a good balance in both treatment arms according to sex, age, HLA-type, LDH, Karnofsky index as well as AJCC M classification (except patients with lung metastases were more frequent in the DTIC arm, $P = 0.027$, Fisher's exact test). Out of the 108 randomized patients (ITT), 104 were eligible for participation in the study, 99 received study treatment and 93 were eligible for PP analysis (Figure 1).

Grade 3/4 toxicities (CTC) (http://ctep.cancer.gov/reporting/ctc.html) were experienced by seven patients in each treatment arm (Table 2).

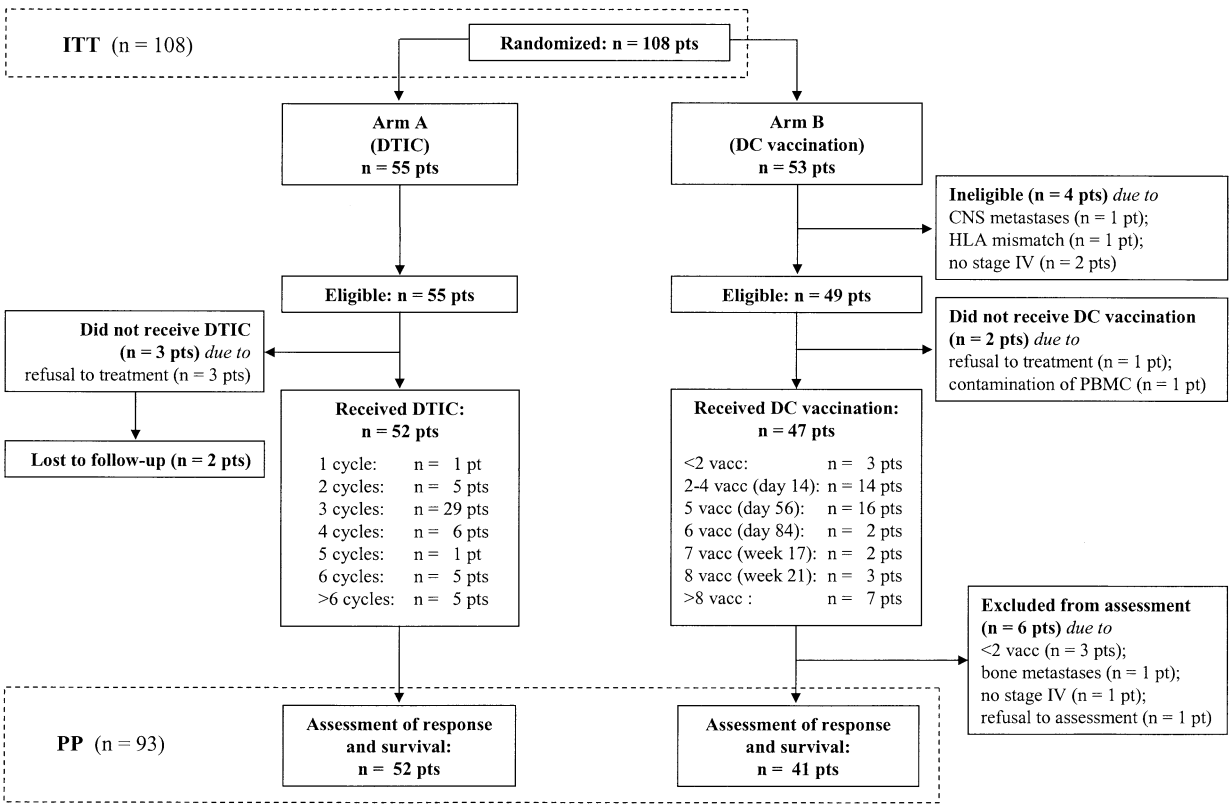

Figure 1. Schematic presentation of the study flow.

Table 1. Patient characteristics

	Arm A (DTIC)	Arm B (DC vaccination)
Randomized ITT (n = 108)	55 (100.0%)	53 (100.0%)
Sex (n = 108)		
Male	37 (67.3%)	31 (58.5%)
Female	18 (32.7%)	22 (41.5%)
Median age (years)	58.0 (range 20.7–79.6)	58.9 (range 19.0–80.0)
HLA[a] (n = 108)		
A1+	17 (30.9%)	15 (28.3%)
A2+	29 (52.7%)	30 (56.6%)
A3+	19 (34.6%)	16 (30.2%)
A24+	13 (23.6%)	6 (11.3%)
B44+	9 (16.4%)	11 (20.8%)
LDH (n = 96)		
≤UNL	23 (47.9%)	24 (50.0%)
>UNL	25 (52.1%)	24 (50.0%)
Karnofsky (n = 103)		
100%	30 (56.6%)	30 (60.0%)
90%	18 (34.0%)	11 (22.0%)
80%	5 (9.4%)	8 (16.0%)
70%	0 (0.0%)	1 (2.0%)
Metastatic sites[a] (n = 107)		
Skin and/or lymph nodes	38 (70.4%)	38 (71.7%)
Lung	39 (72.2%)	27 (50.9%)
Liver	14 (25.9%)	14 (26.4%)
Other visceral	13 (24.1%)	11 (20.7%)
AJCC M category (n = 96)		
M1a	3 (6.3%)	5 (10.4%)
M1b	8 (16.7%)	10 (20.8%)
M1c	37 (77.1%)	33 (68.8%)
Eligible[b] (n = 104)	55 (100.0%)	49 (92.5%)
Received treatment[c] (n = 99)	52 (94.5%)	47 (88.7%)
Full analysis set,[d] PP (n = 93)	52 (94.5%)	41 (77.4%)

[a]Multiple entries possible; [b–d]reasons for ineligibility, no treatment and exclusion from response and survival assessment see study flow (Figure 1).
TT, intention to treat; CI, confidence interval; LDH, lactate dehydrogenase; UNL, upper normal limit; AJCC, American Joint Committee on Cancer; PP, per protocol.

Table 2. Adverse events (grade 3 or 4) during treatment

	Arm A (DTIC)	Arm B (DC vaccination)
Treated patients[a] (n = 99)	52 (100.0%)	47 (100.0%)
Laboratory changes		
Hematology	0 (0.0%)	0 (0.0%)
Other	0 (0.0%)	1 (2.1%)
Heart/circulation		
Thrombosis	0 (0.0%)	1 (2.1%)
Lung/respiration		
Dyspnoea	1 (1.9%)	1 (2.1%)
Kidney/bladder		
Anuria	1 (1.9%)	0 (0.0%)
Neuropathy		
Cramps	0 (0.0%)	1 (2.1%)
Gastrointestine	2 (3.8%)	0 (0.0%)
Fever/infection	1 (1.9%)	0 (0.0%)
Pain	0 (0.0%)	1 (2.1%)
General/lethargy	2 (3.8%)	2 (4.3%)

[a]Patients receiving study treatment see study flow (Figure 1). Toxicity was graded according to CTC criteria.

Table 3. Treatment efficacy

	Arm A (DTIC)	Arm B (DC vaccination)
Randomized ITT (n = 108)	55 (100.0%)	53 (100.0%)
Best overall response		
CR	0 (0.0%)	0 (0.0%)
PR	3 (5.5%)	2 (3.8%)
SD	10 (18.2%)	8 (15.1%)
PD	39 (70.9%)	35 (66.0%)
Non-evaluable[a]	3 (5.5%)	8 (15.1%)
Objective response (CR+PR)	3 (5.5%)	2 (3.8%)

[a]For reasons for non-evaluability see study flow (Figure 1). Best overall response was defined as the best tumor response recorded from the start of treatment until disease progression.
TT, intention to treat; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

OR was low in both arms (3/55, 5.5% in arm A and 2/53, 3.8% in arm B; ITT) without significant difference. Similar efficacies were observed in the PP population (5.8%, arm A; 4.9%, arm B). Detailed data of treatment response are shown in Table 3.

At final survival data analysis (August 2004) the median follow-up time was 22.2 months and a total number of 75 deaths had occurred (ITT) (Table 4). No significant differences between the two arms, either in OS (Figure 2) or in PFS, were found. Several unscheduled, explorative analyses were performed to investigate the lack of efficacy of DC vaccination and to evaluate these data for prognostic factors, which could be used for stratification in future vaccination trials.

Patients with elevated serum LDH showed a trend to a reduced OS (P = 0.105, ITT; Figure 3; Table 4). Patients with distant metastases to skin, lymph nodes or lung (M1a/b) revealed a favorable OS compared with patients with metastases to other organs or an elevated serum LDH (M1c) (Figure 4). Patients with an unimpaired performance status (Karnofsky = 100) randomized to the DC vaccination arm revealed a significantly improved OS compared with DC-treated patients with a Karnofsky <100 (P = 0.007; Figure 5A). In the DTIC arm this difference showed only borderline significance (P = 0.057; Figure 5B).

We hypothesized that the expression pattern of HLA-haplotypes—notably by determining the type and number of peptides loaded onto DC—could have a major impact on vaccination efficacy. Indeed, when probing the HLA class-I haplotypes (either individually or in all possible combinations), we found that the HLA-A2+/B44− patient subset survived significantly longer under vaccination therapy than patients

Table 4. Overall and progression-free survival

	Arm A (DTIC)	Arm B (DC vaccination)
Randomized ITT (n = 108)	55 (100.0%)	53 (100.0%)
Survival status (n = 108)		
Alive	17 (31.0%)	14 (26.4%)
Dead	36 (65.4%)	39 (73.6%)
Unknown[a]	2 (3.6%)	0 (0.0%)
Median time to progression (n = 106)	2.8 months	3.2 months
	(95% CI 2.6–3.0)	(95% CI 2.9–3.4)
Median overall survival (n = 106)	11.6 months	9.3 months
	(95% CI 9.8–14.4)	(95% CI 6.7–14.4)
AJCC M category (n = 96)		
M1a/b	16.3 months	17.9 months
M1c	9.8 months	7.3 months
HLA (n = 106)		
A1+	10.5 months	7.1 months
A2+	11.1 months	9.4 months
A3+	11.6 months	7.6 months
A24+	8.7 months	6.1 months
B44+	10.5 months	8.5 months
A2+/B44−	11.1 months	20.8 months
LDH (n = 95)		
≤UNL	13.6 months	10.4 months
>UNL	9.8 months	7.1 months
Karnofsky (n = 103)		
100%	14.1 months	14.4 months
<100%	10.5 months	7.1 months
Best overall response (n = 106)		
PR	Not reached	Not reached
SD	Not reached	17.5 months
PD	10.1 months	6.7 months
Full analysis set,[b] PP (n = 93)	52 (100.0%)	41 (100.0%)
Survival status (n = 93)		
Alive	16 (30.8%)	10 (24.3%)
Dead	36 (69.2%)	31 (75.6%)
Median time to progression (n = 93)	2.8 months	3.2 months
	(95% CI 2.6–3.0)	(95% CI 2.8–3.4)
Median overall survival (n = 93)	11.6 months	9.3 months
	(95% CI 9.8–14.4)	(95% CI 7.0–15.6)

[a]Unknown survival status due to loss to follow-up (two patients).
[b]For reasons for exclusion from response and survival assessment see study flow (Figure 1).
ITT, intention to treat; CI, confidence interval; AJCC, American Joint Committee on Cancer; LDH, lactate dehydrogenase; UNL, upper normal limit; PP, per protocol.

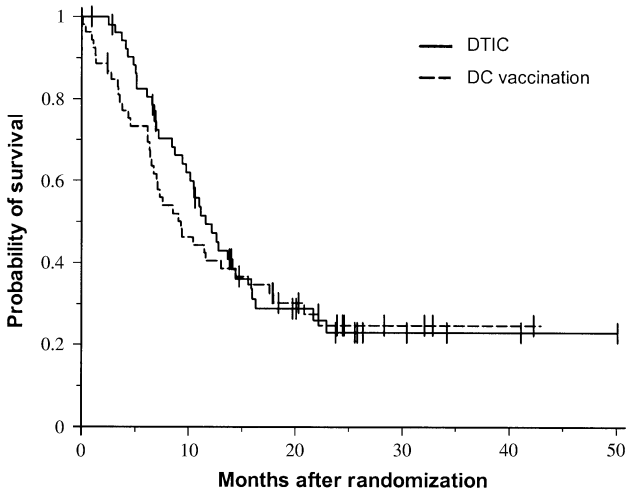

Figure 2. Kaplan–Meier curves of overall survival of the ITT population by treatment arm. Arm A (DTIC), 53 patients and 36 deaths; arm B (DC vaccination), 53 patients and 39 deaths. P = 0.48 by log rank test. Two patients of the ITT population are not presented due to missing data; censored observations are indicated by vertical bars.

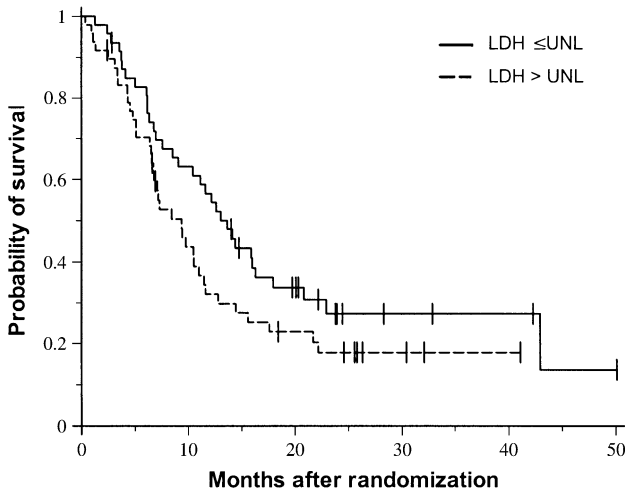

Figure 3. Kaplan–Meier curves showing the overall survival of the ITT population by serum LDH. LDH ≤upper normal limit (UNL), 47 patients and 33 deaths; LDH >UNL, 48 patients and 37 deaths. P = 0.105 by log rank test. Thirteen patients are not presented due to missing data.

displaying other HLA-haplotypes (P = 0.01; Figure 6A), while this difference could not be observed in DTIC-treated patients (P = 0.57; Figure 6B). This observation suggests an HLA-dependent induction of immunological effects in DC-treated patients. The survival advantage of HLA-A2+/B44− patients was puzzling given the high frequency of HLA-B44+ patients (30%) in the 10 patients responding to DC vaccination with PR (n = 2) or SD (n = 8). We therefore followed the individual fate of these responders and observed that all long-term survivors were HLA-B44−, while all deceased patients were HLA-B44+. Cox multivariate regression analysis revealed HLA A2+/B44−, Karnofsky performance status and AJCC M-category as independent prognostic factors for OS. Since no typing for HLA class-II was designated in the study protocol, a potential association of survival with these haplotypes could not be analysed.

We also investigated whether or not the DC preparation had a major influence on OR or OS. This was not the case as there were no apparent differences in the number or quality of DCs administered between responding and non-responding

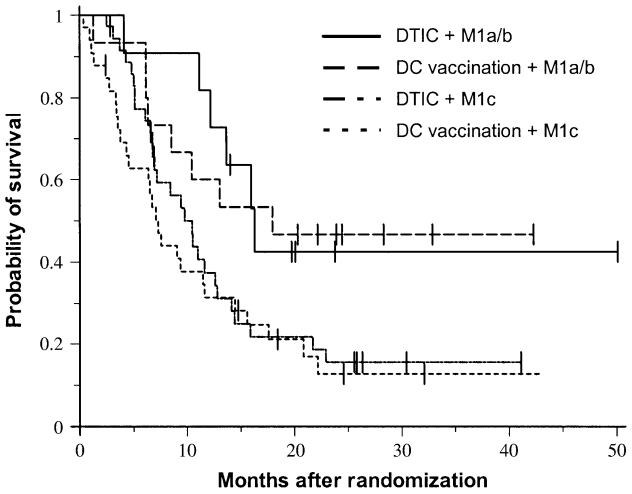

Figure 4. Kaplan–Meier curves showing the overall survival of the ITT population by treatment arm and AJCC M category. Arm A (DTIC) M1a/b, 11 patients and 6 deaths; arm A M1c, 36 patients and 28 deaths; arm B (DC vaccination) M1a/b, 15 patients and 8 deaths; arm B M1c, 33 patients and 28 deaths. $P = 0.019$ by log rank test. Thirteen patients are not presented due to missing data.

patients or short- and long-term survivors, respectively (data not shown). It should be noticed, that both the number of DC administered per peptide and, to a lesser extent, the maturation status of DC was lower than we had aimed at (2.8 ± 1.099 million instead of 4 million DC/peptide; mean CD83 expression 68.1 ± 14.7%; and only 49% of DC vaccines showed CD83 expression >75%). In addition, we observed a large inter- as well as intra-patient variability as well as variations between centers. However, these variations had no impact on treatment response or survival.

discussion

After the first interim analysis the study was prematurely closed based on recommendations of the external Data Monitoring & Safety Board (DMSB), because of an extremely low probability of reaching the planned study goals.

The OR of <6% in both arms was perplexingly low given that several-fold higher RR had been reported previously. The RR reported here are, however, in perfect agreement with very recent data obtained from the largest randomized trial ever performed in metastatic melanoma using DTIC ± Genasense [19], and are presumably reflective of the strict response criteria applied in this study. Albeit disappointing, the current clinical trial does provide several important clues and lessons for future studies. Such additional trials are crucial, as to date no vaccination strategy exists with proven clinical efficacy for any type of metastatic cancer [20].

We discovered that the repetitive generation of DC from frozen PBMC aliquots at multiple centers was variable and did not constantly yield an optimal vaccine quality. Importantly, we could only administer an average of 2.8 million instead of an intended 4 million DC per class-I peptide. This, together with the rather low and variable maturation status, is likely to have

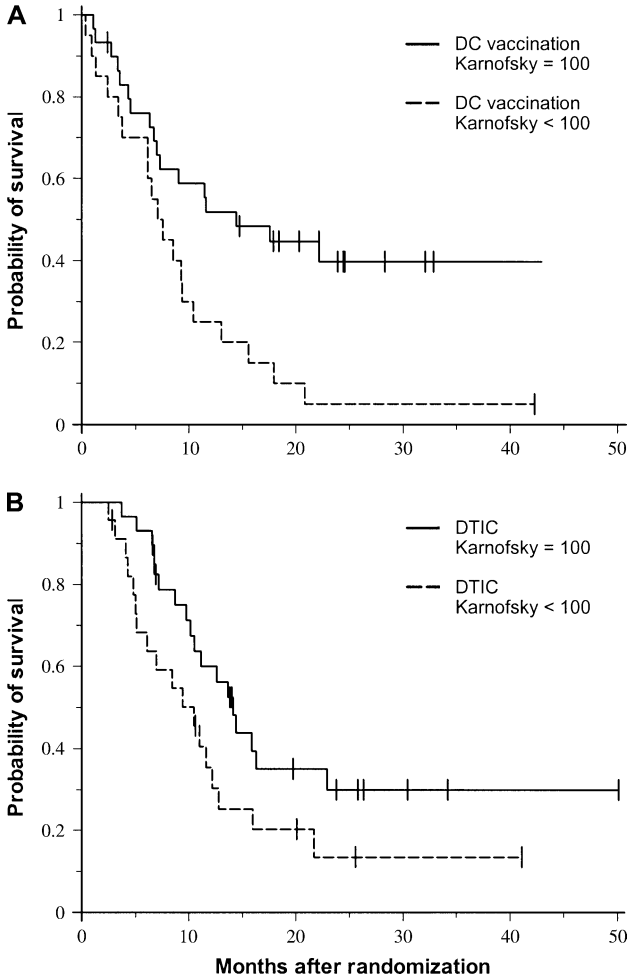

Figure 5. Kaplan–Meier curves for overall survival of the ITT population by treatment arm and performance index (Karnofsky) for the DC vaccination group (**A**) as well as for the DTIC group (**B**). DC vaccination + Karnofsky = 100, 30 patients and 18 deaths; DC vaccination + Karnofsky ≤100, 20 patients and 19 deaths; DTIC + Karnofsky = 100, 29 patients and 18 deaths; DTIC + Karnofsky ≤100, 23 patients and 18 deaths. $P = 0.007$ (**A**) and $P = 0.057$ (**B**), respectively, by log rank test. Six patients are not presented due to missing data.

limited the efficacy of the vaccine. Another drawback of the present study might have been the subcutaneous route, which was chosen to avoid too many intradermal injection sites. The subcutaneous administration is, however, now known to be much less effective than either the intradermal or intranodal route in delivering DC to the regional nodes [21, 22]. Finally, in late 1999 when the design of the study was finalized, we had decided to omit pulsing DC with KLH or tetanus toxoid, as these proteins, which had been included as a control for tolerance induction and potential unspecific help, had caused in part strong vaccination reactions in the initial trials [6, 7]. Data from a recent clinical trial suggest that the inclusion of such strong unspecific helper proteins enhances CTL generation, which is also in perfect accordance with recent animal experiments [23]. In retrospect, the omission of unspecific help might thus also have decreased the effectiveness of the DC vaccine used in this trial.

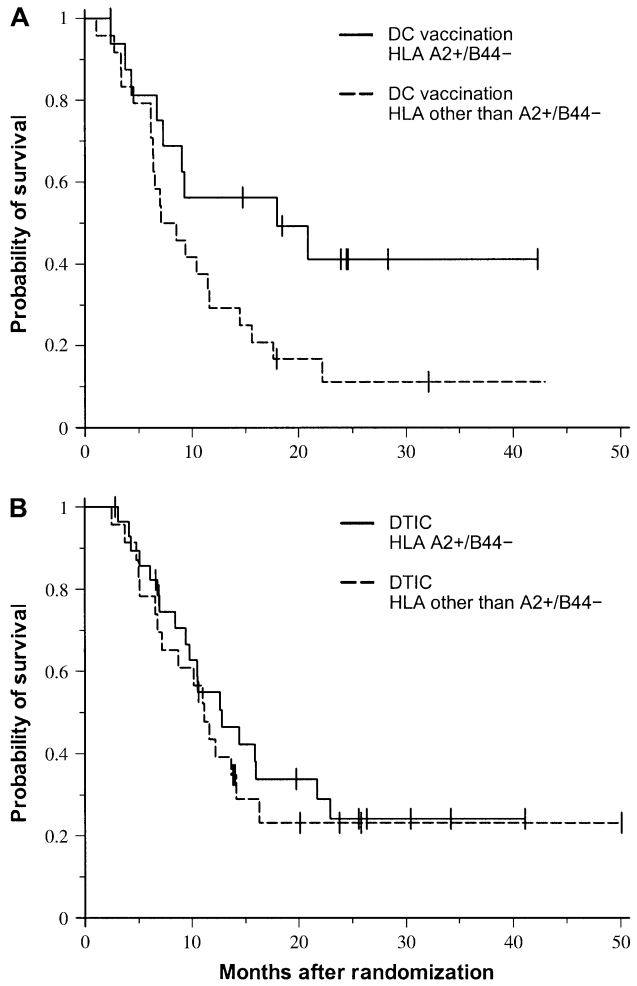

Figure 6. Kaplan–Meier curves showing the overall survival of the ITT population by treatment arm and HLA phenotype for the DC vaccination group (**A**) as well as for the DTIC group (**B**). DC vaccination + HLA A2+/B44−, 22 patients and 11 deaths; DC vaccination + HLA other than A2+/B44−, 31 patients and 28 deaths; DTIC + HLA A2+/B44−, 24 patients and 17 deaths; DTIC + HLA other than A2+/B44−, 29 patients and 19 deaths. $P = 0.01$ (**A**) and $P = 0.57$ (**B**), respectively, by log rank test. Two patients are not presented due to missing data.

In order to gain detailed information about the patients treated in the DC arm, we performed explorative post-hoc subgroup analyses, being well aware that sub-setting the already underpowered dataset further erodes the power to detect differences and can lead to spurious associations [24]. Based on these considerations, however, we detected significant differences between certain subgroups, which allow us to generate hypotheses to be tested in future clinical studies.

An expected finding was that patients in the prognostically favorable AJCC M1a/b category had better survival rates in both study arms. More interesting was the observation that an excellent overall performance status correlated with a significantly improved OS in the DC but not in the DTIC arm. This implies that immune responses might evolve and be more effective under good overall performance.

We subsequently analyzed whether or not the HLA haplotypes influenced clinical responses and subsequent long-term survival. The most relevant finding here was that the HLA-A2+/B44− subset of DC-treated patients survived significantly longer than patients carrying other haplotypes, while no such difference was observed in the DTIC arm. Moreover, we found that all initial responders, who were also long-term survivors, were HLA-B44− while all deceased patients were HLA-B44+. It may be that a gene locus in linkage disequilibrium with HLA-B44 impedes the effectiveness rather than the induction of immunity. It was less surprising to observe that the HLA-A2 haplotype was more frequent in non-progressors than in progressors (80% versus 52% respectively), as it has been reported previously that a beneficial adjuvant effect of an allogeneic tumor vaccine occurred preferably in vaccine patients expressing HLA-A2 [25].

Our findings are in concordance with recently published data showing an association of HLA-B44 with metastatic progression and an unfavorable clinical outcome in a cohort of 382 Italian melanoma patients [26]. However, we do not want to over-interpret these findings regarding the HLA association, particularly in view of the low numbers of patients amenable for analysis. Nevertheless, it is noteworthy that such effects were evident in the DC arm but not in the DTIC arm. We therefore suggest that HLA-haplotypes should be considered as parameters for patient stratification in future vaccination trials.

It has been a traditional deficit in the field of immunotherapy that clinical efficacy has not been put to a timely objective test in randomized trials, even if the therapy was shown to be promising in initial trials. This might have contributed to the fact that to date not a single vaccination approach exists with proven clinical efficacy for the treatment of metastatic cancer.

acknowledgements

Center Investigators: Thomas Berger, Eckhardt Kämpgen, Beatrice Schuler-Thurner, Gerold Schuler (Erlangen, Germany); Andrea Tüttenberg, Alexander Enk (Mainz, Germany); Christine Zimpfer-Rechner, Anette Zimpfer, Robert Figl, Udo Hofmann, Antje Sucker, Selma Ugurel, Dirk Schadendorf (Mannheim, Germany); Carmen Loquai, Stephan Grabbe (Münster, Germany); Kerstin Otto, Petra Keikavoussi, Eckhardt Kämpgen, Jürgen C. Becker, Eva-B. Bröcker (Würzburg, Germany); Tanja Maier, Jiri Kamarachev, Frank Nestle (Zürich, Switzerland).

This investigation was supported by a grant from the German Cancer Aid ('Deutsche Krebshilfe').

The authors wish to thank Robert Sarkany for proof-reading the manuscript.

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