

prior radiation as an independent factor associated with chromosome 3 loss (Damato B, et al, unpublished data, 2016); we plan to publish this study soon.

In conclusion, genetic analysis of choroidal melanoma by MLPA or MSA following completion of PBR distinguishes between disomy 3 and monosomy 3 tumors and produces results that are predictive of metastasis-free survival.

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Adjuvant Dendritic Cell Vaccination in High-Risk Uveal Melanoma



Uveal melanoma (UM) is the most common primary intraocular malignancy in adults with an annual incidence of 4 to 10 per million in the white population. The 5-year overall survival (OS) rate is approximately 70% to 80%. Up to 50% of patients with UM develop metastases, usually after a long disease-free interval (2–5 years). If metastatic disease is present, the prognosis is dismal with a 1-year OS rate of 10% to 40%. Currently, no effective systemic treatment improving OS is available for patients with metastatic UM, nor has any adjuvant treatment shown survival benefit.

Our research group and others have performed several prospective dendritic cell (DC) vaccination studies in patients with cutaneous melanoma showing little toxicity and promising immunologic and clinical results. Dendritic cells are antigen-presenting cells with the unique capacity to activate naïve antigen-specific T cells, and thus are suitable to induce antitumor immune responses. The tumor antigens gp100 and tyrosinase, used in our DC vaccination studies for patients with cutaneous melanoma, are both expressed in human UM tumor cells and thus constitute an appropriate target for immunotherapy in UM. We recently showed that DC vaccination is feasible in metastatic UM, and no safety concerns were detected. Furthermore, DC vaccination showed the potential to enhance the host's antitumor immunity and may be associated with longer than average OS in metastatic UM.¹ Dendritic cell vaccination may have a more pronounced effect in the adjuvant setting because high tumor burden in metastatic patients may hamper the induction of effective immune responses. Preferably, patients with a high risk for development of metastatic disease are selected for adjuvant treatment. In primary UM, monosomy 3 correlates strongly with the development of metastases and decreased survival (3-year OS rate 60% with monosomy 3 vs. 95%–100% with disomy 3) and is identified in approximately 50% of patients.²

Therefore, we performed an open-label phase II study in high-risk patients with UM with monosomy 3, investigating immunologic responses after adjuvant DC vaccination. Inclusion criteria included human leukocyte antigen (HLA)-A*02:01 positivity, interval since local treatment <12 months, and age 18 to 75 years. Patients with distant metastases were excluded. Patients received autologous, monocyte-derived DC transfected with mRNA encoding the tumor-antigens gp100 and tyrosinase according to a schedule of 3 biweekly intradermal and intravenous vaccinations. In the absence of disease recurrence, patients received a maximum of 2 maintenance cycles at 6-month intervals. Ethics Committee approval was obtained, and written informed consent was obtained from all patients (NCT00929019). Because of low accrual rates, mainly caused by the rarity of the tumor, older age at diagnosis, HLA restriction, and the increase of eye-conserving treatments interfering with the availability of tumor material for genetic testing, the trial was stopped prematurely. Still, 23 patients received at least 1 cycle of adjuvant DC vaccination and were considered evaluable; 18 patients completed all 3 cycles of vaccinations. Baseline characteristics are shown in Table 1 (available at www.aaojournal.org). Dendritic cell vaccinations were well tolerated. Side effects associated with DC vaccination were transient flu-like symptoms in 91% of patients and erythema at the site of injection in 87% of patients. Vitiligo occurred in 1 patient. No treatment-related grade 3 or 4 toxicity was observed.

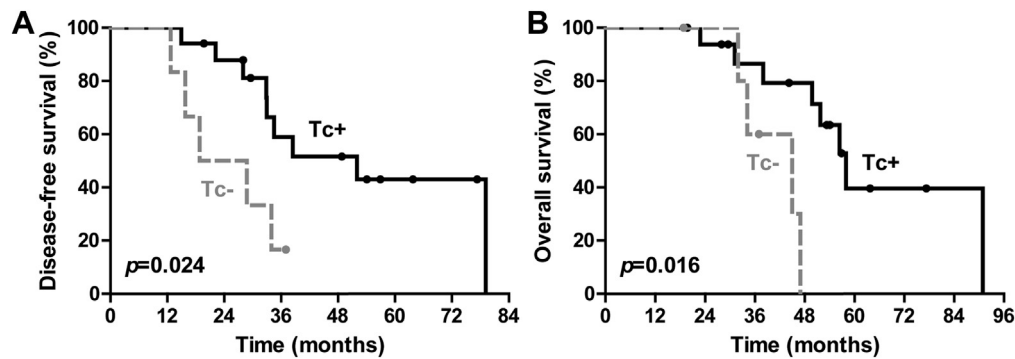


Figure 1. Survival in correlation with the presence of tumor antigen-specific T cells after adjuvant dendritic cell (DC) vaccination. Kaplan–Meier curves of disease-free survival (DFS) (A) and overall survival (OS) (B) for patients with high-risk uveal melanoma (UM) who received adjuvant DC vaccination after treatment of the primary tumor according to the presence (Tc+; $n = 17$; solid black line) or absence (Tc–; $n = 6$; dashed grey line) of tumor antigen-specific T cells in skin-test infiltrating lymphocytes. Survival was calculated from the treatment of the primary tumor. Statistical significance was determined by a log-rank test.

To test the capacity of the patients in this study to generate an immune response on vaccination, DCs were loaded with keyhole limpet hemocyanin, a control antigen. All patients tested showed a cellular response to keyhole limpet hemocyanin, indicating that the vaccine induced *de novo* immune responses. Previously, we showed that the presence of tumor-specific T cells in cultures of skin-test infiltrating lymphocytes positively correlated with the clinical outcome in patients with metastatic cutaneous melanoma. Therefore, skin tests were performed after each vaccination cycle, and the presence and functionality of tumor-specific T cells induced by DC vaccination were analyzed. Tumor-specific T cells in the skin tests were present in 17 patients (74%), demonstrating the effectiveness of these type of vaccines. Our previous findings in patients with metastatic UM showed a lower tumor-specific immunologic response rate, because only 29% of patients with metastatic UM showed tumor-specific CD8+ T cells after DC vaccination. Even if we only take the first skin test into account, the difference remains remarkable. We observed similar differences in the rates of patients with cutaneous melanoma in the metastatic and adjuvant setting.³ Therefore, the hypothesis that DC vaccination might be more potent in the adjuvant setting is further supported by this study.

Up to April 2016, 9 patients (39%) are free of melanoma relapse and 14 patients (61%) developed metastatic disease after DC vaccination, of whom 12 patients have died (52%). The median disease-free survival (DFS) was 34.5 months (95% confidence interval, 27.2–41.8), with a 3-year DFS rate of 47%. The median OS was 51.8 months (95% confidence interval, 42.1–62.7), with a 3-year OS rate of 79%. When patients were analyzed separately, on the basis of the presence or absence of tumor-specific T cells in the skin test, patients with tumor-specific T cells had better DFS and OS. No large differences were seen in their baseline characteristics (Table 1, available at www.aaojournal.org). In patients with tumor-specific T cells after DC vaccination, the median DFS was 51.9 versus 18.8 months in patients with whom we could not detect tumor-specific T cells ($P = 0.024$) (Fig 1A). Median OS was 45.0 months with a 3-year OS rate of 60% for patients without detectable tumor-specific T cells and 58.0 months and 87% for patients in whom tumor-specific T cells were found ($P = 0.016$) (Fig 1B).

By taking the restrictions of comparing results of small studies with historical data into account, the 3-year OS rate of the DC-vaccinated patients (79%) compared well with the literature

(~60% in high-risk UM).^{2,4} In theory, HLA-A*02:01 phenotype could be a confounding factor, but no correlation with survival is shown in a large cohort of patients with UM.⁵ Of course, a randomized trial is needed to provide a definitive conclusion on the effect of DC vaccination in high-risk patients with UM, which is currently opened elsewhere (NCT01983748).

Adjuvant treatment with DC vaccination in high-risk patients with UM gives little toxicity and correlates with favorable OS in patients with a detectable tumor antigen-specific immune response after DC vaccination. Further evidence for the clinical efficacy of adjuvant DC vaccination should be obtained from prospective randomized clinical trials.

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