Clinical Cancer Research

Long-Term Protective Effect of Mature DC-LAMP⁺ Dendritic Cell Accumulation in Sentinel Lymph Nodes Containing Micrometastatic Melanoma

Bruce Elliott, Richard A. Scolyer, Stefan Suciu, et al.

Updated Version	Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-07-0358

Cited Articles	This article cites 40 articles, 13 of which you can access for free at: http://clincancerres.aacrjournals.org/content/13/13/3825.full.html#ref-list-1
Citing Articles	This article has been cited by 4 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/13/13/3825.full.html#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.

Imaging, Diagnosis, Prognosis

Long-Term Protective Effect of Mature DC-LAMP+ Dendritic Cell Accumulation in Sentinel Lymph Nodes Containing Micrometastatic Melanoma

Bruce Elliott,[1,2] Richard A. Scolyer,[3] Stefan Suciu,[5] Serge Lebecque,[6] Donata Rimoldi,[7] Oliver Gugerli,[8] Elena Musat,[5] Raghwa N. Sharma,[3,4] Danielle Lienard,[8] Ulrich Keilholz,[9] Alessandro Testori,[10] Alexander Eggermont,[11] Rona MacKie,[12] Caroline Robert,[13] Martin Cook,[1] John F. Thompson,[3] Eric Angevin,[13] and Alain Spatz[13] on behalf of the European Organization for Research and Treatment of Cancer Melanoma Group

Abstract

Purpose: In a previous immunohistochemical study of dendritic cells (DC) in sentinel lymph nodes (SLN) draining regressing melanomas, we found that the accumulation of mature DC-LAMP+ DCs in SLNs was associated with local expansion of antigen-specific memory effector CTLs and the absence of metastasis in downstream lymph nodes. The aim of this study was to investigate the prognostic importance of the maximal density of mature DCs in SLNs.

Experimental Design: A total of 458 consecutive patients with micrometastatic melanoma within SLNs were eligible for analysis. The maximal density of mature DC-LAMP+ DCs was evaluated by three independent observers and categorized into three classes (<100, 100 to <200, and $\geq 200/mm^2$).

Results: There was excellent interobserver reproducibility for maximum density of mature DC-LAMP+ DC scores (κ score = 0.82). There were differences in the maximal density scores and staining intensity according to the treating melanoma center ($P < 0.001$). The higher the mature DC density in the SLN is, the longer is the duration of survival [$P = 0.047$; hazard ratio, 0.70; 95% confidence interval, 0.50-1.00]. Adjusted by thickness and ulceration, the prognostic importance of DC density was lower ($P = 0.36$).

Conclusion: This study is the first to report the prognostic value of DC-LAMP+ DC counts in SLNs containing metastatic melanoma. Patients with a high density of mature DCs ($\geq 200/mm^2$) have the lowest risk of death. It also provides evidence that a lack of maturation in the SLNs is important in biological facilitation of melanoma progression.

Dendritic cells (DC) are antigen-presenting cells (APC) whose function, location, and morphology are highly dependent on their degree of maturation (1–3). Mature DCs are the most potent APCs capable of priming tumor-specific T cells. It is generally postulated that following a cutaneous inflammatory reaction, epidermal Langherhans cells that represent immature DCs can migrate to regional lymph nodes where they mature further (4–6). Mature DCs express MHC class I and II, CD80, CD86, CD54, CD83, and CD208/DC-LAMP molecules. The mature DCs contribute to antigen presentation and T-cell costimulation, which results in the induction or restimulation of antigen-specific immune responses, including IFN-γ–producing T helper-1 (Th-1) responses that are desirable for antimetastatic responses (7, 8). Conversely, it has been recognized that Th-2 responses, which result in interleukin-4 (IL-4) and IL-10 production, and regulatory T-cell lymphocyte responses, characterized by CD4-positive/CD25-positive cells and Foxp3-expressing cells, can result in immune suppression (9–11).

Imaging, Diagnosis, Prognosis

DC-LAMP, the human lysosome-associated membrane, protein-3, was originally described as a molecule specifically expressed by mature dendritic cells (12, 13). Its expression is induced in the later stages of maturation. A definite function of DC-LAMP has not yet been established. DC-LAMP is most homologous to CD68, a lysosomal glycoprotein expressed by macrophages (12). Besides mature dendritic cells, DC-LAMP mRNA has been found in human and murine lungs and recently in pulmonary adenocarcinomas with Clara cell differentiation (13, 14).

The extent of SLN involvement by melanoma metastasis is one of the most powerful independent predictive factors for melanoma survival, and 30% to 40% of patients with SLN micrometastases will eventually die of their disease, compared with 5% to 10% of patients without SLN involvement (15). Melanoma SLNs are privileged sites of T-cell priming and cross-talk between cells of the immune system. In a previous study, we showed that the accumulation of DC-LAMP$^+$ mature DCs (mature DCs$^{DC-LAMP+}$) was clustered with tumor cells and lymphocytes and was associated with the local expansion of antigen-specific memory effector CTLs and spontaneous regression of melanoma (16). Furthermore, this study also showed that in SLNs containing micrometastatic melanoma, there was a significant correlation between the density of DC-LAMP$^+$ DC infiltrates in SLNs and the absence of metastasis in downstream nonsentinel lymph nodes (16).

In the current study, we investigated whether the accumulation of mature DCs$^{DC-LAMP+}$ in melanoma containing SLNs controlled melanoma progression and was an important prognostic variable. A population of 458 micrometastatic melanoma containing SLNs with detailed follow-up information was used to perform the study.

Patients and Methods

Patient population. This retrospective study included all patients with a diagnosis of a cutaneous melanoma who were evaluated and treated between 1992 and 2002 in one of the institutions participating in the European Organization for Research and Treatment of Cancer (EORTC) Melanoma Group Pathology Committee. The inclusion criteria were (*a*) histologic diagnosis of primary melanoma; (*b*) a sentinel node procedure was done in the participating institution; (*c*) the SLN pathologic examination was based, as a minimum standard, on the guidelines published by the EORTC Melanoma Group (protocol 1; ref. 17); (*d*) at least two unstained slides taken from sequential SLN slides set were available for the study; and (*e*) the patients either died or were followed up for at least 3 years after SLN excision.

The study was conducted in accordance with local regulations and with the approval of all relevant local institutional review boards. Patients were identified through institutional databases, which provided demographic, clinicopathologic, treatment, and outcome information. In addition, medical records were reviewed to obtain details regarding patient characteristics, surgical treatment, and chemotherapy.

A total of 458 patients were included in the first study phase that aimed to determine the immunostaining intercenter variability. These patients came from the following institutions: the Royal Prince Alfred Hospital (RPAH), Sydney, Australia (center 1, *n* = 219); Royal Surrey County Hospital, Guildford, United Kingdom (center 2, *n* = 73); Gustave-Roussy Institute, Villejuif, France (center 3, *n* = 50); Glasgow University, Glasgow, United Kingdom (center 4, *n* = 55); Erasmus Hospital, Rotterdam, the Netherlands (center 5, *n* = 37); and the European Instituto for Oncologia, Milan, Italy (center 6, *n* = 24). In total, 1,126 SLN sections corresponding to 537 SLNs were available.

On average, each patient had 1.13 (range, 1-3) micrometastatic SLN; all positive SLNs were available for the study. Only the patients from RPAH were included in the second study phase that aimed to study the impact on survival of mature DC$^{DC-LAMP+}$ maximum (max) density in the SLNs. Table 1 summarizes the characteristics of these patients.

Immunohistochemical staining. One slide per SLN section was pretreated in sodium citrate at 68°C (pH, 6) for 20 min. After a blocking step (Blocking agent, Ultra-tech HRP kit, Beckman-Coulter Immuno-tech), slides were incubated with the primary antibodies at 1:300 for anti–DC-LAMP monoclonal antibody (Dendritics) for 1 h at room temperature followed with the incubation of secondary antibodies and detection (Ultra-tech HRP kit). The slides were assessed by one observer (B.E.) without knowledge of the center nor the clinical information. As a quality assurance process, 10% of all the slides were randomly selected and reexamined independently by two other observers (D.R. and A.S.). Semiquantitative evaluation of mature DC$^{DC-LAMP+}$ maximum (mature DC max) density was made as follows: (*a*) the slide was examined at intermediate magnification (×100) to select the area containing the maximum number of DC-LAMP$^+$ DCs; (*b*) the number of DC-LAMP$^+$ cells per square millimeter was then determined at high magnification (×400) in 3 mm^2 in high-density areas. When a patient had more than one positive SLN, the mature DC max density was counted in each SLN, and the highest score was used for determining the prognostic significance. Results are reported as number of cells per square millimeter.

Statistical analysis. κ statistics were used for assessment of interobserver agreement of mature DC-LAMP$^+$ DC density scores with the statistical package, SPSS for Windows (version 11.0.1 [2001], SPSS, Inc.). This method includes correction for chance agreement. Interob-server reproducibility has been inferred in the published literature to be "very good" to "excellent" when the κ statistics exceed 0.80 (18). Distribution of DC maximum densities according to the center was tested by the Kruskall-Wallis test. Association between age, tumor thickness, number of excised SLNs, number of positive SLNs, and maximum cell densities were evaluated by the Pearson's test. Association between ulceration, Clark's level, sex, and maximum

Table 1. Characteristics of included patients

Variable	All patients (*N* = 219)
Age (y) at SLN excision	
Median	53
Range	5-81
Sex (%)	
Female	74 (33.8)
Male	145 (67.2)
Sentinel nodes basin (%)	
Axillary	105 (45.7)
Groin	89 (38.7)
Neck	33 (14.3)
Other	3 (1.3)
Number of positive/removed sentinel nodes	
Median	1/2
Range	0-4/1-8
Breslow thickness (%)	
<1.01 mm	7 (3.2)
1.01-2.00 mm	62 (28.3)
2.01-4.00 mm	91 (41.6)
>4.00 mm	59 (26.9)
Clark's level (%)	
III	40 (18.4)
IV	153 (70.1)
V	25 (11.5)
Ulceration (%)	
No	121 (55.3)
Yes	86 (39.3)
Not available	12 (5.5)

mature DC-LAMP$^+$ DC densities were tested by the Spearman's rank correlation test.

The overall survival time was defined as time from SLN biopsy until death, whatever the cause; the follow-up of patients still alive has been censored at their latest date of follow-up. The actuarial curves were computed using the Kaplan-Meier technique, and the SEs of the estimates were computed using the Greenwood formula. The prognostic value of mature DC$^{DC-LAMP+}$ (coded as 0, <100/mm^2; 1, 100 to <200/mm^2; 2, ≥200/mm^2) and Breslow thickness (coded as 0, ≤1; 1, 1-2; 2, 2.1-4; 3, > 4 mm), considered as ordered categorical variables, and of ulceration (presence versus absence) were assessed using the Cox's proportional hazards model. This model provides an estimate and the 95% confidence interval (95% CI) of the hazard ratio (HR) of the death intensity per time unit of a category versus the one of baseline category of the same variable. In the case of an ordered variable (DC$^{DC-LAMP+}$, Breslow thickness), a smooth estimate of the HR corresponding to one category versus the previous one was obtained. The same model was used for multivariate analysis to determine whether several variables were of independent prognostic importance. The Wald test was used to determine the prognostic importance of each variable included in the model. All analyses were done according to the intent-to-treat principle. The SAS 9.1 software (SAS Institute Inc.) was used for the survival analysis. All statistical tests were two sided.

Results

DC-LAMP+ DCs maximum density in melanoma SLNs varied according to center. The mean mature DCs max density in melanoma containing SLNs from each of the melanoma treatment centers is summarized in Table 2. The interobserver reproducibility for DC counting was excellent with a κ score of 0.82. However, qualitative assessment of the DC-LAMP–immunostained sections revealed important variations in staining intensity of mature DC-LAMP$^+$ DCs in SLNs from patients treated at different centers, leading to intercenter variation in positivity thresholds. This was also reflected in a significant difference between the mature DC$^{DC-LAMP+}$ maximal density counts ($P < 0.001$) from patients treated at different centers. The same effect was observed when mature DC$^{DC-LAMP+}$ mean instead of maximum densities were studied ($P < 0.001$). To avoid the effect of immunostaining variation on survival analyses between different centers, the RPAH population of 219 patients, representing 47.8% of the study population, was used for survival analysis.

DC-LAMP$^+$ DC maximum density correlates with melanoma ulceration but not with thickness. Table 3 summarizes the clinical and histopathologic variables according to DC count categories. The maximum mature DC$^{DC-LAMP+}$ density correlated significantly and inversely with ulceration of the primary melanoma ($P = 0.0005$). Ulcerated melanomas were associated with lower mature DC$^{DC-LAMP+}$ density in the SLNs. There was no significant correlation between Breslow's thickness and maximal DC density ($P = 0.26$).

DC-LAMP+ DCs maximum density in melanoma SLNs is associated with overall survival. Maximum mature DC densities were categorized in three groups: <100/mm^2 ($N = 30$), 100 to <200/mm^2 ($N = 89$), and ≥200/mm^2 ($N = 100$). These thresholds were based on a previous pilot study of 20 SLNs where it was found that the 100/mm^2 threshold was the most robust to categorize the DC densities counts in micrometastatic SLNs according to non-SLNs status (16). During the follow-up time, death was observed in 13 cases (43%) in category 1, 27 cases (30%) in category 2, and 21 cases (21%) in category 3.

Table 2. Distribution of mature dendritic cell maximal density

Center	Maximal density of mature DCs	n	SD	Median
1	157.19	219	60.90	153
2	184.95	73	77.27	190
3	214.75	50	66.21	221
4	169.44	55	66.02	151
5	134.21	37	31.05	128
6	170.13	24	48.41	166
Total	168.19	458	62.98	164

The estimated 5-year survival rates (SE, %) were 45.8% (10.8) in category 1, 56.4% (7.6) in category 2, and 61.5% (7.8) in category 3. Overall, there was a significant association between mature DC$^{DC-LAMP+}$ maximal density and survival ($P = 0.047$, Fig. 1). The higher the mature DC$^{DC-LAMP+}$ maximal density is, the lower is the risk of death rate: estimated HR was 0.70, and 95% CI was 0.50-1.00. The protector effect of mature DC accumulation (category 3) in the SLN seems to persist more than 10 years after diagnosis.

Univariate analysis indicated that Breslow's thickness (P = 0.029) and ulceration (presence versus absence: P = 0.002) were both of prognostic importance. Multivariate analysis, done on 207 patients with known ulceration status, including mature DC$^{DC-LAMP+}$ density, Breslow's thickness, and tumor ulceration, identified ulceration as the only independent prognostic factor (presence versus absence; $P = 0.02$), whereas Breslow's thickness was only borderline ($P = 0.07$), and mature DC$^{DC-LAMP+}$ density was not significant ($P = 0.36$). In the entire population ($n = 219$), when max DC mature DC$^{DC-LAMP}$ density and tumor thickness were included in the model, they were both of borderline significance (respectively, $P = 0.06$ and 0.07).

Discussion

Exploration of the immunologic status of SLNs, including DC maturation and T-cell responses, is one of the most promising issues in tumor immunology. We reported here the first evidence that high maximum density of mature dendritic cells expressing the DC-LAMP molecule in melanoma-draining SLN is associated with significant overall survival benefit. This benefit is maintained more than 10 years after SLN procedure in the category of highest maximal mature DC density. These results clearly indicate that the preservation of the maturation ability of DCs in SLNs containing melanoma is crucial for the long-term antimetastatic host immune response.

Previous studies have addressed DC status in SLNs in several types of cancers (16, 19–32). However, to the best of our knowledge, this study is the first to have correlated mature DCs in the SLN with overall survival. In melanomas, it was suggested that invasion of lymph nodes by tumor cells results in suppression of lymph node functions with alterations in immune cell ratio and activity (20, 25, 33). Cochran et al. reported that the paracortex of lymph nodes with melanoma metastases contained decreased numbers of DCs (33, 34) that were phenotypically less mature as compared with DCs in nonmetastatic lymph nodes (33). They observed that the SLNs were more affected than the non-SLNs and suggested that there may be a direct effect of tumor or tumor-associated factors on

DC maturation and function. They also showed that a higher frequency of DCs in SLNs was correlated with reduced likelihood of recurrence and death from melanoma (34). The marker they used in that study (S100) identifies DCs at different stages of maturation, including mature DCs. More recently, Lee et al. provided molecular evidence of cytokine-mediated SLN immunosuppression associated with the presence of melanoma cells and showed that SLN immunosuppression may be potentially reversed by cytokine therapy using granulocyte macrophage-colony stimulating factor (25). In breast carcinoma studies, Huang et al. compared S100-positive DCs in SLNs and non-SLNs and showed that the SLNs had reduced frequencies of S100-positive DCs with a predominance of immature DCs (35). Recently, Matsuura et al. (21) compared SLNs and non-SLNs in patients with breast carcinoma. They observed that metastasis-free SLNs contained significantly less mature DCs and a lower degree of Th-1 response than metastasis-free non-SLNs, suggesting that cellular immune responses are less active in the direct tumor-draining node than in distant nodes. They also observed that the metastasis-positive SLNs showed higher expression of CD83 by DCs than the metastasis-free SLNs, suggesting that once metastasis is established, DC maturation is triggered and is followed by the up-regulation of Th-1 responses. Gannon et al. (36) reported that the metastatic pelvic LN from prostate cancer patients contained decreased numbers of $CD20^+$, $CD38^+$, and $CD68^+$ cells compared with nonmetastatic LN. These studies all point to the development of an immunosuppressive microenvironment in the tumor-draining lymph nodes. However, they have only compared micrometastatic SLNs to either non-metastatic SLNs or metastatic non-SLNs. In contrast, we have observed that within the micrometastatic SLNs category, the degree of host response is highly variable with 15% of the SLNs containing very few mature DCs and 45% containing a high mature DCs density. Therefore, it is of utmost importance to study the clinical relevance of these variations between individuals.

Visual counting of DC-LAMP–positive DCs was associated in this study with excellent interobserver reproducibility. However, variability in staining intensity was noticed between participating centers, leading to intercenter variations in the positivity thresholds and to a significant center effect on the $DC^{DC-LAMP+}$ maximal density scores. The same center effect was observed when $DC^{DC-LAMP+}$ mean densities were studied ($P <$ 0.001). This might be explained either by a fixative effect and/ or by different protocols used among the six participating pathology departments to process the SLNs. For instance, at least three different fixatives were used to process SLNs samples. In a previous pilot study, we observed a strong variation in staining intensity in samples fixed with buffered formalin, AFA (acetic acid, formalin, alcohol), and Bouin liquid (data not shown). This illustrates that multicenter studies for biomarker developments are difficult to carry on due to uncontrolled parameters leading to intercenter variability.

The extent of SLN involvement is one of the most powerful independent predictive factor for melanoma survival, and 30%

Table 3. Clinical and histopathologic variables according to dendritic cell count categories

Variable	Maximum count (categories)			P
	<100 (N = 30)	100 to <200 (N = 89)	≥200 (N = 100)	
Age (y) at LND				
Median	55	54	53	NS
Range	17-81	17-80	5-79	
Sex, n (%)				
Female	11 (36.7)	28 (31.5)	35 (35.0)	
Male	19 (63.7)	61 (68.5)	65 (65.0)	NS
Number of removed SLNs				
Mean	1.97	2.06	2.18	NS
Number of positive SLNs				
Mean	1.23	1.24	1.14	NS
Sentinel node basin, n (%)				
Axillary	32 (42.1)	39 (52.0)	34 (43.0)	
Groin	33 (43.4)	27 (36.0)	29 (36.7)	
Neck	10 (13.1)	9 (12.0)	14 (17.7)	
Other	1 (1.4)	0 (0)	2 (2.6)	NS
Breslow thickness, n (%)				
<1.0 mm	0 (0)	3 (3.3)	4 (4.0)	
1.0-2.0 mm	6 (20.0)	30 (33.3)	26 (26.3)	
2.1-4.0 mm	14 (47.7)	32 (36.7)	45 (44.4)	
>4.0 mm	10 (33.3)	24 (26.7)	25 (25.3)	0.043
Clark's level, n (%)				
III	6 (20)	19 (21.3)	15 (15.0)	
IV	19 (63.3)	57 (64.0)	77 (77.0)	
V	5 (16.7)	13 (14.6)	7 (7.0)	NS
NA	0 (0)	0 (0)	1 (1.0)	
Ulceration, n (%)				
No	9 (30.0)	48 (53.9)	64 (64.0)	
Yes	18 (60.0)	34 (38.2)	34 (34.0)	0.0005
NA	3 (10.0)	7 (7.9)	2 (2.0)	

Abbreviation: NS, not significant.

Fig. 1. Survival curves according to the dendritic cells maximal count (<100, 100 to <200, and $\geq 200/mm^2$). The 5-y survival rates (SE %) were 45.8% (10.8) in category 1, 56.4% (7.6) in category 2, and 61.5% (7.8) in category 3. Overall, there was a significant association between mature $DC^{DC\text{-}LAMP+}$ maximal density and survival ($P = 0.047$). *N*, number of patients; *O*, observed number of deaths.
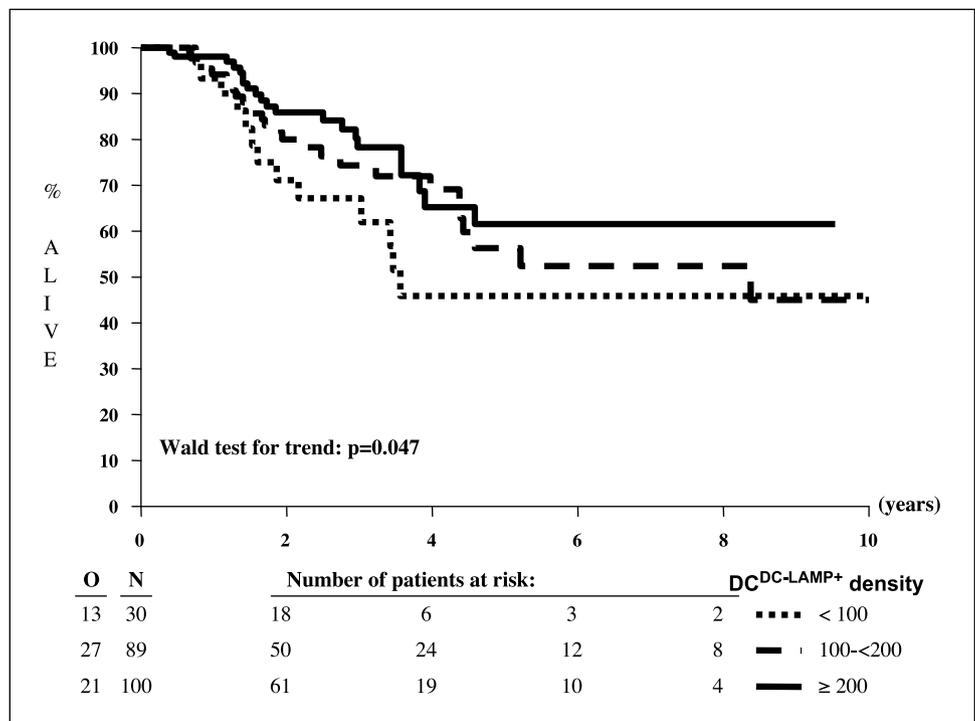
to 40% of patients with SLN micrometastasis will eventually die of their disease, compared with 5% to 10% of patients without SLN involvement (15). Moreover, SLNs draining cutaneous melanoma site are privileged sites of both T cell priming and initial metastasis, and therefore, evaluation of SLNs provides a unique opportunity to study the early phases of tumor-lymph node immune interaction. However, the respective roles of the biological characteristics of micrometastatic tumor cells and SLN immune response remain largely unknown. In a genomics study of primary melanomas, we found that primary melanomas with metastatic evolution were characterized by a dysregulation in key pathways driving replication, including the replication origins firing (ROF) system and *survivin* gene involved in chromatid separations (37, 38). We found no difference in expression signature between primary melanomas and metastases, illustrating once more that the genetic machinery for melanoma metastases evolution is acquired early in melanoma development.

Interestingly, in this study, we observed an inverse correlation between ulceration of the primary melanoma, a factor associated with worse prognosis, and mature $DCs^{DC\text{-}LAMP+}$ density in the draining SLNs. It is still unknown whether tumor ulceration can directly lead to a decreased DC response in the primary melanoma and, therefore, participates to the creation of an immunosuppressive environment, or whether ulceration is an associated phenotype in tumor cells that are poorly immunogenic. In several recent studies, including large population-based studies, it has been shown that ulceration is dependent on mitotic activity (39, 40). Therefore, there might be a relation between melanoma replication and further immune response. The interaction between the micrometastasis biology and immune response is of particular interest for the development of new treatment strategies that combine immunotherapy (e.g., with antiregulatory T cells) and targeted therapy.

In conclusion, this study shows that high maximum density of mature dendritic cells expressing DC-LAMP in melanoma-draining SLNs is associated with significant and prolonged overall survival benefit, reinforcing the notion that the immune system plays an active role in limiting the spread of melanoma. Ulceration of the primary melanoma is associated with low mature DC density in the draining SLNs. These results indicate that the preservation of DC maturation ability in the draining LN, an early event in the development of immune responses, is strongly associated with the establishment of long-term antimetastatic host response. The interactions between mature DCs and other immune cells, as well as the relationship between cellular responses and biological characteristics of micrometastases, are under investigation.

References

1. Germeau C, Ma W, Schiavetti F, et al. High frequency of antitumor T cells in the blood of melanoma patients before and after vaccination with tumor antigens. J Exp Med 2005;201:241–8.
2. Bancereau J. The long arm of the immune system. Sci Am 2002;287:52–9.
3. Bancereau J, Briere F, Caux C, et al. Immunobiol-ogy of dendritic cells. Annu Rev Immunol 2000;18: 767–811.
4. Geissmann F, Dieu-Nosjean MC, Dezutter C, et al. Accumulation of immature Langerhans cells in human lymph nodes draining chronically inflamed skin. J Exp Med 2002;196:417–30.
5. Jacobs JJ, Lehe CL, Hasegawa H, et al. Skin irritants and contact sensitizers induce Langerhans cell migration and maturation at irritant concentration. Exp Dermatol 2006;15:432–40.
6. Stoitzner P, Tripp CH, Eberhart A, et al. Langerhans cells cross-present antigen derived from skin. Proc Natl Acad Sci U S A 2006;103:7783–8.
7. Reinhardt RL, Hong S, Kang SJ, et al. Visualization of

IL-12/23p40 *in vivo* reveals immunostimulatory dendritic cell migrants that promote Th1 differentiation. J Immunol 2006;177:1618 – 27.

8. Schiller M, Metze D, Luger TA, et al. Immune response modifiers-mode of action. Exp Dermatol 2006;15:331 – 41.

9. Halak BK, Maguire HC, Jr., Lattime EC. Tumor-induced interleukin-10 inhibits type 1 immune responses directed at a tumor antigen as well as a non-tumor antigen present at the tumor site. Cancer Res 1999; 59:911 – 7.

10. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003;299:1057 – 61.

11. Sakaguchi S, Ono M, Setoguchi R, et al. Foxp3+ CD25$^+$ CD4$^+$ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev 2006;212:8 – 27.

12. de Saint-Vis B, Vincent J, Vandenabeele S, et al. A novel lysosome-associated membrane glycoprotein, DC-LAMP, induced upon DC maturation, is transiently expressed in MHC class II compartment. Immunity 1998;9:325 – 36.

13. Akasaki K, Nakamura N, Tsukui N, et al. Human dendritic cell lysosome-associated membrane protein expressed in lung type II pneumocytes. Arch Biochem Biophys 2004;425:147 – 57.

14. Zhu LC, Yim J, Chiriboga L, et al. DC-LAMP stains pulmonary adenocarcinoma with bronchiolar Clara cell differentiation. Hum Pathol 2007;38:260 – 8.

15. Balch CM, Buzaid AC, Soong SJ, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol 2001; 19:3635 – 48.

16. Movassagh M, Spatz A, Davoust J, et al. Selective accumulation of mature DC-LAMP+ dendritic cells in tumor sites is associated with efficient T-cell – mediated antitumor response and control of metastatic dissemination in melanoma. Cancer Res 2004; 64:2192 – 8.

17. Cook MG, Green MA, Anderson B, et al. The development of optimal pathological assessment of sentinel lymph nodes for melanoma. J Pathol 2003; 200:314 – 9.

18. Sepucha KR, Belkora JK, Tripathy D, et al. Building bridges between physicians and patients: results of a pilot study examining new tools for collaborative decision making in breast cancer. J Clin Oncol 2000;18: 1230 – 8.

19. Essner R. Experimental frontiers for clinical applications: novel approaches to understanding mechanisms of lymph node metastases in melanoma. Cancer Metastasis Rev 2006;25:257 – 67.

20. Shu S, Cochran AJ, Huang RR, et al. Immune responses in the draining lymph nodes against cancer: implications for immunotherapy. Cancer Metastasis Rev 2006;25:233 – 42.

21. Matsuura K, Yamaguchi Y, Ueno H, et al. Maturation of dendritic cells and T-cell responses in sentinel lymph nodes from patients with breast carcinoma. Cancer 2006;106:1227 – 36.

22. Straten P, Dahl C, Schrama D, et al. Identification of identical TCRs in primary melanoma lesions and tumor free corresponding sentinel lymph nodes. Cancer Immunol Immunother 2006;55:495 – 502.

23. Molenkamp BG, Vuylsteke RJ, van Leeuwen PA, et al. Matched skin and sentinel lymph node samples of melanoma patients reveal exclusive migration of mature dendritic cells. Am J Pathol 2005;167:1301 – 7.

24. Kohrt HE, Nouri N, Nowels K, et al. Profile of immune cells in axillary lymph nodes predicts disease-free survival in breast cancer. PLoS Med 2005;2: e284.

25. Lee JH, Torisu-Itakara H, Cochran AJ, et al. Quantitative analysis of melanoma-induced cytokine-mediated immunosuppression in melanoma sentinel nodes. Clin Cancer Res 2005;11:107 – 12.

26. Sakakura K, Chikamatsu K, Sakurai T, et al. Infiltration of dendritic cells and NK cells into the sentinel lymph node in oral cavity cancer. Oral Oncol 2005; 41:89 – 96.

27. Poindexter NJ, Sahin A, Hunt KK, et al. Analysis of dendritic cells in tumor-free and tumor-containing sentinel lymph nodes from patients with breast cancer. Breast Cancer Res 2004;6:R408 – 15.

28. Orui H, Ishikawa A, Okada K, et al. Dendritic cell and effector cell infiltration in soft tissue sarcomas with reactive lymphoid hyperplasia. J Orthop Sci 2003;8: 669 – 77.

29. Ishigami S, Natsugoe S, Uenosono Y, et al. Infiltration of antitumor immunocytes into the sentinel node in gastric cancer. J Gastrointest Surg 2003; 7:735 – 9.

30. Essner R, Kojima M. Dendritic cell function in sentinel nodes. Oncology (Huntingt) 2002;16:27 – 31.

31. Cochran AJ, Morton DL, Stern S, et al. Sentinel lymph nodes show profound downregulation of antigen-presenting cells of the paracortex: implications for tumor biology and treatment. Mod Pathol 2001;14:604 – 8.

32. Vermaelen KY, Carro-Muino I, Lambrecht BN, et al. Specific migratory dendritic cells rapidly transport antigen from the airways to the thoracic lymph nodes. J Exp Med 2001;193:51 – 60.

33. Cochran AJ, Pihl E, Wen DR, et al. Zoned immune suppression of lymph nodes draining malignant melanoma: histologic and immunohistologic studies. J Natl Cancer Inst 1987;78:399 – 405.

34. Cochran AJ, Wen DR, Huang RR, et al. Prediction of metastatic melanoma in nonsentinel nodes and clinical outcome based on the primary melanoma and the sentinel lymph node. Mod Pathol 2004;17:747 – 55.

35. Huang RR, Wen DR, Guo J, et al. Selective modulation of paracortical dendritic cells and T-lymphocytes in breast cancer sentinel lymph nodes. Breast J 2000; 6:225 – 32.

36. Gannon PO, Alam Fahmy M, Begin LR, et al. Presence of prostate cancer metastasis correlates with lower lymph node reactivity. Prostate 2006;66: 1710 – 20.

37. Winnepenninckx V, Debiec-Rychter M, Belien JA, et al. Expression and possible role of hPTTG1/securin in cutaneous malignant melanoma. Mod Pathol 2006; 19:1170 – 80.

38. Winnepenninckx V, Lazar V, Michiels S, et al. Gene expression profiling of primary cutaneous melanoma and clinical outcome. J Natl Cancer Inst 2006;98: 472 – 82.

39. Barnhill RL, Katzen J, Spatz A, et al. The importance of mitotic rate as a prognostic factor for localized cutaneous melanoma. J Cutan Pathol 2005;32: 268 – 73.

40. Berwick M, Armstrong BK, Ben-Porat L, et al. Sun exposure and mortality from melanoma. J Natl Cancer Inst 2005;97:195 – 9.