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Original Article

Contrary effect of two types of spatial distribution (diffuse *versus* marginal) of CD8-positive lymphocytes on clinical outcome in melanoma patients

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Abstract: Malignant melanoma is one of the most immunogenic human neoplasms; immune response of the host impedes disease development, especially at its early phase. Immunohistochemical staining was used to visualize $CD4^+$ and $CD8^+$ T cells and to examine the prognostic significance of their proportion and spatial distribution within 68 melanoma primary tumors with non-brisk and brisk tumor-infiltrating lymphocytes (TILs). We revealed that a high ratio of $CD8^+$ cells among TILs and their diffuse distribution were associated with longer melanoma-specific survival. Moreover, diffuse localization of $CD8^+$ lymphocytes correlated with a significantly longer disease-free survival. Neither the percentage nor spatial distribution of $CD4^+$ TILs influenced patient survival. Our results underscore the role of TILs in melanoma pathobiology. We demonstrated that the clinical outcome in skin melanoma patients is related to the phenotypic composition of TILs and their spatial relation to the tumor mass.

Keywords: Malignant melanoma, prognosis, lymphocyte distribution, $CD8^+$ lymphocytes

Introduction

Malignant melanoma is one of the most immunogenic human neoplasms; immune response of the host impedes disease development, especially at its early phase. The presence of lymphocytes among cancer cells, termed tumor-infiltrating lymphocytes (TILs) in 1975 by Mihm and associates, is a morphologic sign of this response [1]. Immune cells within melanoma tumors include a wide array of cytotoxic, helper and regulatory T lymphocytes, natural killer cells, B lymphocytes, dendritic cells, macrophages, granulocytes and mast cells. In common belief a heterogeneous group of cytotoxic lymphocytes, comprising Tαβ $CD8^+$ cells, some of Tαβ $CD4^+$ cells, Tγδ lymphocytes, NKT and NK cells, is the main weapon of the immune system against malignant melanocytes.

Existing possibilities for patients with advanced-stage disease present many drawbacks, namely toxicity, rapidly emerging resistance to classical chemotherapeutics and new drugs e.g. BRAF inhibitors, restricted application of the latter, and only modest clinical benefit. Therefore, modulation of antitumor immune response seems to be a promising option. One of the key mechanisms of novel strategies is to enhance T cell-mediated immunity. Scientists pin great hopes on adoptive cell transfer techniques: reinfusion of previously harvested autologous TILs, expanded and conditioned *in vitro*, demonstrated remarkable effects in multiple clinical trials in advanced-stage melanoma patients [2, 3]. Another approach to reinforcing patient's immunity against cancer are various types of anti-melanoma vaccines. Administration of melanoma-related peptides and cell lysates in combination with adjuvants and immunomodulators was beneficial in some experiments [4]. Moreover, TILs are affected by some of the therapies that are already approved for clinical use. In an experiment by Wilmott et al., selective inhibitors of mutant BRAF induced pronounced

Table 1. Correlations between percentage and spatial distribution of $CD8^+$ T cells, and clinical parameters

Clinical parameters	$CD8^+$ lymphocytes					
	Percentage of TILs			Spatial distribution		
	<70%	≥70%	*p* value	Marginal	Diffuse	*p* value
Age in years (28-79)[a] mean, 60±13.1; median, 60.5			0.288			0.391
Gender[b]						
Female	19	18	0.013	17	20	0.532
Male	6	25		11	20	
Primary tumor location[c]						
Head/neck	5	3	0.219	4	4	0.491
Extremities	13	19		11	21	
Hand/foot	0	1		1	0	
Trunk	7	20		12	15	
Primary tumor (pT)[a]						
pT1	9	18	0.052	7	20	0.040
pT2	3	10		4	9	
pT3	9	4		9	4	
pT4	4	11		8	7	
Regional lymph nodes status (pN)[b]						
No metastases (pN-)	20	38	0.559	21	37	0.097
Metastases present (pN+)	5	5		7	3	
Distant metastases (pM)[b]						
No metastases (pM-)	23	43	0.255	26	40	0.324
Metastases present (pM+)	2	0		2	0	
Sentinel lymph node biopsy status (SNLB)[b] (40 patients)						
No metastases (SNLB-)	9	25	0.499	9	25	0.143
Metastases present (SNLB+)	3	3		4	2	
Recurrence[b]						
No	23	40	1.000	24	39	0.174
Yes	2	3		4	1	

[a]*p* value of Wilcoxon two sample test; [b]*p* value of Fisher's exact test; [c]*p* value of chi^2 test; Statistically significant results ($P<0.05$) are in bold text.

infiltration of melanomas by $CD4^+$ and $CD8^+$ lymphocytes [5]. However, clinical parameters remained unaffected by the observed increase in TIL rates [5]. Similarly, Frederic and colleagues reported augmented tumor infiltration by $CD8^+$, but not $CD4^+$ cells, due to treatment with BRAF inhibition [6]. Treatments with interferon alfa2b, PD-1 and CTLA-4 blocking antibodies, and interleukin 12 were also associated with modulation of TIL infiltrates [7-10].

While a nonredundant role of TILs in melanoma pathology is generally recognized, discrepancies exist with regard to the significance of particular lymphocyte phenotypes and especially their differential localization patterns remain elusive. The utility of particular TIL categorization (diffuse *vs* marginal) applied in this study has not been previously systematically analyzed. We used immunohistochemical staining to visualize $CD4^+$ and $CD8^+$ T cells and examined the prognostic significance of their proportion and spatial distribution within melanoma primary tumors with non-brisk and brisk TILs.

Material and methods

Patients

The study group consisted of 68 patients with cutaneous melanoma with non-brisk and brisk TILs diagnosed between 2005 and 2010 and treated in the Lower Silesian Oncology Center in Wroclaw, Poland. The group was selected on

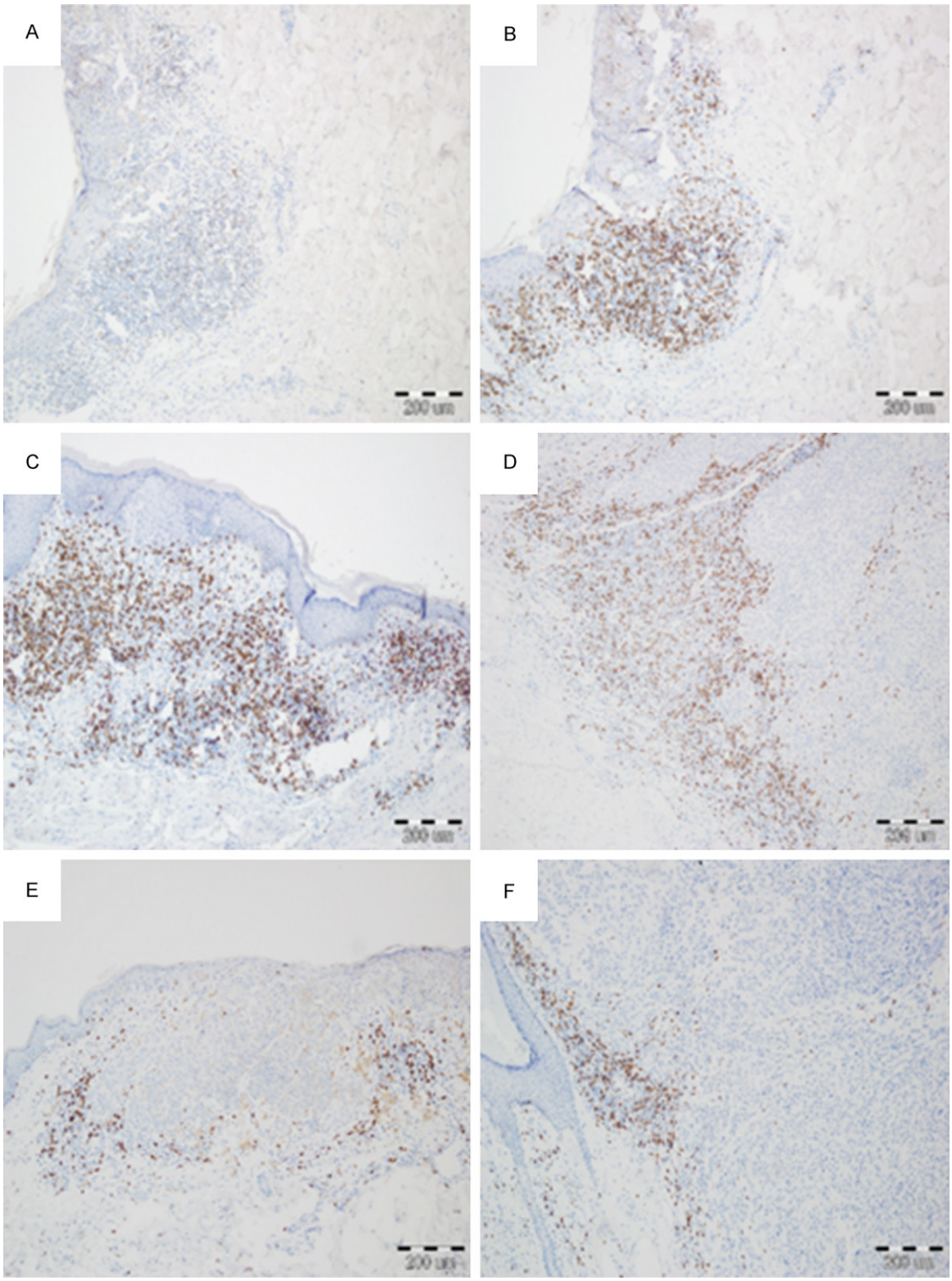

Figure 1. Immunohistochemically visualized expression of CD4 and CD8 in cutaneous melanoma. Lack of $CD4^+$ lymphocytes in invasive melanoma (A, 100×, hematoxylin); high percentage of $CD8^+$ lymphocytes with diffuse pattern of spatial distribution (B and C, 100×, hematoxylin); high percentage of $CD8^+$ lymphocytes with marginal spatial distribution (D, 100×, hematoxylin); low marginal distribution of $CD8^+$ observed only at the base of tumor (E and F, 100×, hematoxylin).

Table 2. Correlations between percentage and spatial distribution of $CD8^+$ T cells, and pathological parameters

Histopathological parameters	$CD8^+$ lymphocytes					
	Percentage of TILs			Spatial distribution		
	<70%	≥70%	*p* value	Marginal	Diffuse	*p* value
Breslow thickness[a]						
≤1 mm	9	18	0.017	7	20	0.018
1.01-2.00 mm	2	10		3	9	
2.01-4.00 mm	10	4		10	4	
>4 mm	4	11		8	7	
Clark level[a]						
I	-	-		-	-	
II	3	10		4	9	
III	10	21	0.372	8	23	0.014
IV	10	10		14	6	
V	2	2		2	2	
Histologic type[b]			0.730			0.096
Superficial spreading melanoma (SSM)	19	31		17	33	
Nodular malignant melanoma (NMM)	6	11		10	7	
Acral-lentiginous melanoma (ALM)	0	1		1	0	
Mitotic rate[a]						
0	11	22	0.204	8	25	0.002
≥1	14	21		20	15	
Ulceration[c]						
No	10	28	0.078	9	29	0.002
Yes	15	15		19	11	
TILs[c]						
Non-brisk	11	16	0.768	16	11	0.027
Brisk	14	25		12	29	
Microsatellitosis[c]						
No	23	42	0.627	25	40	0.129
Yes	2	1		3	0	
Lymphatic invasion[c]						
No	15	37	0.032	16	36	0.004
Yes	10	6		12	4	
Tumor regression[c]						
No	22	39	1.000	25	36	1.000
Yes	3	4		3	4	

[a]*p* value of Wilcoxon two sample test; [b]*p* value of chi^2 test; [c]*p* value of Fisher's exact test; Statistically significant results ($P<0.05$) are in bold text.

the basis of the availability of tissue material (paraffin blocks and histopathology slides) and medical documentation. Comprehensive clinical data were obtained from archival medical records. The diagnostic and therapeutic procedures utilized were determined based on the medical records held by the oncology outpatient clinic of the Lower Silesian Oncology Center and the data provided by the Lower Silesian Cancer Registry and Civil Register Office. The study was approved by the ethical committee of the Wroclaw Medical University, Poland.

The clinicopathological profile of patients included the following parameters: age and gender, primary tumor location, tumor stratification according to American Joint Committee

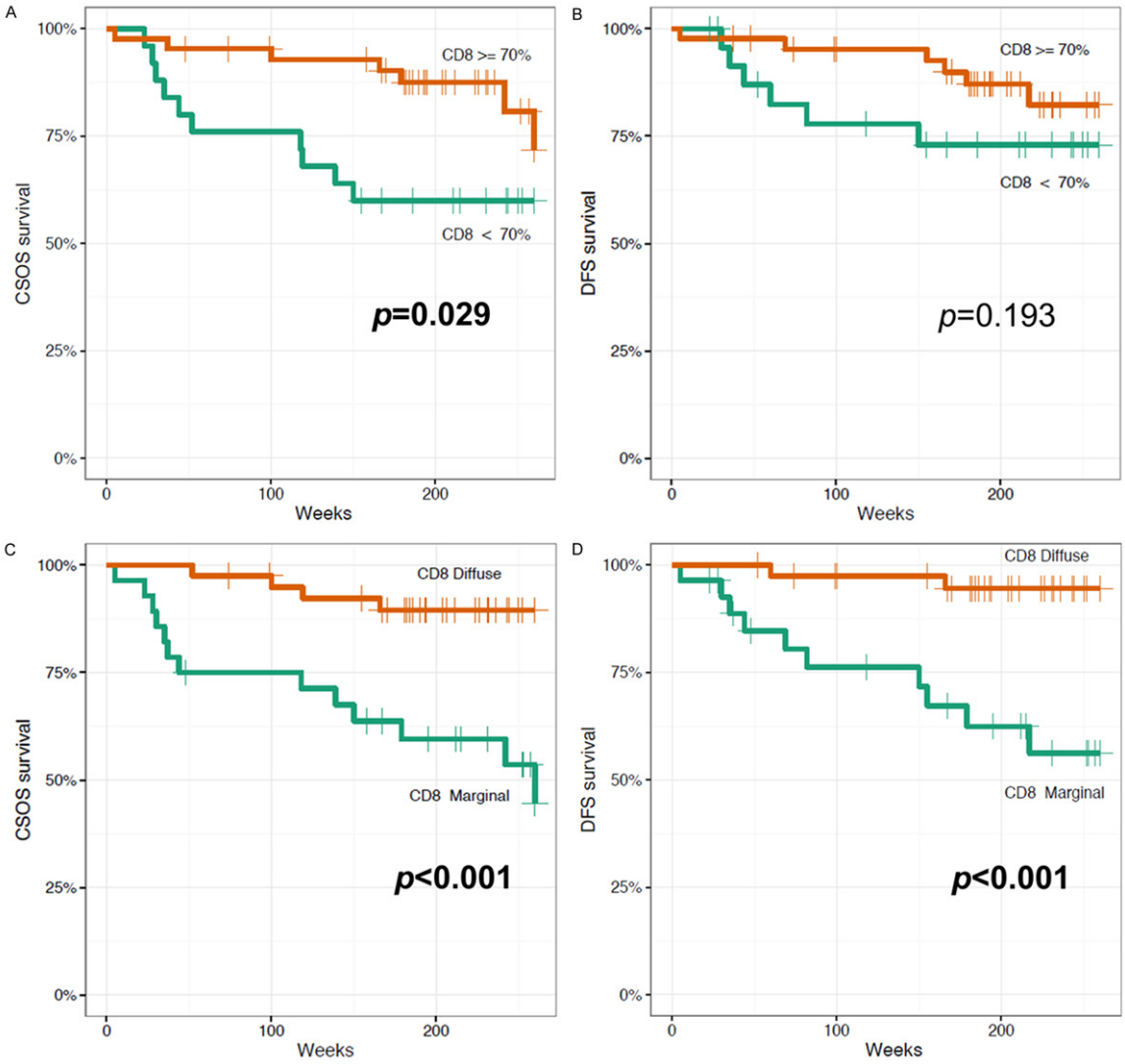

Figure 2. Kaplan-Meier analysis of the prognostic impact of $CD8^+$ lymphocytes and their spatial distribution in cutaneous melanoma patients. High percentage of $CD8^+$ lymphocytes was associated with longer cancer-specific overall survival (CSOS) (A); however, proportion of $CD8^+$ TILs had no impact on disease-free survival (DFS) (B); spatial location of $CD8^+$ cells also demonstrated prognostic significance-diffuse type of distribution strongly correlated with longer CSOS and DFS (C and D, respectively).

on Cancer (pT), presence or absence of nodal (pN) and distant (pM) metastases, information on disease recurrence and sentinel lymph node biopsy (SLNB) procedures (**Table 1**).

Tumor samples and histopathological evaluation

Tumor specimens were fixed in 10% buffered formalin and embedded in paraffin. All haematoxylin and eosin stained sections were examined by two pathologists. The parameters of the primary tumor recorded in pathology reports were Breslow thickness, Clark level, growth phase, histologic type, mitotic rate (number of mitotic figures per 1 mm^2), presence of ulceration, lymphangio-invasion, microsatellitosis, intensity of lymphocytic inflammatory infiltrate (TILs, tumor-infiltrating lymphocytes) and microscopic evidence of regression (**Table 1**).

TILs were assessed in a semiquantitative way as defined below. Absence of TILs: there are no lymphocytes present or lymphocytes are present but they do not infiltrate tumor at all. Non-brisk TILs: lymphocytes infiltrate melanoma

only focally or not along the entire base of the vertical growth phase. Brisk TILs: lymphocytes diffusely infiltrate the entire base of the vertical growth phase or the entire invasive component of the melanoma.

Immunohistochemistry

CD8 and CD4 immunoreactivity was analyzed in 68 cutaneous melanoma patients with non-brisk and brisk TILs. Immunohistochemical determination of CD8 expression (clone C8/144B IR623, DAKO; Glostrup, Denmark) and CD4 expression (clone 4B12 IR649, DAKO; Glostrup, Denmark) was performed on 4 μm-thick paraffin sections mounted on sialinized slides (DAKO, code number S 3003), which were then subjected to deparaffinization, rehydration and heat induced epitope unmasking performed using PT Link, with EnVision™ Target Retrieval Solution used for 20-40 minute incubation at 97°C. Autostainer Link was used to perform immunological test using detection reagents Dako EnVision™ FLEX/HRP (SM802).

Evaluation of immunohistochemistry

The percentage of $CD4^+$ and $CD8^+$ T lymphocytes was counted in 5 microscopic fields per each section (slide) at total magnification of ×400, independently by two histopathologists. Additionally, two categories of spatial distribution were identified for each of the analyzed antigens, namely diffuse with lymphocytes both along the front of melanoma invasion and interspersed within the tumor mass, and marginal with lymphocytes localized only at the base of tumor. Only positive immunohistochemical signals with clear lymphocyte morphology were evaluated (**Figure 1**).

Statistical analysis

All statistical analysis was performed using the R language [http://www.R-project.org]. Continuous variables like age or proportions of lymphocytes were summarized with the use of the mean, median, min and max values. Expression of TILs was dichotomized into two levels: lower than 70% vs. larger or equal 70%. For cancer-specific overall survival (CSOS) and disease-free survival (DFS) we performed log-tests and Kaplan-Meier curves, all such analyses were conducted with the survival package for R. To assess the relation between dichotomized TILs expression and continuous variables, the Wilcoxon two sample test was used. The relation of TILs and binary variable was assessed by exact Fisher test while with other categorical variables were assessed by chi-square test. All relations were summarized by a suitable *p*-value, and all *p*-values smaller than 0.05 were considered as significant.

Results

TIL subpopulations of $CD4^+$ and $CD8^+$ lymphocytes

Analysis of immunoreactivity for CD4 and CD8 surface molecules demonstrated a marked domination of $CD8^+$ lymphocytes in analyzed tumors. Mean proportion of $CD8^+$ lymphocytes was 69% (median: 70%, min.: 30% max.: 95%) and of $CD4^+$ cells was 11% (median: 5%, min.: 0%, max.: 50%). No $CD4^+$ lymphocytes were detected in 15 (9 with TILs: brisk and 6 with TILs: non-brisk) out of 68 cases (22%) whereas $CD8^+$ lymphocytes were present in every analyzed tumor.

Different patterns of spatial distribution of TILs were observed: diffuse (lymphocytes both along the front of melanoma invasion and interspersed within the tumor mass) and marginal (only at the base of tumor). Distribution of $CD8^+$ cells was predominantly diffuse (40/68; 58.8%). Conversely, $CD4^+$ cells were located marginally in most cases (44/53; 83% of tumors containing $CD4^+$ lymphocytes).

Analysis of correlations between proportions and spatial distribution of T cell subpopulations, and clinicopathological parameters

High percentage of $CD8^+$ lymphocytes was statistically linked with male gender, thinner tumors and lack of lymphatic invasion (**Tables 1**, **2**).

Tumors with diffuse pattern of $CD8^+$ cell distribution were thinner and presented lower mitotic activity. Marginal location of $CD8^+$ cells correlated with ulceration and lymphatic invasion within the primary tumor. High intensity (brisk) TIL infiltration was observed more often in melanomas with diffuse pattern of $CD8^+$ distribution (**Table 2**).

We found no association between the proportion and distribution patterns of $CD4^+$ cells, and analyzed clinicopathological parameters (data not shown).

Characteristics of lymphocytic infiltrate within the primary tumor and patient survival-Kaplan-Meier analysis

We revealed that high percentage of $CD8^+$ lymphocytes was associated with longer CSOS, but had no impact on DFS (**Figure 2A**, **2B**). Analogously, a positive relationship between the proportion of $CD8^+$ TILs and CSOS, but not DFS, was observed in patients without nodal metastases (data not shown).

Spatial location of $CD8^+$ cells also demonstrated prognostic significance-diffuse type of distribution strongly correlated to longer CSOS and DFS (**Figure 2C**, **2D**).

Patient prognosis appeared independent of the proportion and distribution patterns of $CD4^+$ cells (data not shown).

Discussion

In this study we reported that a high ratio of $CD8^+$ cells among TILs and their diffuse distribution are associated with a favorable prognosis in patients with melanoma of the skin. Marginal localization of $CD8^+$ lymphocytes correlated with a significantly shorter melanoma-specific survival. Neither the percentage nor spatial distribution of $CD4^+$ TILs influenced patient survival.

The topic of local immune response in melanoma has been investigated for many years [1], but data referring to TIL localization patterns (diffuse *versus* marginal) applied in our study are scarce. Hansen and McCarten analyzed differences in patient survival in relation to the presence or absence of lymphocytic infiltration at the base of the lesion [11]. They revealed better outcome in the former group, but diffuse TILs were not considered [11]. Ladanyi et al. reported a survival advantage related to a high density of activated (CD25 and OX40 positive) peritumoral, and not intratumoral T lymphocytes [12]. McGovern reported that TILs localized predominantly at the base of a tumor nodule associate with better survival [1]. In our opinion, peritumoral/marginal location of TILs may rather be an indication of weakness or fading of local immunity against melanoma. This type of TIL infiltrate, besides correlation with worse survival (compared to patients with diffuse TILs), characterized tumors with aggressive histology, i.e. thick, proliferating melanomas with lymphatic invasion and superficial ulceration (**Table 2**). Hence, it seems possible that marginal TIL pattern reflects inability of lymphocytic response to keep up with high mitotic rate of the tumor bulk. Acquired mechanisms of immune escape-another factor potentially involved-might contribute to limiting intratumoral/diffuse gathering of TILs. Expression of certain proteins by melanocytes, e.g. Fas (Apo-1/CD95) ligand [13, 14] or PD-L1 [15], may induce apoptosis in adjacent lymphocytes. Indoleamine 2, 3-dioxygenase [16] and matrix metalloproteinase-23 [17] diminish accumulation of TILs by impairing their activation and possibly a proliferation arrest.

In their ultrastructural analysis, Dvorak et al. noticed that immunologic reaction to melanoma resembles human delayed hypersensitivity responses like allergic contact dermatitis and allograft skin rejection [18]. The researchers demonstrated robust activation of lymphocytes and macrophages, influx and partial degranulation of mast cells, and marked changes in microvasculature (swelling and focal necrosis of endothelial cells, thickening of basal lamina) [18]. TILs maintained intimate contact with individual melanoma cells-viable and dead-often wrapping them inside rosette structures [18]. This observation of lymphocytic satellitosis endorses the authors' opinion that effective immune response against melanoma largely depends on direct interactions between lymphocytes and malignant melanocytes. Another support comes from early experiments with vaccinations, in which an abscopal effect and pyknosis of melanocytes were associated with direct contact between neoplastic and mononuclear immune cells [1].

It may come as a surprise that $CD4^+$ lymphocytes (their proportion among TILs and distribution amid melanoma cells) seem irrelevant for patients' prognosis. We suspect that the heterogeneity of cells expressing CD4 might be a key for the interpretation of this finding. Classical types of T helper cells, named Th1 and Th2 and both expressing CD4, have a radically different activity [19]. The former secrete IFN-γ, IL-2, TNF-β, and drive cell-mediated

responses, while the latter produce IL-4, IL-10, IL-13, and participate in humoral defense and allergies [19]. Differential significance of these subtypes has been observed in melanoma [20]. CD4+CD25+FOXP3+ phenotype is a hallmark of regulatory T cells, a population controlling self-tolerance and abrogating undue immune responses. They feature in various cancer settings [21], but their influence on melanoma is unclear [22-24]. Several other subtypes that go beyond the classical categorization of $CD4^+$ cells outlined above, e.g. Th17, Tr1 or cytotoxic $CD4^+$ cells, play a role in immunologic response to melanoma [25-27]. Complex interplay between the subsets may obscure and counterbalance their individual actions rendering total $CD4^+$ pool outwardly passive.

Great heterogeneity of TILs and a complex immunological homeostasis dysregulated during the evolution of cancer are very problematic aspects of melanoma research, especially considering that appropriate analytical methods are lacking. Isolation of peripheral blood cells provides a very poor insight into localized and multifaceted immunological reaction against melanoma. Especially in the early stages, when a number of cancer cells triggering immune response is limited, alterations in peripheral immune profile may remain undetectable even when sensitive methods are employed. Immunohistochemistry is an important, yet imperfect tool. It enables the scientists to discern individual cells, but the complexity of epitope expression profiles of numerous lymphocyte subpopulations warrants caution with interpretation of results.

We demonstrated that a higher ratio of $CD8^+$ TILs is correlated with longer melanoma-specific survival. In addition, not only a phenotype of lymphocytes, but also their spatial relation to the tumor mass seems to be a significant factor for patient's outcome. It would be interesting to analyze, in analogy to breast cancer [28], prognostic differences between stromal TILs and those in direct contact with malignant melanocytes. Our results underscore the role of TILs in melanoma pathobiology, but future studies are needed to translate knowledge into clinical practice.

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Disclosure of conflict of interest

None.

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References

[1] Mihm MC Jr and Mule JJ. Reflections on the Histopathology of Tumor-Infiltrating Lymphocytes in Melanoma and the Host Immune Response. Cancer Immunol Res 2015; 3: 827-835.

[2] Svane IM and Verdegaal EM. Achievements and challenges of adoptive T cell therapy with tumor-infiltrating or blood-derived lymphocytes for metastatic melanoma: what is needed to achieve standard of care? Cancer Immunol Immunother 2014; 63: 1081-1091.

[3] Sim GC, Chacon J, Haymaker C, Ritthipichai K, Singh M, Hwu P and Radvanyi L. Tumor-infiltrating lymphocyte therapy for melanoma: rationale and issues for further clinical development. BioDrugs 2014; 28: 421-437.

[4] Ozao-Choy J, Lee DJ and Faries MB. Melanoma vaccines: mixed past, promising future. Surg Clin North Am 2014; 94: 1017-1030, viii.

[5] Wilmott JS, Long GV, Howle JR, Haydu LE, Sharma RN, Thompson JF, Kefford RF, Hersey P and Scolyer RA. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. Clin Cancer Res 2012; 18: 1386-1394.

[6] Frederick DT, Piris A, Cogdill AP, Cooper ZA, Lezcano C, Ferrone CR, Mitra D, Boni A, Newton LP, Liu C, Peng W, Sullivan RJ, Lawrence DP, Hodi FS, Overwijk WW, Lizee G, Murphy GF, Hwu P, Flaherty KT, Fisher DE and Wargo JA. BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. Clin Cancer Res 2013; 19: 1225-1231.

[7] Moschos SJ, Edington HD, Land SR, Rao UN, Jukic D, Shipe-Spotloe J and Kirkwood JM. Neoadjuvant treatment of regional stage IIIB melanoma with high-dose interferon alfa-2b induces objective tumor regression in association with modulation of tumor infiltrating host cellular immune responses. J Clin Oncol 2006; 24: 3164-3171.

[8] Curran MA, Kim M, Montalvo W, Al-Shamkhani A and Allison JP. Combination CTLA-4 blockade and 4-1BB activation enhances tumor rejection by increasing T-cell infiltration, prolifera-

tion, and cytokine production. PLoS One 2011; 6: e19499.
[9] Mortarini R, Borri A, Tragni G, Bersani I, Vegetti C, Bajetta E, Pilotti S, Cerundolo V and Anichini A. Peripheral burst of tumor-specific cytotoxic T lymphocytes and infiltration of metastatic lesions by memory CD8+ T cells in melanoma patients receiving interleukin 12. Cancer Res 2000; 60: 3559-3568.
[10] Curran MA, Montalvo W, Yagita H and Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A 2010; 107: 4275-4280.
[11] Hansen MG and McCarten AB. Tumor thickness and lymphocytic infiltration in malignant melanoma of the head and neck. Am J Surg 1974; 128: 557-561.
[12] Ladanyi A, Somlai B, Gilde K, Fejos Z, Gaudi I and Timar J. T-cell activation marker expression on tumor-infiltrating lymphocytes as prognostic factor in cutaneous malignant melanoma. Clin Cancer Res 2004; 10: 521-530.
[13] Kim R, Emi M, Tanabe K, Uchida Y and Toge T. The role of Fas ligand and transforming growth factor beta in tumor progression: molecular mechanisms of immune privilege via Fas-mediated apoptosis and potential targets for cancer therapy. Cancer 2004; 100: 2281-2291.
[14] Hahne M, Rimoldi D, Schroter M, Romero P, Schreier M, French LE, Schneider P, Bornand T, Fontana A, Lienard D, Cerottini J and Tschopp J. Melanoma cell expression of Fas (Apo-1/CD95) ligand: implications for tumor immune escape. Science 1996; 274: 1363-1366.
[15] Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu G, Tamada K, Lennon VA, Celis E and Chen L. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002; 8: 793-800.
[16] Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N, Boon T and Van den Eynde BJ. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med 2003; 9: 1269-1274.
[17] Moogk D, da Silva IP, Ma MW, Friedman EB, de Miera EV, Darvishian F, Scanlon P, Perez-Garcia A, Pavlick AC, Bhardwaj N, Christos PJ, Osman I and Krogsgaard M. Melanoma expression of matrix metalloproteinase-23 is associated with blunted tumor immunity and poor responses to immunotherapy. J Transl Med 2014; 12: 342.
[18] Dvorak AM, Mihm MC Jr, Osage JE and Dvorak HF. Melanoma. An ultrastructural study of the host inflammatory and vascular responses. J Invest Dermatol 1980; 75: 388-393.
[19] Romagnani S. T-cell subsets (Th1 versus Th2). Ann Allergy Asthma Immunol 2000; 85: 9-18; quiz 18, 21.
[20] Lowes MA, Bishop GA, Crotty K, Barnetson RS and Halliday GM. T helper 1 cytokine mRNA is increased in spontaneously regressing primary melanomas. J Invest Dermatol 1997; 108: 914-919.
[21] Mougiakakos D, Choudhury A, Lladser A, Kiessling R and Johansson CC. Regulatory T cells in cancer. Adv Cancer Res 2010; 107: 57-117.
[22] Hillen F, Baeten CI, van de Winkel A, Creytens D, van der Schaft DW, Winnepenninckx V and Griffioen AW. Leukocyte infiltration and tumor cell plasticity are parameters of aggressiveness in primary cutaneous melanoma. Cancer Immunol Immunother 2008; 57: 97-106.
[23] Ladanyi A, Mohos A, Somlai B, Liszkay G, Gilde K, Fejos Z, Gaudi I and Timar J. FOXP3+ cell density in primary tumor has no prognostic impact in patients with cutaneous malignant melanoma. Pathol Oncol Res 2010; 16: 303-309.
[24] Viguier M, Lemaitre F, Verola O, Cho MS, Gorochov G, Dubertret L, Bachelez H, Kourilsky P and Ferradini L. Foxp3 expressing CD4+CD25 (high) regulatory T cells are overrepresented in human metastatic melanoma lymph nodes and inhibit the function of infiltrating T cells. J Immunol 2004; 173: 1444-1453.
[25] Muranski P, Boni A, Antony PA, Cassard L, Irvine KR, Kaiser A, Paulos CM, Palmer DC, Touloukian CE, Ptak K, Gattinoni L, Wrzesinski C, Hinrichs CS, Kerstann KW, Feigenbaum L, Chan CC and Restifo NP. Tumor-specific Th17-polarized cells eradicate large established melanoma. Blood 2008; 112: 362-373.
[26] Strauss L, Bergmann C, Szczepanski MJ, Lang S, Kirkwood JM and Whiteside TL. Expression of ICOS on human melanoma-infiltrating CD4+CD25 high Foxp3+ T regulatory cells: implications and impact on tumor-mediated immune suppression. J Immunol 2008; 180: 2967-2980.
[27] Morisaki T, Morton DL, Uchiyama A, Yuzuki D, Barth A and Hoon DS. Characterization and augmentation of CD4+ cytotoxic T cell lines against melanoma. Cancer Immunol Immunother 1994; 39: 172-178.
[28] Ali HR, Provenzano E, Dawson SJ, Blows FM, Liu B, Shah M, Earl HM, Poole CJ, Hiller L, Dunn JA, Bowden SJ, Twelves C, Bartlett JM, Mahmoud SM, Rakha E, Ellis IO, Liu S, Gao D, Nielsen TO, Pharoah PD and Caldas C. Association between CD8+ T-cell infiltration and breast cancer survival in 12,439 patients. Ann Oncol 2014; 25: 1536-1543.