Avens Publishing Group
Inviting Innovations

Journal of Surgery

Special Issue

Surgical Oncology

Editors:

[1]Dr. Marlon A Guerrero

[2]Dr. James A Warneke

[1]Assistant Professor of Surgery, University of Arizona, USA
[2]Associate Professor of Surgery, University of Arizona, USA

Avens Publishing Group
J Surgery
Special Issue 2014

Tumor Infiltrating Lymphocytes and their Role as a Prognostic Indicator for Melanoma

Open Access Case Report

Journal of Surgery

Sanjay S. Reddy*, Stefanos Boukovalas, Victor Barnica, Fang Zhu, Hong Wu and Jeffrey M. Farma

Department of Surgical Oncology, Fox Chase Cancer Center, Philadelphia, PA, USA

***Address for Correspondence**

Sanjay S. Reddy, M.D., Department of Surgical Oncology, Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111, USA, Tel: 201-290-6739; Fax: 215-728-2773; E-mail: sanjay.reddy@fccc.edu

Submission: 26 September 2014
Accepted: 31 October 2014
Published: 05 November 2014

Keywords: Melanoma; Tumor infiltrating lymphocytes; Prognosis

Abstract

Background: Tumor infiltrating lymphocytes (TIL) are associated with spontaneous regression and improved survival in patients with melanoma. The aim of our study was to evaluate if histopathological detection of TIL is a reliable indicator to predict aggressive tumor biology.

Methods: We retrospectively analyzed 241 patients in a single institution from 2009-2011. The presence or absence of TIL was compared to preoperative white blood cell counts (WBC), age, tumor depth, mitotic rates, sentinel lymph nodes (SLN), and pathologic stage.

Results: Of 241 patients, 53% were male. Age was categorized as <65 or >65 years, with 55% being under 65 (mean=61). Patients >65 with lower WBC counts were more likely to have absence of TIL (p=0.03). Thick melanomas >4 mm were seen in 15% of our population. Patients with thinner melanomas had TIL present 48% of the time, compared to only 22% with thicker tumors (p=0.02). Of patients with positive SLNs, TIL was identified in 46% vs. 38% with a negative SLN (p=0.38); a mitotic rate >4 mm^2 was identified in 31% vs. 21%, respectively (p<0.001). Preoperative mitotic rates were compared to overall stage, and the presence of low mitotic rates correlated to a lower stage (p <0.001). Preoperative TIL was compared to overall stage, and the presence of TIL did not correlate with an earlier staged melanoma (p=0.29). Multivariate analysis comparing clinical stage, TIL, and mitotic rate was performed, and the combination of TIL and degree of mitoses did not predict earlier staged melanoma when compared to mitotic rate alone (p=0.39).

Conclusion: Known factors such as positive SLNs, mitotic rates, and depth of invasion serve as reliable prognostic indicators independently. In our series, we found the presence of TIL to correlate with tumors <4 mm. When comparing TIL with mitosis, SLN, and early staged melanomas, there was no statistical significance to suggest benefit as a reliable prognostic indicator.

Introduction

Despite public awareness warning against increased sun exposure, cutaneous melanoma continues to be one of the leading causes of death of all the skin cancers. Both incidence and mortality rates have increased steadily worldwide [1]. Significant advances in adjuvant therapies have been made in the past ten years. One of the earliest publications documenting prognostic factors in patients with melanoma was published thirty five years ago, and tumor thickness, along with anatomical site, were felt to be dominant factors [2]. Additional factors included: pigmentation, lymphocyte infiltration, growth pattern and regression; however, these factors were found to have either indirect or no influence on survival. In 2001, the American Joint Committee on Cancer proposed major revisions on the staging system, and with the aid of a multi-institutional effort, several key prognostic indicators for melanoma were agreed upon; tumor thickness, ulceration, presence of positive lymph nodes, and visceral versus non visceral metastatic disease [3]. Accurately predicting prognosis aids in guiding appropriate management, determining the need for further investigation, and to assign risk status for patients that may benefit from closer surveillance, adjuvant therapy, or consideration for a clinical trial [1].

The presence of tumor infiltrating lymphocytes (TIL) and their roles as a prognostic indicator for malignant melanomas has been a topic of much controversy. There currently is no consensus regarding the presences of TIL in melanoma; nonetheless, the general trend suggests that tumor infiltrating lymphocytes portend an added survival benefit. Tumor infiltrating lymphocytes are histopathological markers of the host immune response to melanoma [4]. Several studies have been able to correlate the presence of TIL with improved overall survival [1,4-6], however others have found no such relationship [7,8]. There have been numerous prognostic indicators that are known to play a role in melanoma, and depth of invasion has been one of the most sensitive factors [2]. As advances have been made, the presence of ulceration, mitosis, and sentinel lymph node positivity have added even more information on outcomes [3]. As much controversy continues to exist regarding tumor infiltrating lymphocytes as a potential mediator in prognosis, we aimed to evaluate if TILs correlated with prognosis in patients with malignant melanoma.

Methods

We performed an IRB approved retrospective analysis on 241 patients treated at a tertiary cancer center from 2009-2011. We compared the absence or presence of tumor infiltrating lymphocytes correlated to preoperative white blood cells counts, age, tumor depth, mitotic rates, sentinel lymph node positivity and pathological stage. Tumor infiltrating lymphocytes were quantified by hematoxylin and eosin (H&E) staining by an experienced dermatopathologist. They were globally characterized as being absent or present. They were further sub-classified, if able, into brisk or non-brisk categories. The primary aim of our study was to identify if the presence of TIL served as a reliable indicator to predict aggressive tumor biology, and to review the literature comparing TIL and melanoma, as both a prognostic indicator, and a potential modality for treatment.

Results

Patient distribution was fairly equal between male and female, and the average age slightly older for men at 63, compared to 59 for women. The overwhelming majority of patients were Caucasian,

ISSN: 2332-4139

at 93%. Anatomical site of the melanoma varied from the upper extremity, lower extremity, head and neck, trunk or genitourinary regions. The majority of the lesions were found to be on the trunk, followed by the extremities (Table 1). Preoperative biopsies were performed by a shave, excision or punch method. Pathological staging ranged from in-situ lesions to patients with stage IV disease. Of the patients with pathological staging available, 5% were diagnosed with in-situ disease, 23% IA, 25% IB, 22% II, 8% IIIA, 6% IIIB, 6% IIIC, and 5% IV.

A wide array of tumor biology was seen, ranging from superficial spreading, nodular, acral, lentigo, metastatic, in-situ, and a sub-set of unknown. The superficial spreading variant was seen in 38%, nodular in 12%, lentigo in 9%, and acral in 3% of the population. Fifteen percent of the patients had thick melanomas over 4 mm in depth. Fifty-two percent of patients had mitotic rates between 1-4 mm^2, and 22% had ulceration. The presence of tumor infiltrating lymphocytes was determined by a single pathologist, and was categorized as either being absent or present. When present, they were subdivided into classifications of brisk or non-brisk. Forty-two percent of patients had no TIL present, 32% had TIL present, and in 25% TIL status was not recorded (Table 2) (Figure 1 and 2). Of the patients found to have superficial spreading, 42% had absent TIL, 8% brisk, and 28% non-brisk. The remaining 22% were recorded as being present, with no specification on type of TIL, or not reported. Of the cohort found to have a nodular type, 46% had absent TIL, 4% brisk, and 39% non-brisk. The remaining 11% were not recorded.

Only 15% of the patients had thick melanomas greater than 4 mm. Patients with thin melanomas were found to have TIL in 48% versus 22% with thick melanomas, suggesting a potential indicator for prognosis (p=0.020). Mitotic rate has been a proven indicator for prognosis, with higher rates leading to decreased survival [9]. Attempts were made to correlate mitosis with the presence or absence of TIL. When TIL was identified, 8% of patients had mitosis > 4 mm^2, and 14% when TIL was absent. Although not reaching statistical significance, the trend suggests that presence of TIL may be associated with lower mitotic rates, and thus improved survival. Similar to mitosis, sentinel lymph node positivity has been found to correlate with worse survival trends [10]. This was corroborated in our study, showing that positive sentinel lymph nodes were found in 31% versus 21% of patients when mitosis was > 4 mm^2 (p=0.001). When comparing lymph node positivity, TIL was identified in 38% of patients, and negative node status yielded the presence of TIL in 21% (p=0.388); as such, no correlation can be made between lymph node positivity and TIL.

Comparisons were made independently between the presence of TIL, degree of mitosis and survival, to overall stage. The presence of TIL did not correlate with an earlier staged melanoma (p=0.294); when the degree of mitosis were compared, a strong correlation was found to stage (p=<0.001). Independently, the presence of TIL did not correlate with increased survival (p=0.140) (Figure 3). Using multivariate analysis, stage was compared to both TIL and mitotic rate; however no statistical significance was found when compared to mitotic rate alone (p=0.39). The only association of TIL to prognosis that was found in our series was the trend for TIL to be identified in thinner melanomas. When comparing TIL with mitosis, SLNs, and early staged melanomas, there was no statistical significance to suggest benefit as a reliable prognostic indicator.

Table 1: Patient demographics.

Demographics:	N (%)
Sex (n):	
Male	120 (50)
Female	108 (45)
Unknown	13 (5)
Age (avg):	
Male	63 (25-87)
Female	59 (23-98)
Race:	
Caucasian	224 (93)
Indian	1
Hispanic	1
African American	1
Asian	1
Unknown	13
Site:	
Upper Extremity	47 (20)
Lower Extremity	57 (24)
Head and Neck	21 (8)
Trunk	104 (43)
Genitourinary	5 (2)
Other	7 (3)

Table 2: Tumor characteristics.

Tumor Characteristics:	N (%)
Pathology:	
Superficial Spreading	92 (38)
Nodular	28 (12)
Lentigo	21 (9)
Acral	8 (3)
In-Situ	5 (2)
Metastatic	15 (6)
Other	72 (30)
Mitotic Rate (mm^2):	
<1	51 (21)
1-4	125 (52)
> 4	26 (11)
Unknown	39 (16)
Tumor Infiltrating Lymphocytes:	
Present:	11 (5)
Brisk	57 (24)
Non-Brisk	9 (4)
Unknown	102 (42)
Absent	61 (25)
N/A	
Ulcerations:	
Present	52 (22)
Absent	152 (63)
N/A	37 (15)

Discussion

The prognostic significance of tumor infiltrating lymphocytes in patients with melanoma remains controversial. It is believed that the number of TIL may be indicative of a potent immune response against tumor antigens, and therefore portend a more favorable prognosis. One could hypothesize that TIL may be altered in older patients.

While our study found that high numbers of TIL were inversely related to tumor depth of invasion, the degree of T-cell infiltration of the tumor was not correlated with survival benefit. Balch et al. performed a retrospective study of 339 melanoma patients in order to evaluate the important factors affecting survival. Pathological stage, ulceration, surgical treatment, tumor thickness and location

ISSN: 2332-4139

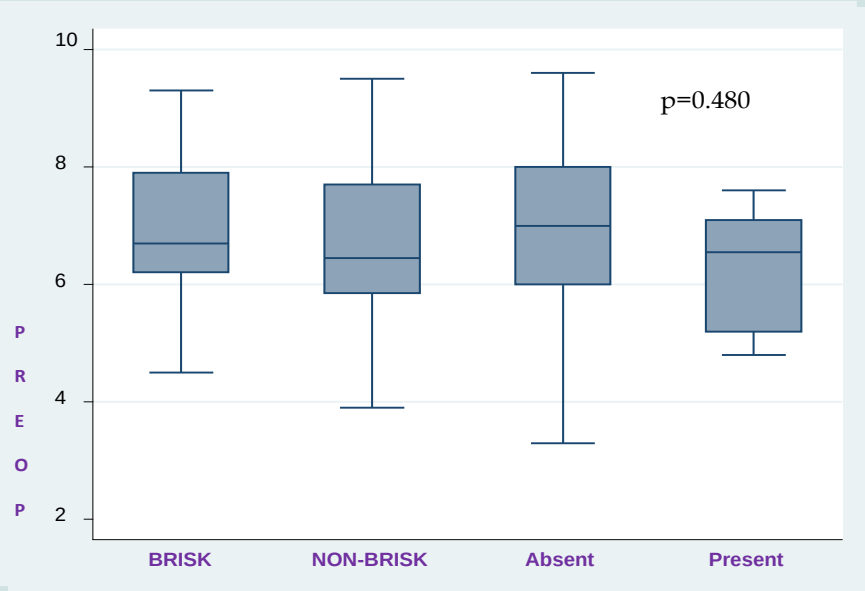
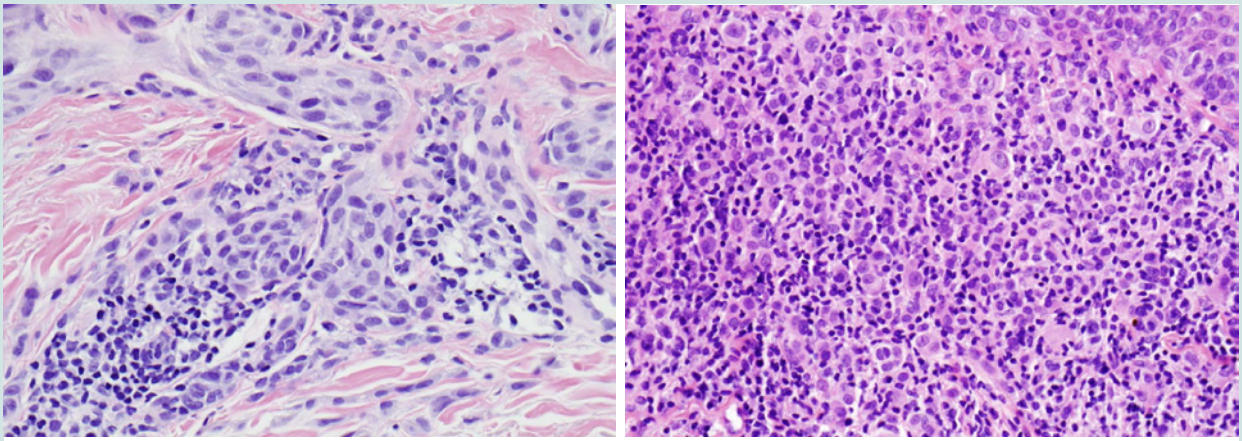


Figure 1: WBC correlation to TIL. The presence or absence of tumor infiltrating lymphocytes correlated to preoperative WBC counts.



(a) Non-brisk tumor infiltrating lymphocytes (high power 40x) (b) Brisk tumor infiltrating lymphocytes (high power 40x)

Figure 2: Tumor infiltrating lymphocytes microscopy.

were found to be independent prognostic factors of 5-year survival. In patients with stage I disease, TIL concentration was inversely correlated with tumor thickness (P=0.003), however differences in TIL magnitude and depth of invasion did not have an impact on survival [2]. Burton et al. studied 515 patients where TIL responses were classified as being brisk and non-brisk. Using multivariate analysis, a brisk TIL response was not found to be a significant independent factor for disease-free survival [6].

On the contrary, several studies reported in the literature have found a positive correlation with TILs as a prognostic indicator in melanoma. Grotz et al. performed a retrospective study of 250 patients, and found that non-brisk or absent TIL was independently associated with recurrence (P< 0.0001), decreased 5-year disease-free survival (76 vs. 91%, P = 0.0006), as well as, a decreased 5-year melanoma-specific survival (82 vs. 95%, P=0.0008) [11]. Knol et al. suggested a benefit in both progression free survival (PFS) and overall survival (OS) with the presence of tumor reactive T cells, which was increased with the injection of melanoma reactive TIL (PFS: p=0.00289 and OS: p=0.00279) [12]. Although our series did not find a relationship between TIL and stage, earlier stage III tumors were associated with a greater number of tumor-reactive T cells than late stage III tumors [12].

In a study stratifying TIL classification, Mihm et al. examined the histology from 99 resected metastatic lymph nodes. The pattern of TIL was investigated and 16 tumors were classified as brisk, 37 as non-brisk, and 46 as absent. The disease-free survival was 81.3% for brisk cases, 46.8% for non-brisk cases, and 29.3% for absent groups (P=0.007) [13]. This suggests that patients, who express TIL, versus those who express none at all, do have improved survival. The results of this study are similar to the results published by Clark et al., who showed that the 8-year survival rate for patients with melanoma was

ISSN: 2332-4139

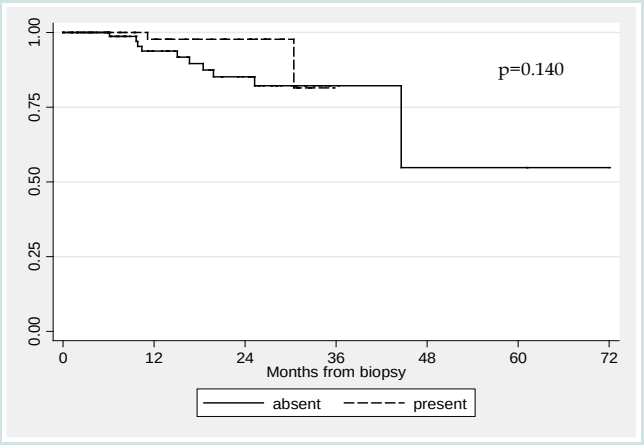


Figure 3: Survival correlation to TIL. The presence or absence of tumor infiltrating lymphocytes correlated to overall survival.

88.5% when TIL were brisk, 75% for non-brisk, and 59.3% for absent [14]. Clemente et al. published 5-year survival rates for patients with melanomas following similar patterns; 77% for brisk, 53% for non-brisk, and 37% for absent [5].

Although it is evident that patients with TIL seem to have better survival than when absent, there continues to be no standardized means of pathological analysis to classifying tumor infiltrating lymphocytes. Busam et al. performed a study which found that educating pathologists on how to prepare and analyze specimens allowed for more precise identification of TILs [15]. The current terminology for the classification and description of TIL deposits as "brisk", "non-brisk" and "absent," has also been challenged. It has been suggested that terms such as "diffuse" versus "focal" may be more accurate, and could potentially facilitate better reproducibility [15].

Questions have arisen regarding the specific TIL antigen receptor, and how that may influence overall prognosis. As the role of TILs remains uncertain, one fact remains true, that immune response likely plays a critical role. A variety of studies have looked into TIL antigen receptor structure and the antigen to which melanoma-specific T cells react. Recent data has revealed that the composition of TIL is not homogenous, but rather comprised of many lymphocytic subsets [16]. Although the majority of the aforementioned studies correlate TIL to lymph node positivity, depth of invasion, and even survival; few studies have looked into the specific immunophenotype of TILs.

As the majority of research deals with immunology, hypothesizes have been made linking impaired lymphatic function, which may be more prominent in the elderly, to the lack of melanoma cells from spreading to the SLN. This could potentially prevent the induction of the adaptive immune response to the melanoma, manifested as a non-brisk or absent TIL response [11]. Our series tried to find a correlate between the elderly, WBC, and the presence or absence of TIL, however no statistical significance was reached. These findings suggest that more research needs to occur; looking into the composition and function of TIL before conclusions can be made.

It has been established that TIL plays some role with overall prognosis in patients with melanoma. Depending on what type of TIL, "brisk," "non-brisk," or "absent," each can affect survival differently. A new area that has brought much excitement to the field is the use of immunotherapy. Immunotherapy using TIL was first described in 1988 and has emerged as a promising treatment option for metastatic melanoma. Mature lymphocytes specific to the tumor antigen are isolated in vitro, processed, cultured and re-infused, allowing for targeted therapy.

A variety of cells participate in the immune response against the tumor. $CD8^+$, $CD4^+$ T cells, B cells, NK, and innate type T cells expressing the γδ TCR have been described as playing a role in TIL [17]. It appears that the main cells are $CD\text{-}8^+$ T cells, demonstrating critical effects in anti-tumor response, known to mediate the antigen-specific cytolysis of cancerous cells [17,18]. The role of $CD4^+$ cells is debatable but studies have shown that they might have a role in the adaptive immunity, and may also play a role in a small percentage of patients in which $CD4^+$ dominant TIL were found [18-20]. Different techniques have been suggested to improve the isolation and expansion of the lymphocytes. Some examples include the "young TIL" protocol, lymphodepletion, irradiation, trogocytosis, and the use of IL-2, IFN-γ or other co-stimulatory molecules [17,21-26].

Another interesting area revolves on the discovery of corticotropin-releasing factors, and there involvement in the "skin stress response system". It is well documented that CRFR-1 receptors are expressed in melanoma, so targeting of this is a rational approach to treatment of this disease [27]. Slominsky et al. proved on immunohistochemistry that CFR and CRFR-1 were detected in melanoma specimen, however differences in their expression level did not correlate to melanoma progression [27]. Again we see a potential tool for measuring prognosis in patients with melanoma fall short. Taking a step back, we should look at the skin as a natural immune barrier to toxins in the environment. The skin is a steroidogenic organ which can regulate local and systemic immune activities. Dysregulation of cutaneous steroidogenesis could be linked to various skin diseases [28]. An example of another epidermal product that has been shown to be involved with melanomas is pro-opiomelanocortin (POMC). It is suggested that progression of melanoma is associated with increased POMC-peptide expression due to generation of a "tumor-favorable environment" [27]. The use

ISSN: 2332-4139

of POMC in determining if the melanoma is progressing on or off therapy seems promising. Steroidogenesis is not the only method which affects melanogenesis. L-tyrosine and L-DOPA have also been studied as intermediates that affect melanogenesis. Slominsky et al. reports that in addition to serving as substrates and intermediates for melanogenesis, L-tyrosine and L-DOPA also act as inducers and positive regulators of the melanogenic pathway [29]. Therapies directed to control regulators of melanogenesis could lead to new drugs that fight melanoma at the immunogenic level.

Many factors have been shown to affect the efficacy of TIL therapy. It has been observed that the age and gender of patients, as well as stage and primary site of tumor play a role [11,12,25,30,31]. In addition, the temporal relationship of systemic therapy to the time of resection, the number of activated lymphocytes, and the expansion rate of the cells isolated from the patient impact efficacy [11,31,32]. The role of TIL in the treatment of melanoma is currently in clinical trials, and results appear promising. Further research is currently being done in order to elucidate the exact role and the optimal timing for TIL. Additional studies need to be done so protocols can be established to make this potentially life-saving therapy more accessible and cost effective.

There remains inconsistency in the literature as to the prognostic significance of TIL in melanoma. The overwhelming majority of these studies are retrospective in nature, and there are few randomized prospective trials investigating the relationship between TIL and prognosis. It has been well established that tumor thickness, lymph node involvement, and the presence of ulcerations are key prognostic indicators. From current literature, results have been published stating that the presence of TIL provides for a better prognosis, and increased 5-year survival rates; however just as many articles say the contrary. Results suggest that TIL may in fact predict sentinel lymph node positivity, but then no significance is found for survival benefit. Each study obviously carries its own flaws and biases. After a thorough review of the literature, our series suggests that the use of tumor infiltrating lymphocytes as a prognostic factor for cutaneous melanoma shows no statistical significance when compared to mitosis, SLN status or early staged melanomas. Our results support the current standards of mitotic rates, lymph node positivity, and depth of invasion as independent predictors of prognosis, with further research needed to elucidate the true significance of TIL.

References

1. Azimi F, Scolyer RA, Rumcheva P, Moncrieff M, Murali R, et al. (2012) Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. J Clin Oncol 30: 2678-2683.
2. Balch CM, Murad TM, Soong SJ, Ingalls AL, Halpern NB, et al. (1978) A multifactorial analysis of melanoma: prognostic histopathological features comparing Clark's and Breslow's staging methods. Ann Surg 188: 732-742.
3. Balch CM, Soong SJ, Gershenwald JE, Thompson JF, Reintgen DS, et al. (2001) Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. J Clin Oncol 19: 3622-3634.
4. Rao UN, Lee SJ, Luo W, Mihm MC Jr, Kirkwood JM (2010) Presence of tumor-infiltrating lymphocytes and a dominant nodule within primary melanoma are prognostic factors for relapse-free survival of patients with thick (t4) primary melanoma: pathologic analysis of the e1690 and e1694 intergroup trials. Am J Clin Pathol 133: 646-653.
5. Clemente CG, Mihm MC Jr, Bufalino R, Zurrida S, Collini P, et al. (1996) Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer 77: 1303-1310.
6. Burton AL, Roach BA, Mays MP, Chen AF, Ginter BA, et al. (2011) Prognostic significance of tumor infiltrating lymphocytes in melanoma. Am Surg 77: 188-192.
7. Taylor RC, Patel A, Panageas KS, Busam KJ, Brady MS (2007) Tumor-infiltrating lymphocytes predict sentinel lymph node positivity in patients with cutaneous melanoma. J Clin Oncol 25: 869-875.
8. Larsen TE, Grude TH (1978) A retrospective histological study of 669 cases of primary cutaneous malignant melanoma in clinical stage I. 3. The relation between the tumour-associated lymphocyte infiltration and age and sex, tumour cell type, pigmentation, cellular atypia, mitotic count, depth of invasion, ulceration, tumour type and prognosis. Acta Pathol Microbiol Scand A 86A: 523-530.
9. Hale CS, Qian M, Ma MW, Scanlon P, Berman RS, et al. (2013) Mitotic rate in melanoma: prognostic value of immunostaining and computer-assisted image analysis. Am J Surg Pathol 37: 882-889.
10. Mozzillo N, Pennacchioli E, Gandini S, Caracò C, Crispo A, et al. (2013) Sentinel node biopsy in thin and thick melanoma. Ann Surg Oncol 20: 2780-2786.
11. Grotz TE, Vaince F, Hieken TJ (2013) Tumor-infiltrating lymphocyte response in cutaneous melanoma in the elderly predicts clinical outcomes. Melanoma Res 23: 132-137.
12. Knol AC, Nguyen JM, Pandolfino MC, Quéreux G, Brocard A, et al. (2012) Tissue biomarkers in melanoma patients treated with TIL. PLoS One 7: e48729.
13. Mihm MC Jr, Clemente CG, Cascinelli N (1996) Tumor infiltrating lymphocytes in lymph node melanoma metastases: a histopathologic prognostic indicator and an expression of local immune response. Lab Invest 74: 43-47.
14. Clark WH Jr, Elder DE, Guerry D 4th, Braitman LE, Trock BJ, et al. (1989) Model predicting survival in stage I melanoma based on tumor progression. J Natl Cancer Inst 81: 1893-1904.
15. Busam KJ, Antonescu CR, Marghoob AA, Nehal KS, Sachs DL, et al. (2001) Histologic classification of tumor-infiltrating lymphocytes in primary cutaneous malignant melanoma. A study of interobserver agreement. Am J Clin Pathol 115: 856-860.
16. Oble DA, Loewe R, Yu P, Mihm MC Jr (2009) Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human melanoma. Cancer Immun 9: 3.
17. Wu R, Forget MA, Chacon J, Bernatchez C, Haymaker C, et al. (2012) Adoptive T-cell therapy using autologous tumor-infiltrating lymphocytes for metastatic melanoma: current status and future outlook. Cancer J 18: 160-175.
18. Radvanyi LG, Bernatchez C, Zhang M, Fox PS, Miller P, et al. (2012) Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. Clin Cancer Res 18: 6758-6770.
19. Friedman KM, Prieto PA, Devillier LE, Gross CA, Yang JC, et al. (2012) Tumor-specific CD4+ melanoma tumor-infiltrating lymphocytes. J Immunother 35: 400-408.
20. Muranski P, Restifo NP (2009) Adoptive immunotherapy of cancer using CD4(+) T cells. Curr Opin Immunol 21: 200-208.
21. Dudley ME, Gross CA, Somerville RP, Hong Y, Schaub NP, et al. (2013) Randomized selection design trial evaluating CD8+-enriched versus unselected tumor-infiltrating lymphocytes for adoptive cell therapy for patients with melanoma. J Clin Oncol 31: 2152-2159.
22. Chacon JA, Wu RC, Sukhumalchandra P, Molldrem JJ, Sarnaik A, et al. (2013) Co-stimulation through 4-1BB/CD137 improves the expansion and function of CD8(+) melanoma tumor-infiltrating lymphocytes for adoptive T-cell therapy. PLoS One 8: e60031.
23. Donia M, Hansen M, Sendrup SL, Iversen TZ, Ellebæk E, et al. (2013)

ISSN: 2332-4139

Methods to improve adoptive T-cell therapy for melanoma: IFN-gamma enhances anticancer responses of cell products for infusion. J Invest Dermatol 133: 545-552.

24. Eisenberg G, Uzana R, Pato A, Frankenburg S, Merims S, et al. (2013) Imprinting of lymphocytes with melanoma antigens acquired by trogocytosis facilitates identification of tumor-reactive T cells. J Immunol 190: 5856-5865.

25. Lee S, Margolin K (2012) Tumor-infiltrating lymphocytes in melanoma. Curr Oncol Rep 14: 468-474.

26. Yang S, Ji Y, Gattinoni L, Zhang L, Yu Z, et al. (2013) Modulating the differentiation status of *ex vivo*-cultured anti-tumor T cells using cytokine cocktails. Cancer Immunol Immunother 62: 727-736.

27. Slominski AT, Zmijewski MA, Zbytek B, Tobin DJ, Theoharides TC, et al. (2013) Key role of CRF in the skin stress response system. Endocr Rev 34: 827-884.

28. Slominski A, Zbytek B, Nikolakis G, Manna PR, Skobowiat C, et al. (2013) Steroidogenesis in the skin: implications for local immune functions. J Steroid Biochem Mol Biol 137: 107-123.

29. Slominski A, Zmijewski MA, Pawelek J (2012) L-tyrosine and L-dihydroxyphenylalanine as hormone-like regulators of melanocyte functions. Pigment Cell Melanoma Res 25: 14-27.

30. Zippel DB, Besser M, Shapira R, Ben-Nun A, Goitein D, et al. (2012) Adoptive cell therapy with autologous tumor-infiltrating lymphocytes and high-dose interleukin-2 for metastatic melanoma: The surgeon's perspective. Exp Ther Med 3: 898-902.

31. Joseph RW, Peddareddigari VR, Liu P, Miller PW, Overwijk WW, et al. (2011) Impact of clinical and pathologic features on tumor-infiltrating lymphocyte expansion from surgically excised melanoma metastases for adoptive T-cell therapy. Clin Cancer Res 17: 4882-4891.

32. Pilon-Thomas S, Kuhn L, Ellwanger S, Janssen W, Royster E, et al. (2012) Efficacy of adoptive cell transfer of tumor-infiltrating lymphocytes after lymphopenia induction for metastatic melanoma. J Immunother 35: 615-620.