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REVIEW ARTICLE

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Sentinel node biopsy in melanoma

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Abstract There has, for a long time, been an ongoing discussion on whether the prophylactic removal of lymph nodes ("elective lymph node dissection") is of benefit for melanoma patients. More recently, "selective" lymphadenectomy ("sentinel node biopsy", SNB) has been proposed to evaluate the status of the first draining lymph node ("sentinel node") of the regional basin. Several studies now demonstrate that the sentinel node evaluation for underlying metastatic disease reflects the status of the entire lymph node region and is therefore a useful prognostic factor. A multi-institutional study highlighted SNB status as the most significant prognostic factor, superior to measurement of tumor thickness in primary melanoma. Different techniques to detect micrometastasis within the lymph node are under current evaluation. Histology and immunohistology using antibodies against melanoma-associated antigens are routinely performed in SNs. The clinical value of reverse-transcriptase polymerase chain reaction (RT-PCR)-based search for minimal melanoma disease in lymph nodes remains unclear.

Keywords Melanoma · Sentinel node · Lymphadenectomy · Metastasis · RT-PCR

Introduction

A rapid increase in the incidence of melanoma among Caucasians has been demonstrated in most countries of the world [1]. For instance, in the United States and in Germany, approximately 38,000 [39, 47] and 10,000 new cases [23], respectively, are diagnosed per year. Despite several efforts in the treatment of malignant melanoma (MM), surgery remains as the standard of care. An excision of the primary tumor with prognosis-adapted side margins is recommended worldwide as a basic therapeutic approach [53].

Whereas this surgical intervention has found wide acceptance, elective ("prophylactic") lymph node dissection (ELND) was discussed controversially over the last two decades. Beneficial outcome for affected patients was only seen in studies with a retrospective design. Prospective randomized trials (Table 1) were not able to demonstrate prolonged survival for patients receiving ELND and, furthermore, melanoma patients suffered from extensive prophylactic surgical interventions, including tissue damage [3, 15, 56, 67]. Therefore, a minimal invasive surgical technique appeared attractive. The concept of a step-wide progression of melanoma was described by Reintgen and co-workers [42, 50]. To minimize surgical damage, a "selective" "lymph node evaluation" ["sentinel node biopsy" (SNB), "lymphatic mapping", and "nodal staging"] was promoted by Morton and others 10 years ago and subsequently published in 1992 [43].

Very recently, a new cancer staging system for cutaneous melanoma was proposed by the American Joint Committee on Cancer (AJCC) [4]. Among other modifications of the current staging system, dominant independent prognostic factors involving melanoma patients have been incorporated in the new classification proposal published in March 2000 (Table 2, Table 3, and Table 4).

Because in almost all studies the number or the percentage of metastatic lymph nodes was the strongest predictor of survival outcome, this criterion was considered as relevant now. Secondly, the "N classification" distinguishes between macroscopic and microscopic metastases [4]. With the increased use of SNB as a microscopic staging tool, a substantial number of patients are identified as having clinically occult lymph node disease. In the following, a brief review of the historical background, different methods of lymph node examination, remaining questions, and future perspectives on SNB will be discussed.

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Table 1 Prospective-randomized trials on elective lymph node dissection (ELND) in melanoma patients. *WHO* World Health Organization

Trial/group	Study design	Survival analysis	Reference
WHO melanoma group trial no. 1	Extremity melanomas of all tumor thicknesses	No improved overall survival	[67]
Mayo clinic surgical trial	Mostly extremity melanomas of all tumor thicknesses	No improved overall survival	[56]
Intergroup melanoma surgical trial	1.5–4.0 mm Tumor thickness of all anatomic sites	No improved overall survival (subgroup analysis: benefit for patients younger than 60 years)	[3]
WHO melanoma group trial no. 14	Trunk melanomas of more than 1.5 mm tumor thickness	No improved overall survival (subgroup analysis: benefit for 1.5–4.0 mm melanomas)	[15]

Table 2 Current melanoma staging system (1998). American Joint Committee on Cancer/ Union Internationale Contra le Cancer (AJCC/UICC) classification regarding lymph node status (N-classification)

Lymph node (N)	
N_x	Regional lymph nodes cannot be assessed
N_0	No regional lymph node metastasis
N_1	Metastasis ≅ 3 cm in greatest dimension in any regional lymph node(s)
N_2	Metastasis >3 cm in greatest dimension in any regional lymph node(s) and/or in transit metastasis
N_{2a}	Metastasis >3 cm
N_{2b}	In transit metastases
N_{2c}	Both (N_{2a} and N_{2b})

Stage grouping

I	pT_1	N_0	M_0	pT_2	N_0	M_0
II	pT_3	N_0	M_0	pT_4	N_0	M_0
III	Any pT	N_1	M_0	Any pT	N_2	M_0
IV	Any pT	Any N	M1			

Table 3 Proposed "new" melanoma staging system [4]. Proposal of the American Joint Committee on Cancer (AJCC; 2000) regarding lymph node status (N-classification). Micrometastases are diagnosed after elective or sentinel lymphadenectomy. Macrometastases are defined as clinically detectable lymph node metastases confirmed by therapeutic lymphadenectomy or when any lymph node metastasis exhibits gross extracapsular extension

Lymph node (N)		
N_1	One lymph node	Micrometastasis Macrometastasis
N_2	Two–three lymph nodes	Micrometastasis Macrometastasis In-transit metastasis(es)/satellite(s) without metastatic lymph nodes
N_3	Four or more metastatic lymph nodes, matted lymph nodes, or combinations of in-transit met(s)/satellite(s), or ulcerated melanoma and metastatic lymph nodes	

Historical background of SNB

The concept of SNB [45] was developed over 20 years ago. In 1977, Holmes et al. described a study in patients with melanoma, where radioactive colloidal gold scanning was used to label regionally draining lymph nodes [30]. Cabanas used this procedure at the same time for carcinomas of the penis to evaluate affected regional lymph nodes in the groin [14]. The term "sentinel node" to describe the first draining lymph node was coined in 1977 by Cabanas [14].

Morton and colleagues have been pioneers of SNB in melanoma patients since they built up systematic studies on the technique and its clinical usefulness [43, 44, 45]. In the meantime, extensive experience has been gained for SNB in breast cancer [7, 40, 68, 70]. Furthermore, a limited number of clinical studies and case reports have been published on SNB technique in cutaneous B-cell lymphoma [58], thyroid cancer [7, 36], cancer of the vulva [7, 21], Merkel cell carcinoma of the skin [7, 69], squamous cell cancer of the head and neck [7], and pharyngeal and laryngeal carcinomas [71]. Thus, SNB was widely tried in different disciplines.

Clinical results in melanoma

In 1992, Morton's group [43] provided detailed information on the technical procedure of SNB in melanoma patients with clinically non-suspicious lymph nodes (pT_x; N_0;

Table 4 Proposed "new" melanoma staging system [4]. Proposal of the American Joint Committee on Cancer (AJCC; 2000) regarding lymph node status. Clinical staging includes microstaging of the primary melanoma and clinical/radiologic evaluation for metastases. By convention, it should be used after complete excision of the primary melanoma with clinical assessment for regional and distant metastases. Pathologic staging includes microstaging of the primary melanoma and pathologic information about the regional lymph nodes after partial or complete lymphadenctomy, except for pathologic stage 0 or stage 1A patients, who do not need pathologic evaluation of their lymph nodes

Proposed stage groupings							
Clinical staging				Pathologic staging			
0	Tis	N_0	M_0	0	Tis	N_0	M_0
IA	T1a	N_0	M_0	IA	T1a	N_0	M_0
IB	T1b	N_0	M_0	IB	T1b	N_0	M_0
T2a	N_0	M_0		T2a	N_0	M_0	
IIA	T2b	N_0	M_0	IIA	T2b	N_0	M_0
T3a	N_0	M_0		T3a	N_0	M_0	
IIB	T3b	N_0	M_0	IIB	T3b	N_0	M_0
T4a	N_0	M_0		T4a	N_0	M_0	
IIC	T4b	N_0	M_0	IIC	T4b	N_0	M_0
IIIA	Any T1–4a	N1	M_0	IIIA	T1-4a	N1a	M_0
IIIB	Any T1–4a	N2b	M_0	IIIB	T1-4a	N1b	M_0
			M_0		T1-4a	N2a	M_0
IIIC	Any T	N2c	M_0	IIIC	Any T	N2b, N2c	M_0
	Any T	N3	M_0		Any T	N3	M_0
IV	Any T	Any N	Any M	IV	Any T	Any N	Any M

M_0). They proposed to label the skin around the primary melanoma with blue dye, because it was known that a particular site of the skin drains to specific initial lymph nodes (SNs). Thus, it was important to identify this node(s) intraoperatively. Later, several investigators demonstrated that a preoperative lymphoscintigraphy and a combination of blue dye with radioactive tracers, such as technetium 99m increase the diagnostic accuracy of the SN identification [2, 12, 22, 62, 63]. Several authors reported on sufficient identification rates of SNs between 90% and 100% [25, 44, 60]. However, there is a clear learning curve associated with this methodology [44, 59]. Both surgical techniques and the detection of the SN using a gamma probe, in cooperation with the nuclear medicine, proved successful. Due to variations of the lymphatic drainage, SNB in the head and neck area is more difficult. Identification rates varied between 70% and 90% in this region of the body [32]. Another difficult issue associated with SNB is wide local excisions of the primary melanoma prior to SNB [35]. Since lymphatic channels could be destroyed due to previous surgical interventions, blue dye injections and lymphoscintigraphy failed to demonstrate reproducible results in some cases. An ideal scenario for reliable identification rates of the SN are previously untreated melanoma patients [35].

In a recently published multicenter trial, Gershenwald and co-workers [25] were able to convincingly demonstrate the clinical relevance of SNB. In 580 melanoma patients with at least 1.0-mm tumor thickness according to Breslow and no clinical or radiological evidence of loco-regional or distant metastasis SNB was performed. In 85 patients (15%), SN were positive for metastatic cells, whereas in 495 patients (85%), SNB status was negative using conventional histology [25]. These results correspond with similar findings of other studies on SNB and ELND.

Moreover, Gershenwald et al. compared the effect of SNB status with known "conventional" prognostic factors for primary melanoma with regard to recurrences and survival times [25]. Interestingly, the SNB status was the most significant prognostic factor by means of univariate and multivariate analyses. Although tumor thickness and ulceration of the primary tumor influenced survival in SN-negative patients, these markers provided no additional prognostic information in SN-positive melanoma patients. The 3-year survival rates for SN-positive patients accounted for 55.8%, in contrast to 88.5% for SN-negative melanoma patients [25]. Thus, an impressive distinction between patients with favorable and poor outcome is feasible using SNB.

Examination of SNs

Conventional histology/immunohistochemistry

Despite a large number of single- and multi-institutional studies on the use of SNB in malignant melanoma and breast cancer, technical details used in conventional histology were infrequently reported [2, 43, 44, 70]. Most publications on a detailed description of lymph node examination resulted from early studies using ELND in pathological stage I/II melanoma patients (Table 1).

In their earlier reports, Morton and co-workers described the detection of melanoma cells in 40 of 194 (21%) SNs upon examination of routine hematoxylin and eosin (HE)-stained slides [43]. During the development phase of the SNB technique, these authors and others used frozen-section analysis for routine histology. Later, most centers moved to permanent sections to minimize loss of diagnostic material during "facing up". The interpretation is more accurate, and immunohistochemistry will be optimized [44].

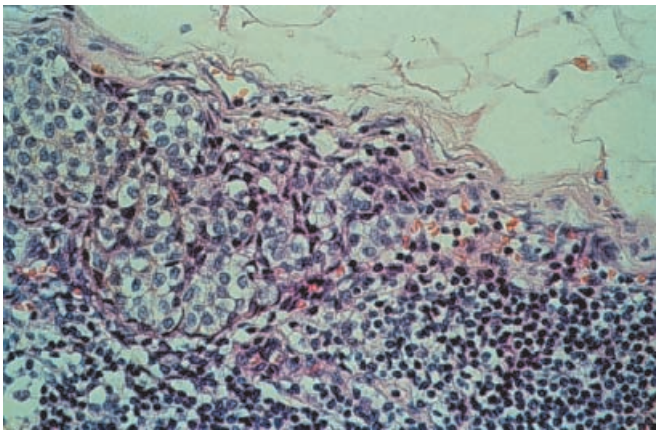

Fig. 1 Micrometastasis of melanoma in sentinel node. (Hematoxylin and eosin staining; courtesy of Dr. Hans-Jürgen Blaheta, Department of Dermatology, University of Tübingen, Germany)

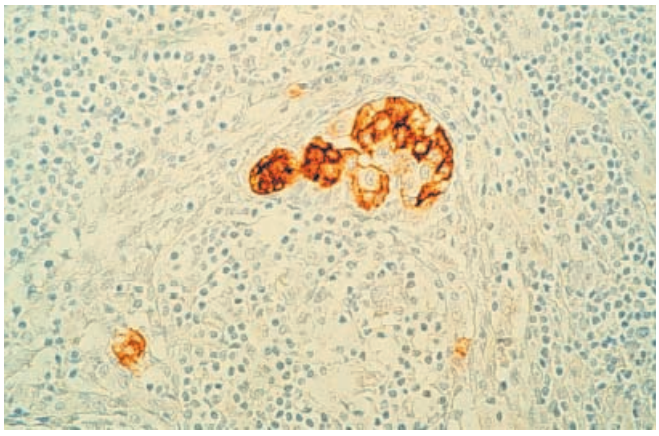

Fig. 2 Peritrabecular micrometastasis of melanoma cells in a sentinel node (immunostaining with HMB-45; Courtesy of Dr. Hans-Jürgen Blaheta, Department of Dermatology, University of Tübingen, Germany)

Table 5 Sensitivity and specificity of markers against melanoma-associated antigens detectable in sentinel nodes. +++ 90–100%; ++ 60–90%; + <60%

Antigen/marker	Sensitivity	Specificity
S-100	+++	Moderate
HMB-45	++	Moderate
NKI/C_3	+++	Moderate
MART 1/Melan-A	++	High
Tyrosinase	++[a]	High
MAGE3	+	High

[a] Weak staining of melanoma cells; unspecific background staining expression pattern in melanoma

Theoretically, each SN should be serially sectioned to extinction, but this appears expensive and clearly impractical. Therefore, Morton et al. recently recommended the following more practical procedure of SN examination [44], which is currently used at one of the most experienced SNB centers, the John Wayne Cancer Institute in Santa Monica (Calif.).

The lymph node will be cut into two halves through its longest circumference. These halves should be placed cut-face down in cassettes and fixed for a minimum of 24 h. The technician is instructed to minimize "facing up". As soon as full-faced sections can be obtained, ten serial sections are removed. Sections number 1, 3, 5, and possibly 10 are stained using HE. Sections 2 and 4 are recommended for immunohistochemical staining, while sections 6 and 7 serve as negative controls. Sections 8 and 9 could be used in cases where the technique was unsatisfactory or for additional staining [44].

Very recently, the pathologist's role in SN evaluation was described in detail [20]. Cochran emphasized not only to carefully examine the nodes for micrometastasis but, furthermore, stressed the importance of distinguishing melanoma cells from interdigitating dendritic cells, pigment containing macrophages, and nevus cells [20].

Some other authors reported previously on the importance of routine histopathology to distinguish between melanoma metastasis and melanocytic nevus cells in lymph nodes [2, 16]. Melanocytic nevi are a potential source for false-positive results in the examination of SN for metastatic melanoma. Carson et al. investigated 4821 nodes from 208 melanoma patients and revealed so-called "nodal nevi" in 22% of the cases, in contrast to previously reported frequencies of approximately 2–5% [16]. Obviously, there is a huge difference of nodal nevi in lymph nodes obtained, for instance, from female patients with breast cancer with a frequency of 0.3% compared with lymph nodes from melanoma patients [51].

Nodal nevi are located in the peripheral capsule and in the internal trabecula in 93% and 7%, respectively [16]. The detection of such nevi was feasible using HE sections alone in 78% and exclusively by means of immunohistochemistry in 22% [16]. Thus, it is important that one is aware about the appearance and morphology of nodal nevi to avoid misinterpretation as melanoma metastasis in SNs.

Immunohistochemistry in addition to HE staining (Fig. 1) is considered essential by most groups. The accuracy of the routine histology will be improved and, furthermore, a substantial number of metastatic nodes (approximately 10–15%) will be missed without immunostaining [2, 8, 19, 20, 25, 44]. As an example, in the first publication on SNB, 9% of metastatic affected SN would have been overlooked with HE staining alone [43]. Thus, the sensitivity of the detection of occult micrometastasis will increase significantly with the addition of immunohistochemistry.

Several mono- and polyclonal antibodies directed against melanoma-associated antigens (MAA) can be used for routine evaluation of paraffin-embedded specimens [61]. The sensitivity and specificity of widely used antibodies against MAA is illustrated in Table 5.

Polyclonal antibody to protein S-100 [17, 24] is expressed by virtually all benign and malignant melanocytic lesions [66]. In addition, it recognizes other neurally derived cells and antigen-presenting cells (i.e., interdigita-

ting reticular cells and Langerhans' cells) [6, 46]. Thus, misinterpretations might occur in the examination of SNs with S-100 alone.

Therefore, a monoclonal antibody to HMB-45 (gp 100) [72] with higher specificity for melanoma is used in parallel to S-100 staining [18] by most pathologists (Fig. 2). However, even HMB-45 may lead to misinterpretation since, for instance, HMB-45 positive breast carcinomas have been described [55].

Recently, Jarrett et al. reported on a comparative immunohistochemistry study in SNs from melanoma patients [33]. Antibodies to S-100 and NKI/C3, both well established with a high sensitivity [61], were compared with two new markers. Antibodies to Melan-A and tyrosinase, useful in paraffin-embedded tissue from lymph nodes, were investigated [33]. Melan-A, as a product of the MART-1 gene and a melanocytic differentiation antigen and tyrosinase, an enzyme of the melanin production pathway, did not produce additional information to S-100 and NKI/C3 alone. Whereas Melan-A demonstrated clean and effective staining, tyrosinase was less helpful, with weaker staining of melanoma cell deposits. Furthermore, a significant, unspecific background staining was present [33].

An immunohistological study on the use of monoclonal antibody 57 B, specifically detecting MAGE-3 gene protein, expression in tissue sections from melanoma revealed a high specificity (100%) but unfortunately a low sensitivity of only 44% [29]. In conclusion, S-100 and HMB-45 remain as standard markers for immunohistology, but NKI/C3 and Melan-A are further attractive candidates for the detection of melanoma cells in paraffin-embedded tissue of SN.

Molecular examination of SNs

Minimal residual metastatic disease detection is of great clinical interest, because it allows insights into the biology of metastasis and would ideally implicate clinical decisions (i.e., treatment). A sensitive polymerase chain reaction (PCR)-based technique is able to detect isolated tumor cells. Numerous studies on the detection of tyrosinase mRNA identified by reverse transcriptase (RT)-PCR were performed in peripheral blood from melanoma patients [13, 26, 31, 34, 38, 49, 54, 57]. Tyrosinase, as the key enzyme of melanin biosynthesis, is exclusively expressed by melanocytes and their malignant variant, melanoma cells. Since melanocytes are not found in peripheral blood (artificial venipuncture contamination excluded), the presence of tyrosinase RT-PCR positive cells in blood would reflect disseminated malignant melanoma.

Interestingly, the assay demonstrated a high in vitro sensitivity since single melanoma cells could be identified in 10 ml of blood in most studies, including a two-center trial from Kiel and Heidelberg [26]. However, clinical studies with melanoma patients in different stages of disease produced discrepant results. In advanced metastatic melanoma (stage IV; Table 4), the frequency of tyrosinase RT-PCR positive patients varied between 0% and 100% [13, 26, 31, 34, 49, 54]. Different techniques in the RT-PCR protocols or patient selection might have contributed to the wide range of results in different groups. Since until now no standardization has been available, RT-PCR results from peripheral blood should be considered with some caution, and tyrosinase RT-PCR should be considered "experimental".

More consistent results were reported from trials focusing on the detection of melanoma-associated antigens through RT-PCR in SNs. Blaheta et al. examined 794 regional lymph nodes from 79 different melanoma patients with a tumor thickness of at least 0.75 mm [9]. Patients with non-melanoma underlying diseases serving as negative controls were uniformly RT-PCR negative. Furthermore, this highly sensitive and specific approach was compared with immunohistology [8]. Tyrosinase RT-PCR (49% positivity) significantly increased the number of patients who expressed S-100 B and/or HMB-45 histochemically in SNs (18%) [8]. A clear correlation between RT-PCR status and Breslow's tumor thickness was reported [8, 9]. Blaheta and co-workers extended their clinical study on RT-PCR in SNs from melanoma patients and confirmed earlier results [10]. In a multivariate analysis, histopathology and status of RT-PCR remained the only significant prognostic factors for predicting disease-free survival in observed melanoma patients [10]. They concluded that tyrosinase RT-PCR in SNs may serve as a powerful tool and furthermore might be of value in future classification systems of primary cutaneous melanoma [10].

Van der Velde-Zimmermann and colleagues [64, 65] obtained similar results in a study comparing immunohistochemistry and RT-PCR in SNs. In another study published by Goydos et al., 50 melanoma patients with primary tumors underwent SNB [27]. Using standard histopathology, 10 patients (20%) showed positive SN. All of these nodes were also positive for MART-1 and tyrosinase RT-PCR. Three negative patients based on HE staining examination demonstrated a positive RT-PCR status. The authors concluded that both markers are promising for the detection of occult metastatic disease [27].

The mRNA expression of three melanoma-associated antigens (MART-1, MAGE-3, and tyrosinase) was tested by Bostick and co-workers [11]. Of 17 patients, 16 (94%) positive for HE and/or immunostaining expressed two or three mRNA markers. In addition, 20 of 55 histopathologically negative (36%) patients expressed at least two mRNA markers [11]. The authors summarized that HE- and immunohistochemical staining underestimate the true incidence of melanoma involvement in SNs. Furthermore, a survival analysis revealed that molecular diagnosis is a more powerful predictor of disease relapse than conventional methods alone [11]. Table 6 represents an estimation of survival according to the type of metastatic lymph node detection.

Table 6 An estimation of prognosis in lymph node-"positive" melanoma patients according to the type of detection. *ELND* elective lymph node dissection; *SNB* sentinel node biopsy; *LN* lymph node; *RT-PCR* reverse transcriptase polymerase chain reaction

Lymph node status		Estimated survival rates (approximately) (%)
Clinically visible/palpable lymph node metastasis		
Positive LN	1	40
	2–4	25
	5+	10
Lymph node metastasis detected in ELND		45–60
Lymph node metastasis detected in SNB		
With histology/immunohistology		50
With RT-PCR alone		60

It is of great interest that Lukowsky et al. ruled out that the SNB philosophy may miss patients with micrometastasis in non-SNs [41]. In 7 of 24 cases (29%) the SN was found to be negative with all techniques used, but in non-SNs, tyrosinase and/or gp100 (HMB-45) transcripts were observed. This study is probably the only one focusing step by step on all lymph nodes (SN and non-SN) of a given basin using morphology and tyrosinase- and gp100 mRNA detection by means of PCR. Regarding all methods applied, 54% of melanoma patients with primary tumors of more than 0.75 mm demonstrated a metastatic involvement of SNs in this particular study [41].

Remaining questions/future perspectives

Despite all efforts in the detection of micrometastasis in melanoma, the question remains, which number or types of melanoma cells cause progressive disease. The very sensitive molecular diagnosis using RT-PCR enables us to detect single melanoma cells in peripheral blood or SNs. The relatively high number of RT-PCR positive cells in SNs (approximately 50%) in patients with primary melanoma of more than 0.75 mm is surprising. In accordance with the individual distribution pattern of known risk factors in different studies carried out so far, a rate of about 30% progressive patients would have been expected. Thus, a 50% rate of submicroscopic metastatic-involved lymph nodes by far exceeds the rate of patients having relapses later. This issue was rarely discussed in published studies on RT-PCR in SNs.

Whether solitary melanoma cells can be considered as "micrometastasis" is questioned. Since the definition of metastasis includes the capacity for angiogenesis and proliferation [5], the detection of tumor cells alone, without knowledge of the particular biology of these cells, is unsatisfactory. The term "disseminated tumor cells", instead of "micrometastasis", should be preferred unless the biology of melanoma cells becomes more clear. A limited number of melanoma cells has, further, a chance of tumor escape due to immunosurveillance. Thus, interpretation of clinical studies on RT-PCR diagnosis should be considered with some caution. It appears too early to draw therapeutic decisions (i.e., radical lymphadenectomy of the entire basin) on RT-PCR findings alone.

As discussed earlier, misinterpretation of nodal nevi as melanoma cells in SNs may occasionally occur. However, when morphology becomes apparent, the distinction should be feasible in most cases. In summary, the conclusion to introduce RT-PCR in routine examinations of SNs in general appears too early. However, this experimental tool can contribute to a significantly better knowledge and understanding of tumor biology and the metastatic cascade.

SNB is not far away from being accepted as a standard care for melanoma patients. It has been clearly shown that the SNB status distinguishes melanoma patients with different prognosis. Multiple clinical studies demonstrated SNB as an excellent prognostic factor. This selective surgical approach will avoid ELND with the associated damage (i.e., lymph edema) in melanoma patients probably cured [52]. Whether treatment decisions can be drawn from histologic and immunohistologic examinations of the SN is yet unclear. However, SNB status appears as the most attractive stratification factor for adjuvant treatment modalities. Interferon α is registered for the adjuvant treatment of high-risk melanoma patients in most countries, but only roughly 10% of treated patients may profit from therapy [28, 37, 48]. Interferon α-responding patients cannot be identified so far. Therefore, a careful patient selection for interferon treatment schedules is mandatory. Forthcoming clinical trials in the adjuvant setting will probably include SNB status as a stratification factor in the study design.

Lastly, it has to be clarified whether SNB has an impact in the overall survival of melanoma patients [52]. At present, it is too early to speculate on such effects, but prospective-randomized trials (i.e., JWCI-trial and SUNBELT-trial, USA) are currently underway to give more detailed answers on remaining important questions on the outcome of patients.

In the year 2000, SNB can be considered as a relatively "new" interesting diagnostic tool with unclear therapeutic implications. Carefully conducted examinations of the SNs using conventional histology (HE staining) and immunohistology (S-100, HMB-45, MART-1) are necessary to avoid a miss of melanoma cells in SN. The mRNA detection of melanoma-associated antigens (tyrosinase, MART-1, gp100, and others) remains experimental unless conclusive results on its clinical value are available.

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