Defining optimal oncolytic virus treatment and diagnostics in high risk melanoma patients

Stahlie, E.H.A.

CHAPTER 8

The value of lymph node ultrasound and whole body ^{18}F-FDG PET/CT in stage IIB/C melanoma patients prior to SLNB

Emma H.A. Stahlie
Bernies van der Hiel
Annemarie Bruining
Bart van de Wiel
Yvonne M. Schrage
Michel W.J.M. Wouters
Winan J. van Houdt
Alexander C.J. van Akkooi

European Journal of Surgical Oncology 2021 May;47(5):1157-1162.

ABSTRACT

Background
Stage IIB/IIC(8th AJCC) melanoma patients are known to have high-risk primary tumors, however they follow the same routine to sentinel lymph node biopsy(SLNB) as more low risk tumors. Guidelines are not conclusive regarding the use of preoperative imaging for these patients. The aim of this pilot study was to assess the value of ultrasound(US) and ^{18}F-FDG PET/CT prior to lymphoscintigraphy(LSG) and SLNB for stage IIB/C melanoma patients.

Methods
From 2019-04 till 2020-01, all stage IIB/C melanoma patients underwent US of the regional lymph nodes and whole body ^{18}F-FDG PET/CT before their planned LSG and SLNB. Suspected metastases were confirmed with fine needle aspiration (FNA), prior to surgery.

Results
In total 23 patients were screened: six had metastases detected by imaging, two by US, one by ^{18}F-FDG PET/CT and three were detected by both imaging modalities. All metastases were nodal and therefore treatment was altered to lymph node dissection and all but one also received adjuvant therapy. Eight (47%) of the 17 patients without macroscopic disease, still had a positive SN. Sensitivity, specificity and false negative rate for US and ^{18}F-FDG PET/CT were 36%, 89%, 64% and 29%,100% and 71%, respectively.

Conclusion
Preoperative negative imaging does not exclude the presence of SN metastases, therefore SLNB cannot be foregone. However, US detected metastases in 22% of patients, altering their treatment, which suggests it is effective in the work-up of stage IIB/C melanoma. Staging with ^{18}F-FDG PET/CT is not of added value prior to LSG and SLNB and should therefore not be used.

INTRODUCTION

Currently, most guidelines recommend patients with a pT1b melanoma or higher undergo LSG followed by SLNB, which is used to assess the status of the regional lymph node basin [1]. The pathological assessment of the excised SN's is seen as the strongest prognostic factor for disease recurrence or death due to melanoma for patients with stage I or II melanoma, until the SLNB might be replaced in the future by molecular predictors, such as gene expression profiles [2-5].

However, it is known that patients have a higher risk of developing nodal or distant metastases when having a thick and ulcerated primary tumor, which are staged as IIB and IIC melanomas according to 8th American Joint Committee on Cancer (AJCC). Besides this, these recurrences often develop at an early stage. [6, 7]. Therefore, as macroscopic disease is possibly already present in patients with stage IIB/C melanomas, the SLNB might not be the preferred approach for everyone and alternative methods should be considered.

In the follow-up setting, US as imaging modality is routinely used to exclude nodal metastases, as many studies have proven it to be superior to physical examination[8]. In patients with newly diagnosed melanomas, the use of preoperative US for the evaluation of the regional lymph nodes is still doubted [9]. Results of various studies have shown insufficient proof for replacing SLNB with US, whether guided by fine needle cytology (FNAC) or not. However, several of them have also reported an increase in sensitivity for selected patients with thick and/or ulcerated melanomas, as they are susceptible for larger metastases in the lymph nodes [10-13].

As visceral metastases cannot be accurately detected by US, investigating ^{18}F-FDG PET/CT as second preoperative imaging modality could be of interest. Studies have already shown that Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography (^{18}F-FDG PET/CT) has become invaluable for restaging melanoma patients and during follow-up, mostly for finding distant metastases [14]. Although evidence for the use of ^{18}F-FDG PET/CT for primary staging is lacking, many physicians still use this imaging tool to rule out metastases in newly diagnosed stage IIB/C melanoma patients with thick and ulcerated primary tumors [15].

The aim of this pilot study was to investigate the value of US of the regional lymph nodes and whole body ^{18}F-FDG PET/CT prior to LSG and SLNB in patients with thick and ulcerated melanomas. We hypothesize that the use of ^{18}F-FDG PET/CT in this setting has its limitations and by proving this, we hope to reduce unnecessary usage.

PATIENTS AND METHODS

Patients

This prospective pilot study included patients with a histologically proven primary melanoma staged as IIB or IIC according to the 8th AJCC [16]. Stage IIB and IIC were defined as patients with a primary melanoma with a Breslow thickness >2mm with ulceration and with a Breslow thickness >4mm without ulceration. This therefore concerned all patients with a primary tumor staged as pT3b, pT4a or pT4b. Patients with palpable nodal metastases or symptomatic distant metastases detected by S100B prior to LSG and SLNB, were excluded. Included patients were planned for wide local excision and SLNB at the Netherlands Cancer Institute - Antoni van Leeuwenhoek. The study was reviewed by the Medical Ethics Review Committee of our institute and assessed as non WMO (Medical Research Involving Human Subjects Act), therefore no informed consent was needed.

Screening protocol

The screening protocol, introduced in 2019-04, consisted of a whole body ^{18}F-FDG PET/CT and US of the regional lymph node basin which was guided by FNAC in case of suspicious lymph nodes, both performed before the routine LSG and SLNB. In case of suspicious distant lesions detected by ^{18}F-FDG PET/CT, cytologic puncture or histologic biopsy was performed in order to prove metastases. Figure 1 shows a flowchart of the protocol. Only patients who underwent US as well as whole body ^{18}F-FDG PET/CT, were included in this study.

Patients who had no metastases detected by the preoperative ^{18}F-FDG PET/CT or US, proceeded to undergo LSG and SLNB. SN-positive patients (microscopic disease) entered, depending on their tumor burden, follow-up guided by US or started 1-year adjuvant systemic therapy. SN-negative patients entered standard follow-up, consisting of appointments with the nurse practitioner and dermatologist: 3-monthly the first year and 6-monthly the following 2-5 years.

Patients with metastases (macroscopic disease) detected by preoperative ^{18}F-FDG PET/CT or US, confirmed by cytology, did not undergo LSG or SLNB. Patients with regional lymph node metastases underwent lymph node dissection (LND) followed by adjuvant therapy.

Preoperative imaging and interpretation

Whole body ^{18}F-FDG PET/CT imaging was conducted on a cross-calibrated Phillips Gemini TF time-of-flight 16 or Phillips Gemini TF big-bore PET/CT scanner (Cleveland, USA). Abnormal FDG accumulation was evaluated according to location, size and intensity. Images were assessed by experienced nuclear

medicine physicians and the treating surgeon, who took the clinical setting into account.

US examinations were performed using the Philips EPIQ 7 Ultrasound (Bothell, Washington, USA). Lymph nodes that presented with a loss of fatty hilum, cortical nodules, a short axis diameter of >1cm, a convex aspect and/or blurred margins were considered suspicious. In case of suspicious lymph nodes, US-guided FNAC was performed. Images were assessed and FNAC was performed by experienced radiologists.

US images were considered to be true positive (TP) when suspicious lesions were proven metastases by FNAC and false positive (FP) when FNAC failed to prove metastases. US images were considered to be true negative (TN) when US was negative and SLNB failed to detect SN metastases. US images were considered false negative (FN) when US was negative but SLNB did detect SN metastases or when US failed to detect metastases but ^{18}F-FDG PET/CT did detect cytologically or proven metastases.

^{18}F-FDG PET/CT images were considered to be TP when suspicious lesions were proven metastases by FNAC of histological biopsy and FP when these failed to prove metastases. ^{18}F-FDG PET/CT was considered to be TN when ^{18}F-FDG PET/CT was negative and the SLNB failed to detect SN metastases. ^{18}F-FDG PET/CT images were considered FN when ^{18}F-FDG PET/CT was negative but the SLNB did detect SN metastases or when ^{18}F-FDG PET/CT failed to detect metastases but US did detect FNAC-proven metastases.

Histopathological assessment of the SN was done according to the EORTC protocol, described in detail by Cook et al[17].

Statistics

Baseline characteristics were recorded in a prospective database and summarized using descriptive statistics in the Statistical Package for Social Sciences for Windows version 25.0 (IBM). Sensitivity/true positive rate (TPR), specificity/true negative rate (TNR), positive- and negative predictive value (PPV, NPV) were determined using standard definitions.

RESULTS

In total 23 patients entered the screening protocol between 2019-04 and 2020-01. Table 1 summarizes the baseline characteristics. Most patients were male (70%) and the median age was 74 years at the time of presentation. 48% of

patients had a melanoma primary tumor staged as pT4b, 13% staged as pT4a and 39% staged as pT3b. Most patients had a nodular melanoma (39%).

			N	%
Sex	Female		7	30
	Male		16	70
Median age, years (range)		74 (37 – 85)		
T-stage (8th AJCC)	pT3b		9	39
	pT4a		3	13
	pT4b		11	48
Stage (8th AJCC)	IIB		12	52
	IIC		11	48
Location	Extremity		13	57
	Trunk		10	44
	Head/neck		0	0
Subtype	Superficial		4	17
	Nodular		9	39
	Acral lentiginous		8	35
	Other		2	9
Microsatellites	Absent		18	78
	Present		3	13
	Unknown		2	9

Table 1. *Baseline characteristics (n=23). Abbreviations: AJCC, American Joint Committee on Cancer.*

Screening protocol

A summary of outcomes is shown in Figure 1. As each patient underwent both imaging modalities: a total of 23 ^{18}F-FDG PET/CT's and 23 US's were performed. Overall six patients (26%) had macroscopic disease detected by preoperative imaging. Three patients had macroscopic disease that was detected by ^{18}F-FDG PET/CT as well as US. Two patients had metastases discovered only by US, both patients had axillary hypermetabolic lymph nodes on ^{18}F-FDG PET/CT of which no clear distinction could be made between reactive lymph nodes and metastases, with a slight preference for reactive. A single patient had metastases discovered only by ^{18}F-FDG PET/CT. He had two FDG-avid axillary lymph nodes, which were only recognized as pathologically enlarged by US on the second attempt to obtain cytology.

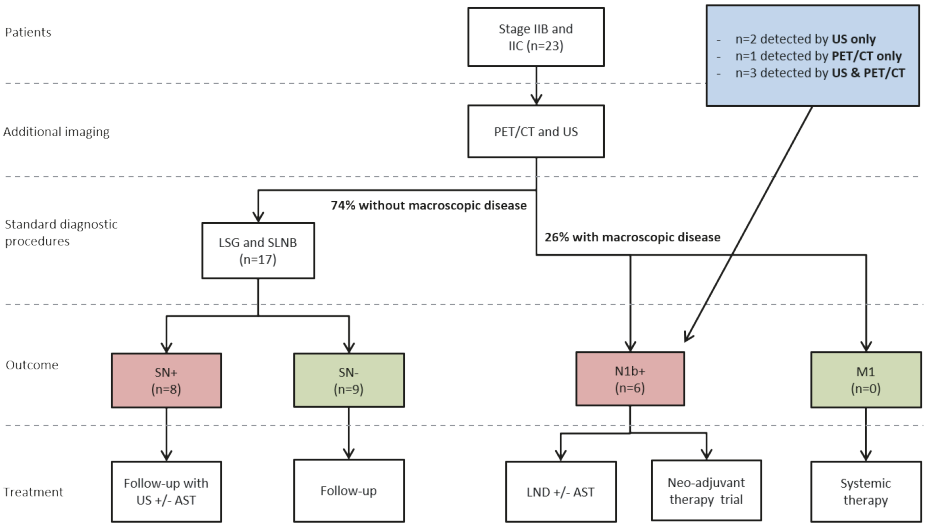

Figure 1. *Flowchart of the order of examinations, procedures and outcomes. Abbreviations: US, ultrasound; LSG: lymphoscintigraphy; SN, sentinel node; SLNB, sentinel lymph node biopsy; N1b+, macroscopic nodal disease; M1, distant metastases; AST, adjuvant therapy; LND, lymph node dissection. (1,5-column fitting image)*

Table 2 summarizes all characteristics of patients with macroscopic disease. All metastases (n=6) detected by preoperative imaging were found in the regional lymph node basin: five in the axilla and one inguinal, all confirmed by US-guided FNAC. The metastases were equally divided over patients with pT3b, pT4a and pT4b primary tumors. Patients had an average of one positive lymph node, most tumor deposits were >4.0mm (Table 3).

		N	%
Detected by	^{18}F-FDG PET/CT	4	67
	US	5	83
	^{18}F-FDG PET/CT and US	3	50
Primary tumor, T-stage	pT3b	2	33
	pT4a	2	33
	pT4b	2	33
Location	Axilla	5	83
	Inguinal	1	17
Treatment	Re-excision + LND	1	17
	Re-excision + LND + adjuvant therapy	5	83

Table 2. *Characteristic of patients and tumor with macroscopic disease detected by preoperative imaging (n=6). Abbreviations: US, ultrasound; ^{18}F-FDG PET/CT, Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography; LND, lymph node dissection.*

A total of 17 patients (74%) had no macroscopic disease detected by ^{18}F-FDG PET/CT or US. Subsequently, these patients underwent LSG followed by SLNB. A positive SN was found in eight patients (47%). Patients had an average of two positive SN and most SN tumor deposits were between >1.0mm and <4.0mm (50%) (Table 3).

		N	%
Number of patients with microscopic disease		8	100
Size SN metastases	<0.1mm	0	0
	>1.0 and <1.0mm	2	25
	>1.0 and <4.0mm	4	50
	>4.0mm	2	25
Number of SN with metastases	1	2	25
	2	3	38
	3	2	25
	>4	1	13
Number of patients with macroscopic disease		6	100
Size LN metastases	<0.1mm	0	0
	>1.0 and <1.0mm	0	0
	>1.0 and <4.0mm	1	17
	>4.0mm	5	83
Number of LN with metastases	1	3	50
	2	1	17
	3	1	17
	>4	0	0

Table 3. *Histopathological tumor burden of largest SN excised during SLNB and largest LN after LND. Abbreviations: SN, sentinel node; LN, lymph node.*

Management of detected metastases

Five patients with macroscopic disease detected by preoperative imaging, underwent wide local excision combined with LND followed by adjuvant therapy. One patient underwent wide local excision combined with LND followed by follow-up, as histopathology of the primary tumor and the LN metastasis, matched with a pigmented epitheloid melanocytoma, which is considered a more indolent type of cancer. The LN metastasis showed mostly tumoral melanosis. Therefore, adjuvant therapy was not advised. These patients were restaged to IIIC melanoma. Patients with a negative SN identified by SLNB entered follow-up. Of the 8 patients with a positive SN, 7 patients started adjuvant therapy. One patient had progression in an axillary lymph node, detected by ^{18}F-FDG PET/CT between surgery and the start of adjuvant therapy, for which he underwent LND. Subsequently, he received adjuvant therapy. This patient was restaged to stage IIID melanoma. The other SN-positive patients were restaged to IIIC melanoma.

Interpretation of imaging

Five (36%) US images were considered to be TP, as there were 5 metastases detected by US. One image (11%) was found to be FP, as it showed suspicious lesions but FNAC failed to prove metastases. Eight US images (89%) were considered to be TN, as these patients also had a negative SN. Finally, 9 images (64%) were considered to be FN: 8 patients had negative US images but a positive SN and for one patient US failed to detect macroscopic disease which was detected by ^{18}F-FDG PET/CT.

Four ^{18}F-FDG PET/CT images (29%) were considered to be TP, as there were 4 metastases detected by ^{18}F-FDG PET/CT. No images (0%) were found to be FP. Nine ^{18}F-FDG PET/CT images (100%) were considered to be TN, as these patients also had negative SN. Finally, 10 images (71%) were considered to be FN: 8 patients had negative ^{18}F-FDG PET/CT images but a positive SN and for 2 patients ^{18}F-FDG PET/CT failed to detect macroscopic disease which was detected by US.

The sensitivity, specificity, PPV and NPV of US to detect regional lymph node metastases in newly diagnosed stage IIB or IIC melanoma patients was 36%, 89%, 83% and 47% respectively. The TPR, TNR, PPV and NPV of ^{18}F-FDG PET/CT to detect regional lymph node and distant metastases was 29%, 100%, 100% and 47% (Table 4).

	Sensitivity (95% CI)	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**
US	36 (13 – 65)	89 (52 – 100)	83 (41 – 97)	47 (36 – 58)
Whole body ^{18}F-FDG PET/CT	29 (8 – 58)	100 (66 – 100)	100	47 (39 – 56)

Table 4. *Diagnostic test accuracy. Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.*

Incidental findings

A total of 14 incidental findings not suspicious of melanoma were detected by ^{18}F-FDG PET/CT. Three patients had aspecific nodes in the lung which required follow-up. In five patients ^{18}F-FDG PET/CT revealed findings that needed further diagnostic examination. These were polyps in the colon, reactive changes in the gastric mucosa, a nonspecific cyst in the thyroid and for two patients benign follicular nodes in the thyroid. Six findings that did not need further examination were described: Schmorl's node, concrement in the kidney, synovitis of the femoral head, dilated aorta ascendens, adrenal adenoma and non-specific skin inflammation.

DISCUSSION

This prospective pilot study is the first to evaluate the use of both US of the regional lymph nodes and whole-body ^{18}F-FDG PET/CT prior to LSG and SLNB in stage IIB/C melanoma patients and demonstrates that they have a high risk of developing macroscopic disease prior to SLNB. Preoperative imaging detected only regional lymph node metastases in six patients (26%), altering their treatment. Negative imaging outcomes did not exclude the presence of SN metastases, emphasizing the role of the SLNB in these patients. However, as five metastases were detected by US, we do believe it may complement the procedure.

Multiple studies have been published about the use of preoperative US +/- FNAC and have concluded that it is not a reliable alternative to SLNB to identify clinically occult nodal metastases. Especially the reported TPR's are low, varying between 7.1-33.8% for the studies with more than 300 patients [9, 11, 12, 18]. Variations between these studies are among other things, due to differences in baseline characteristics and whether studies combined US with FNAC or not. Up to now only the Voit group, using the so-called US Berlin morphology criteria for the assessment of their lymph nodes, has repeatedly succeeded in reporting superior results, with sensitivity rates varying between 51-82% using US-guided FNAC [10, 19, 20]. Unfortunately, to date, others have been unable to reproduce the same outcomes. This study shows a low sensitivity rate of 36% and for US-guided FNAC prior to SLNB. This is because many patients with a negative US, still had a positive SN and these US's were considered FN (n=9). However, the PPV was very high. Therefore, we agree that the SLNB cannot be replaced by the preoperative US we offer patients in our hospital, but we do think this imaging modality can be an adjunct to the procedure, in which it can save unnecessary anesthetics and surgery in 22% of pT3b or higher melanomas.

Although the SLNB is minimally invasive, it has a complication rate of approximately 5-10% worldwide, with studies describing wound infections, seroma and lymphedema[21]. Besides reducing SLNB-related morbidity by avoiding the procedure, costs could be spared by introducing all stage IIB/C melanoma patients to a preoperative US. Van Akkooi et al. calculated that an average SN procedure costs €1254 and a US-guided FNAC costs €169. For each patient that could be spared a SLNB, that is 1 in 4 / 1 in 5 in our study, a total of €1086 can be saved, not to mention the costs from potential additional morbidity associated with it [22].

Another advantage of detecting pathological lymph nodes prior to LSG and SLNB, lie in the possibility of foregoing SLNB and proceeding directly to LND or entering a neo-adjuvant trial. The latter are still under investigation, however

the first published results look very promising: high pathological response rates were achieved in up to approximately 80% of patients and nearly all of these were durable[23-25]. Subsequently, the PRADO trial (NCT #02437279), is investigating if the therapeutic LND can be foregone in patients with a complete pathological response on the index node after having received neo-adjuvant ipilimumab and nivolumab[26]. Therefore, in the future, detecting nodal metastases prior to the SLNB could lead to the omission of two surgical procedures.

In our study, four regional lymph node metastases and no distant metastases were detected by ^{18}F-FDG PET/CT. We showed a low sensitivity of 29% for the detection of metastases by ^{18}F-FDG PET/CT in this patient group. These results are similar to those of a study by Wagner et al., who concluded that ^{18}F-FDG PET/CT does not seem to be an effective imaging modality for detecting distant metastases in patients with an ulcerated melanoma with a Breslow thickness > 1mm or > 4mm [27]. Two studies that investigated the use FDG PET at baseline in patients with melanoma with a Breslow thickness > 1mm only, also failed to prove the utility of this imaging technique for the detection of clinically occult distant metastases [28, 29]. Both studies reported that no distant metastases were revealed by PET, not even in the high-risk patients. This corresponds with our results, as no distant metastases were detected by ^{18}F-FDG PET/CT in a group with only high-risk (pT3b, pT4a and pT4b) melanoma patients. Wagner et al. did however report a high PPV of 100% for nodal involvement in newly diagnosed high-risk melanoma patients. [27] Our results also showed a PPV of 100% for the detection of metastases, but as these were only nodal, they could also be interpreted as the PPV for the identification of regional lymph node metastases. As only 1 of 6 patients had regional metastases detected by ^{18}F-FDG PET/CT only, we recommend using solely preoperative US prior to SLNB. In this pilot study, the addition of ^{18}F-FDG PET/CT was not of added value and only led to unnecessary costs (approximately €1600 per scan) [30].

These conclusions do warrant careful interpretation, as this is a pilot study with a small sample size. One US and no ^{18}F-FDG PET/CT scans were assessed as FP, but one can imagine that these numbers can rise when the sample size is larger. FP scans lead to extra diagnostics tests, which lead to an increase in costs and can create a stressful situation for the patient. In this case, there were a significant number of incidental finding on ^{18}F-FDG PET/CT that were not considered suspicious for melanoma metastases, but did require further diagnostics. It is also important to bear in mind that this study was conducted at the Netherlands Cancer Institute, a specialized center that unintentionally might attract melanomas with a high-risk profile. Therefore, the rate of detected microscopic and macroscopic metastases might be higher than in a population in a general hospital.

We believe that nodal staging with US as adjunct to SLNB is useful in the work-up of stage IIB and IIC melanoma, as it can lead to alterations in treatment and prevent unnecessary surgery. However, as US as well as whole body ^{18}F-FDG PET/CT showed low sensitivity rates of 36% and 29%, we conclude that both are clinically unreliable to perform without SLNB.

REFERENCES

1. Michielin O, van Akkooi ACJ, Ascierto PA, Dummer R, Keilholz U. Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-updagger. *Ann Oncol* 2019; 30(12): 1884-1901.'doi': 10.1093/annonc/mdz411
2. Morton DL, Thompson JF, Cochran AJ, Mozzillo N, Nieweg OE, Roses DF, et al. Final Trial Report of Sentinel-Node Biopsy versus Nodal Observation in Melanoma. *N Engl J Med* 2014; 370(7): 599-609.'doi': 10.1056/NEJMoa1310460
3. Gershenwald JE, Thompson W, Mansfield PF, Lee JE, Colome MI, Tseng CH, et al. Multi-institutional melanoma lymphatic mapping experience: the prognostic value of sentinel lymph node status in 612 stage I or II melanoma patients. *J Clin Oncol* 1999; 17(3): 976-83.'doi': 10.1200/jco.1999.17.3.976
4. Mulder E, Dwarkasing J, Tempel D, van der Spek A, Bosman L, Verver D, et al. Validation of a clinicopathological and gene expression profile model for sentinel lymph node metastasis in primary cutaneous melanoma. *Br J Dermatol* 2020.'doi': 10.1111/bjd.19499
5. Brunner G, Falk TM, Ertas B, Berking C, Schulze H-J, Blödorn-Schlicht N. Validation, in silico and in vitro, of a gene-signature based risk score in cutaneous melanoma. *J Clin Oncol* 2017; 35(15_suppl): 9560-9560.'doi': 10.1200/JCO.2017.35.15_suppl.9560
6. Brauer JA, Wriston CC, Troxel AB, Elenitsas R, Shin DB, Guerry D, et al. Characteristics associated with early and late melanoma metastases. *Cancer* 2010; 116(2): 415-23.'doi': 10.1002/cncr.24724
7. Balch CM, Soong SJ, Gershenwald JE, Thompson JF, Reintgen DS, Cascinelli N, et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol* 2001; 19(16): 3622-34.'doi': 10.1200/jco.2001.19.16.3622
8. Bafounta ML, Beauchet A, Chagnon S, Saiag P. Ultrasonography or palpation for detection of melanoma nodal invasion: a meta-analysis. *Lancet Oncol* 2004; 5(11): 673-80.'doi': 10.1016/s1470-2045(04)01609-2
9. Thompson JF, Haydu LE, Uren RF, Andtbacka RH, Zager JS, Beitsch PD, et al. Preoperative Ultrasound Assessment of Regional Lymph Nodes in Melanoma Patients Does not Provide Reliable Nodal Staging: Results from a Large Multicenter Trial. *Ann Surg* 2019.'doi': 10.1097/sla.0000000000003405
10. Voit CA, Gooskens SL, Siegel P, Schaefer G, Schoengen A, Rowert J, et al. Ultrasound-guided fine needle aspiration cytology as an addendum to sentinel lymph node biopsy can perfect the staging strategy in melanoma patients. *Eur J Cancer* 2014; 50(13): 2280-8.'doi': 10.1016/j.ejca.2014.05.027
11. Chai CY, Zager JS, Szabunio MM, Marzban SS, Chau A, Rossi RM, et al. Preoperative ultrasound is not useful for identifying nodal metastasis in melanoma patients undergoing sentinel node biopsy: preoperative ultrasound in clinically node-negative melanoma. *Ann Surg Oncol* 2012; 19(4): 1100-6.'doi': 10.1245/s10434-011-2172-7
12. Sanki A, Uren RF, Moncrieff M, Tran KL, Scolyer RA, Lin HY, et al. Targeted high-resolution ultrasound is not an effective substitute for sentinel lymph node biopsy in patients with primary cutaneous melanoma. *J Clin Oncol* 2009; 27(33): 5614-9.'doi': 10.1200/jco.2008.21.4882

13. Voit CA, van Akkooi AC, Schafer-Hesterberg G, Schoengen A, Schmitz PI, Sterry W, et al. Rotterdam Criteria for sentinel node (SN) tumor burden and the accuracy of ultrasound (US)-guided fine-needle aspiration cytology (FNAC): can US-guided FNAC replace SN staging in patients with melanoma? *J Clin Oncol* 2009; 27(30): 4994-5000.'doi': 10.1200/jco.2008.19.0033
14. Xing Y, Bronstein Y, Ross MI, Askew RL, Lee JE, Gershenwald JE, et al. Contemporary diagnostic imaging modalities for the staging and surveillance of melanoma patients: a meta-analysis. *J Natl Cancer Inst* 2011; 103(2): 129-42.'doi': 10.1093/jnci/djq455
15. Dinnes J, Ferrante di Ruffano L, Takwoingi Y, Cheung ST, Nathan P, Matin RN, et al. Ultrasound, CT, MRI, or PET-CT for staging and re-staging of adults with cutaneous melanoma. *Cochrane Database Syst. Rev.* 2019(7).'doi': 10.1002/14651858.CD012806.pub2
16. Gershenwald JE, Scolyer RA. Melanoma Staging: American Joint Committee on Cancer (AJCC) 8th Edition and Beyond. *Ann Surg Oncol* 2018; 25(8): 2105-2110.'doi': 10.1245/s10434-018-6513-7
17. Cook MG, Massi D, Szumera-Cieckiewicz A, Van den Oord J, Blokx W, van Kempen LC, et al. An updated European Organisation for Research and Treatment of Cancer (EORTC) protocol for pathological evaluation of sentinel lymph nodes for melanoma. *Eur J Cancer* 2019; 114: 1-7.'doi': 10.1016/j.ejca.2019.03.010
18. Marone U, Catalano O, Caraco C, Anniciello AM, Sandomenico F, Di Monta G, et al. Can high-resolution ultrasound avoid the sentinel lymph-node biopsy procedure in the staging process of patients with stage I-II cutaneous melanoma? *Ultraschall Med* 2012; 33(7): E179-e185.'doi': 10.1055/s-0032-1312827
19. Voit C, Van Akkooi AC, Schafer-Hesterberg G, Schoengen A, Kowalczyk K, Roewert JC, et al. Ultrasound morphology criteria predict metastatic disease of the sentinel nodes in patients with melanoma. *J Clin Oncol* 2010; 28(5): 847-52.'doi': 10.1200/jco.2009.25.7428
20. Voit C, Kron M, Schafer G, Schoengen A, Audring H, Lukowsky A, et al. Ultrasound-guided fine needle aspiration cytology prior to sentinel lymph node biopsy in melanoma patients. *Ann Surg Oncol* 2006; 13(12): 1682-9.'doi': 10.1245/s10434-006-9046-4
21. Moody JA, Ali RF, Carbone AC, Singh S, Hardwicke JT. Complications of sentinel lymph node biopsy for melanoma - A systematic review of the literature. *Eur J Surg Oncol* 2017; 43(2): 270-277.'doi': 10.1016/j.ejso.2016.06.407
22. van Akkooi AC, Voit CA, Verhoef C, Eggermont AM. Potential cost-effectiveness of US-guided FNAC in melanoma patients as a primary procedure and in follow-up. *Ann Surg Oncol* 2010; 17(2): 660-2.'doi': 10.1245/s10434-009-0807-8
23. Amaria RN, Menzies AM, Burton EM, Scolyer RA, Tetzlaff MT, Antdbacka R, et al. Neoadjuvant systemic therapy in melanoma: recommendations of the International Neoadjuvant Melanoma Consortium. *Lancet Oncol* 2019; 20(7): e378-e389.'doi': 10.1016/s1470-2045(19)30332-8
24. Rozeman EA, Menzies AM, van Akkooi ACJ, Adhikari C, Bierman C, van de Wiel BA, et al. Identification of the optimal combination dosing schedule of neoadjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma (OpACIN-neo): a multicentre, phase 2, randomised, controlled trial. *Lancet Oncol* 2019; 20(7): 948-960.'doi': 10.1016/s1470-2045(19)30151-2
25. Blank CU, Rozeman EA, Fanchi LF, Sikorska K, van de Wiel B, Kvistborg P, et al. Neoadjuvant versus adjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma. *Nat Med* 2018; 24(11): 1655-1661.'doi': 10.1038/s41591-018-0198-0

26. Reijers I, Rozeman EA, Menzies AM, Wiel BAVD, Eriksson H, Suijkerbuijk K, et al. Personalized response-driven adjuvant therapy after combination ipilimumab and nivolumab in high-risk resectable stage III melanoma: PRADO trial. *J Clin Oncol* 2019; 37(15_suppl): article number TPS9605.'doi': 10.1200/JCO.2019.37.15_suppl.TPS9605
27. Wagner T, Chevreau C, Meyer N, Mourey L, Courbon F, Zerdoud S. Routine FDG PET-CT in patients with a high-risk localized melanoma has a high predictive positive value for nodal disease and high negative predictive value for the presence of distant metastases. *J Eur Acad Dermatol Venereol* 2012; 26(11): 1431-5.'doi': 10.1111/j.1468-3083.2011.04312.x
28. Clark PB, Soo V, Kraas J, Shen P, Levine EA. Futility of fluorodeoxyglucose F 18 positron emission tomography in initial evaluation of patients with T2 to T4 melanoma. *Arch Surg* 2006; 141(3): 284-8.'doi': 10.1001/archsurg.141.3.284
29. Hafner J, Schmid MH, Kempf W, Burg G, Kunzi W, Meuli-Simmen C, et al. Baseline staging in cutaneous malignant melanoma. *Br J Dermatol* 2004; 150(4): 677-86.'doi': 10.1111/j.0007-0963.2004.05870.x
30. Bastiaannet E, Uyl-de Groot CA, Brouwers AH, van der Jagt EJ, Hoekstra OS, Oyen W, et al. Cost-effectiveness of adding FDG-PET or CT to the diagnostic work-up of patients with stage III melanoma. *Ann Surg* 2012; 255(4): 771-6.'doi': 10.1097/SLA.0b013e31824a5742