Stage III melanoma, a heterogeneous group of patients

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Aspects of diagnosis, staging and treatment

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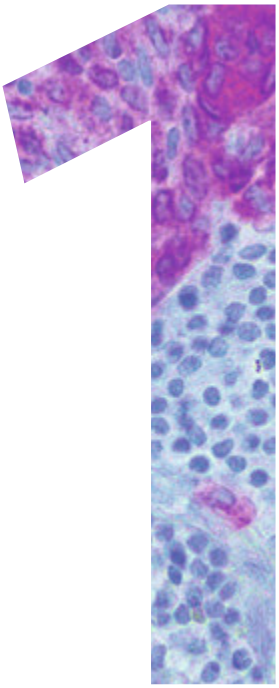
Contents

CHAPTER



Introduction and outline of this thesis

Introduction

It is 1820. A country general practitioner in Stourbridge in the Midlands of England, William Norris, writes about his patient's disease, carefully documenting the family history and describing his patient's deterioration and ultimate demise as the 'fungoid disease' progresses. He even performs an autopsy and describes the trail of disease along the anatomical structures. This is one of the first case reports on melanoma, the most aggressive form of skin cancer, published in a scientific journal.[1] The article provides a fascinating peek into history, when the only treatment was to cut away visible and palpable tumor and alleviate the pain with opium. Nowadays, we have an ever-increasing range of options to diagnose and treat patients with melanoma.[2-4] With such a broad range of tools in our toolbox, selecting the right one for the right patient at the right time is of great importance.

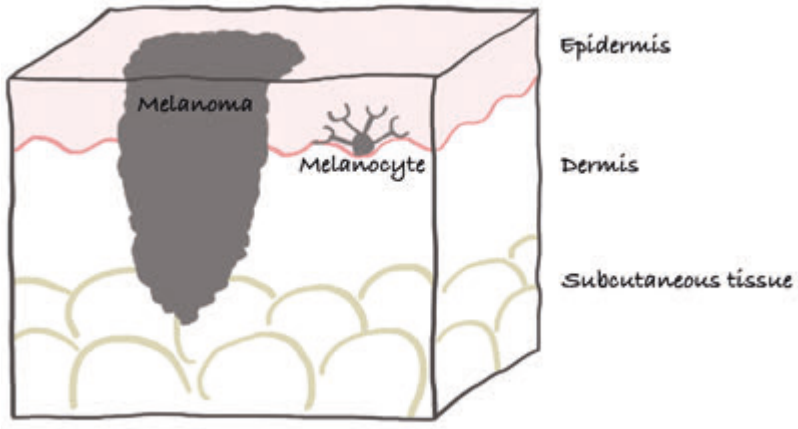


Figure 1.1 Anatomy of the skin and melanoma

Cutaneous melanoma is a cancer that arises in melanocytes and naevus cells.[5] It develops in pre-existing naevi in about 30% of cases, with the remaining 70% of cases thought to arise de novo.[6] Melanocytes are the highly specialised cells located in the upper layer of the skin that are responsible for the production of melanin, the pigment that gives the skin its tan colour. The progression from pigment cells to melanoma is a complex process involving many steps, and mutations in multiple genes.[7,8] Cumulative DNA damage from repetitive excessive UV radiation is considered to be the major causative factor, but melanoma can also be caused by mutations unrelated to UV-radiation and melanoma susceptibility can also be inherited.[9,10] In 2021, 7530 people were newly diagnosed with melanoma in the Netherlands (incidence 32 cases per 100,000 persons) and 17,207 in Australia, (incidence 56 cases per 100,000 persons). These incidence rates have increased steadily over the last 20 years in both countries.[11,12]

Staging and prognosis

After gaining the ability to grow vertically and break through the basement membrane of the skin, tumor cells can travel through the lymphatic and/or vascular system to other areas of the body. The chance of metastasizing depends on several primary tumor characteristics, of which tumor thickness and ulceration are the strongest predictors.[13] Satellite and in-transit metastases are typical for melanoma and represent intralymphatic spread. Satellites are defined as visible or palpable metastases located within 2 cm of the primary tumor. Microsatellites are located adjacent to the primary tumor and only visible under a microscope. In-transit metastases are cutaneous or subcutaneous lymphatic metastases that are more than 2 cm from the primary tumor but not beyond the regional nodal field. Regional lymph node metastases are due to tumor spread to the draining lymph node field(s).[14] If spread to regional lymph nodes occurs, the number of metastatic lymph nodes and their tumor burden (microscopic or macroscopic disease) are the most powerful predictors of outcome.[13]

Figure 1.2 Types of locoregional metastases

The American Joint Committee of Cancer (AJCC) staging system categorises the extent of disease in patients with melanoma.[14] Patients are classified in four stage groups, depending on primary tumor characteristics and on the presence or absence of regional and distant metastases. Staging can be based on clinical assessment (c) or pathological assessment (p). The 8th AJCC staging edition was implemented in January 2018. Patients with early-stage melanoma (stages I and II) generally have a good prognosis with a 10-year melanoma-specific survival (MSS) of between 75%-95%. The 10-year MSS of patients with stage III melanoma (satellite, in-transit and lymph node metastasis) is rather heterogeneous with a range of 24%-88%.[14] Stage IV melanoma patients used to have a very poor prognosis, but their outcome has drastically improved with the implementation of new effective treatment options [15,16]

Staging imaging

Patients with stage III melanoma are at considerable risk of developing distant metastases. Because melanoma can metastasize to virtually any site in the body, imaging techniques such as computed tomography (CT), positron emission tomography (PET), magnetic resonance imaging (MRI), lymphoscintigraphy and ultrasonography are valuable staging tools.

Surgical staging and management of stage III disease

Prior to the introduction of sentinel lymph node biopsy (SLNB), some surgeons used to perform elective lymph node dissection in patients with a primary melanoma to remove any potential lymph node metastases.[17,18] In 1992, Donald Morton proposed the hypothesis that melanoma spreads through the lymphatic system in a sequential fashion.(20) He introduced a new procedure to identify and examine any node that receives afferent lymphatic drainage directly from a primary tumor, a sentinel lymph node (SLN).[19-21] Examination of this node would indicate whether the lymph node field was involved or not. In this way, lymph node dissections could be limited just to those patients who had a positive SLN. The SLNB procedure is straightforward. First, a radiocolloid is administered intradermally at the melanoma site. This tracer travels to a SLN where it accumulates. Through its radioactivity this process can be visualized using lymphoscintigraphy with single photon emission computed tomography with computed radiographic tomography (SPECT/CT). The imaging shows the number of SLNs and their location. During the surgical procedure, a gamma ray detection probe guides the surgeon to the radioactive SLN. Also vital blue dye is injected at the melanoma site at the commencement of the SLNB procedure and drains to the SLN, staining the afferent lymph vessel. Dissecting this lymph vessel will also lead the surgeon to the lymph node at risk, but performing this procedure can sometimes be rather challenging. A thorough pathological assessment of the SLN(s) to identify melanoma cells is another key factor in the success of the procedure.

A large randomized controlled trial – the first Multicenter Selective Lymphadenectomy Trial (MSLT-I) – confirmed the benefit of the procedure: the tumor status of the SLN was found to be the strongest prognostic factor in patients with intermediate-thickness (1.2-3.5mm) primary melanomas.[22,23]

The therapeutic benefit of SLNB has been a topic of fierce discussion. Introduced as a diagnostic test, one may not expect a direct survival benefit for all patients. Lymph node metastases were present in around 20% of patients undergoing the procedure. A survival benefit from SLNB followed by completion regional node dissection (CLND) was established in patients with SLN involvement from an intermediate thickness melanoma.[24]

Consequently, elective lymph node dissection was abandoned. SLNB became a standard staging procedure followed by CLND when metastasis was found. Additional involved nodes

were found in 7–33% of CLND specimens.[25-30] Subsequently, two randomized controlled trials – the German Dermatologic Cooperative Oncology Group Selective Lymphadenectomy Trial (DeCOG-SLT) and the second Multicenter Selective Lymphadenectomy Trial (MSLT-II) – demonstrated that the CLND did not contribute to the survival benefit and this additional procedure is no longer routinely recommended.[31,32] Apparently, the SLN is often the sole site of metastatic disease. Active surveillance with frequent ultrasound examination of the relevant lymph node field(s) detects lymph node recurrence typically before spread to distant sites occurs and is now common practice in patients with a positive SLNB.[33] MSLT-II demonstrated that 75% of SLN positive patients in the observation group did not recur in the regional node basin, proving that the SLNB alone had treated their regional disease.[32] One may thus conclude that SLNB is both a diagnostic staging test and a therapeutic procedure. For patients with palpable lymph node metastases a full therapeutic lymph node dissection is still recommended.[7]

Adjuvant treatment

Patients with melanoma lymph node metastases are at high risk of recurrence of their disease after adequate surgical therapy. High-risk features are the presence of extracapsular extension of the tumor, a greater number of involved lymph nodes and larger size of involved nodes.

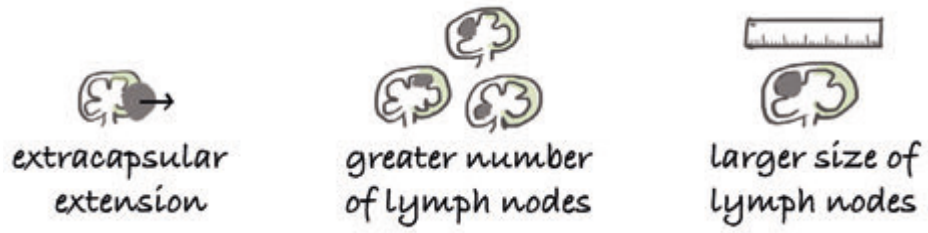


Figure 1.3 High-risk features

There are several adjuvant therapy options to reduce this risk of melanoma recurrence. Radiotherapy to the dissected lymph node field(s) is one of these options. The Australian and New Zealand Melanoma Trials Group (ANZMTG), in collaboration with the Trans-Tasman Radiation Oncology Group (RTOG), demonstrated in a randomised trial that adjuvant radiotherapy of the node field significantly reduced the risk of regional recurrence in high-risk patients. Because of the high risk of – lethal – distant metastases, adjuvant node field radiotherapy did not improve overall survival. In some patients radiotherapy caused long-term side effects such as fibrosis of the skin and lymphedema.[34,35] Because of the lack of effect on overall survival and these potential long-term side effects, the role of adjuvant post-operative radiotherapy remains a subject of discussion.

The recent success of targeted systemic therapies and immunotherapies in patients with stage IV disease prompted their testing in the adjuvant setting in stage III disease. The initial results show prolonged recurrence-free survival (RFS).[36,37] An interesting development has been the discovery of an immunogenic effect when combining radiotherapy with concurrent immunotherapy, especially with the use of hypofractionated radiotherapy.[38-40]

Outline of this thesis

This thesis is focussed on staging and treatment of patients with regionally disseminated (stage III) melanoma. It reports studies on pathological evaluation, staging imaging, and adjuvant radiotherapy in patients with stage III disease. All research data were collected from and in collaboration with Melanoma Institute Australia.

Pathological assessment of non-sentinel lymph nodes

Prior to the final report of the MSLT II trial, several investigators found an excellent survival rate in patients with a minimal metastasis (<0.1 mm) in the SLN.[41-44] Some of them suggested that the minimal metastases in the SLN would be biologically different from larger metastases, in the sense that they would never progress. The term "clinically false positive" was introduced to signify the meaning of their finding. If this notion was accepted, patients could be safely spared the potential morbidity of the – then still standard – CLND. Several arguments could be made to challenge this train of thought; one of these was that without CLND the number of involved lymph nodes might have been underestimated. Chapter 2 describes a detailed pathological assessment of the CLND specimens of patients with a minimal metastasis in the SLN using the protocol that is normally used for the meticulous assessment of SLNs. The frequency of involvement of additional lymph nodes as well as patient outcomes were assessed.

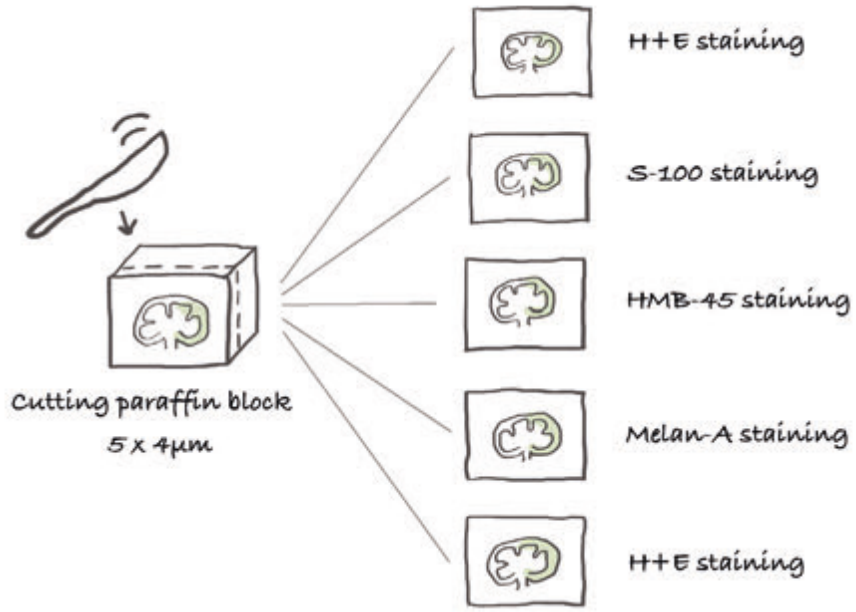


Figure 1.4 Pathological assessment protocol used in chapter 2

Staging imaging

Whole body ^{18}FDG-PET/CT and MRI scans of the brain are common staging tools used in the work-up of melanoma patients. In 37% of those with palpable lymph node metastases, pre-operative staging with the combination of these two techniques was found to change the treatment plan.[45] This prompted the question whether this approach would be also of value in patients with earlier stage III melanoma. Chapter 3 describes a cohort of patients with a positive SLNB who received either CT or whole body ^{18}FDG-PET/CT for staging. Imaging reports and additional investigation results were analysed to determine diagnostic test performance (i.e. sensitivity, specificity and positive predictive value) and number and nature of further diagnostic tests as a result of the staging imaging.

Chapter 4 describes a prospective trial using whole body ^{18}FDG-PET/CT and MRI of the brain to stage melanoma patients with a first presentation of satellite or in-transit metastases. We determined whether the stage of the disease and/or the treatment plan was changed following the staging results. We also evaluated the diagnostic test performance of PET/CT in this situation.

Adjuvant radiotherapy to the lymph node field

The ANZMTG/TROG 01.02/02.01 trial examined the value of conventionally-fractionated adjuvant node field radiotherapy.[46] Conventional radiotherapy is usually given in 20 fractions of 1.8-2.5 Gy per fraction. Laboratory studies in the 1970s showed melanoma cells to be rather radioresistant and this led to the introduction of hypofractionated radiotherapy with higher doses (5-6 Gy) per fraction in six fractions.[47-50] However, a higher dose per fraction is associated with a higher risk of late complications such as fibrosis and lymphedema.[51] Nowadays, both options are being used for adjuvant treatment of lymph node fields, depending on the preference of the treating radiation oncologist and the patient. To our knowledge, there had been no studies directly comparing the two approaches in a strictly adjuvant setting after surgical removal of all known tumor. Chapter 5 describes a large cohort of patients treated with either conventionally fractionated or hypofractionated radiotherapy. Patient characteristics, treatment data and outcome were extracted from the Melanoma Institute Australia (MIA) database and individual patient files were examined. These patients received radiotherapy within 90 days of the lymph node dissection.

Not all patients are routinely offered adjuvant radiotherapy or patients sometimes decline it. Some patients who were not treated with radiotherapy might recur in the previously dissected lymph node field and then be offered radiotherapy after surgical treatment of this recurrence. A thorough analysis of the benefit of adjuvant radiotherapy for this group of patients with a recurrence in the previously dissected lymph node field – in which the tumor cells may have become more biologically aggressive – is lacking. The investigators of the ANZMTG/TROG 01.02/02.01 trial and those of several retrospective studies described the combined results of both patient groups. Only small subgroup analyses were reported in the literature, therefore the MIA database was searched for patients who were treated with adjuvant radiotherapy after they recurred in a previously dissected lymph node field. Chapter 6 describes the oncological outcome of adjuvant node field radiotherapy in 76 melanoma patients after resection of one or more recurrences.

Both Chapters 5 and 6 describe results from an era before effective systemic immunotherapy became available, avoiding its confounding effect.

The overall aim of the studies reported in this thesis was to provide more specific guidance on staging and treatment decisions, with special focus on the use of adjuvant radiotherapy, in selected subgroups of patients with stage III melanoma.

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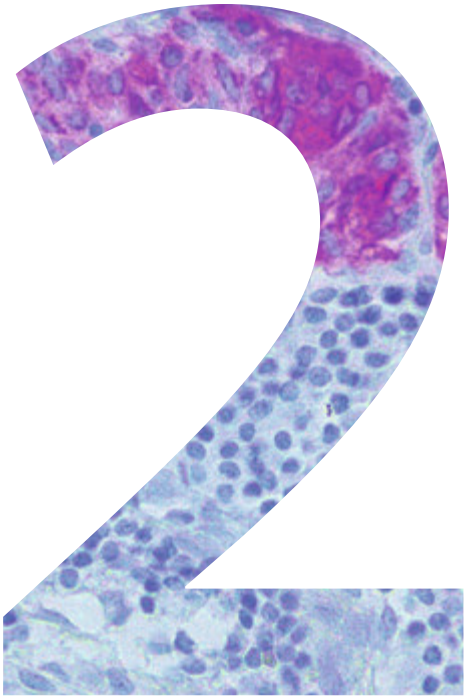
CHAPTER



Detailed pathological examination of completion node dissection specimens and outcome in melanoma patients with minimal (<0.1 mm) sentinel lymph node metastases

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Ann Surg Oncol. 2015; 22:2972-2977

Abstract

Background – Non-sentinel lymph nodes (NSLNs) are rarely involved in patients with minimal volume melanoma metastases in sentinel lymph nodes (SLNs). Therefore, it has been suggested that completion lymph node dissection (CLND) is not required. However, the lack of routine immunohistochemical staining and multiple sectioning may have led to failure to identify additional positive nodes. The present study sought to more reliably determine the tumor status of NSLNs in patients with minimally involved SLNs and their clinical outcome.

Methods – A total of 21 tumor-negative CLND specimens from 20 patients with SLN metastases of <0.1 mm in diameter treated between 1991 and 2013 were examined with a more detailed pathologic protocol (five new sections stained with/for H&E, S-100, HMB45, Melan-A, and H&E). Clinical follow-up data were also obtained.

Results – Of the 343 examined NSLNs, 1 was found to harbor a 0.18-mm subcapsular sinus metastasis. No metastases were identified in the other NSLNs. Median follow-up was 48 months (range 17–130 months). Six patients (30%) developed a recurrence. At the end of follow-up, 15 patients (75%) were alive without sign of melanoma recurrence and 5 patients (25%) had died of melanoma. Estimated 5-year melanoma-specific survival was 64 %. The patient with the additional positive NSLN remains without recurrence after 130 months follow-up.

Conclusions – Although the risk of additional nodal involvement is low, detailed pathologic examination may identify NSLN metastases not identified using routine protocols. Therefore, nodal clearance appears to be the safest option for these patients, pending the results of prospective trials.

Introduction

Sentinel lymph node (SLN) biopsy provides accurate staging of melanoma patients and important prognostic information. It has also been shown that regional disease control is improved in SLN-positive patients who undergo a completion lymph node dissection (CLND).[1] Furthermore, evidence of improved melanoma-specific survival in these patients has been reported.[1] Additional involved nodes are found in only 7–33 % of CLND specimens, raising the possibility that the majority of the patients do not have additional nodal metastases and could potentially be safely spared a node dissection and its associated morbidity.[1] Multiple studies have found that SLN tumor burden is a strong predictor of both non-SLN (NSLN) positivity and patient outcome in SLN-positive melanoma patients.[2] One study of 15 patients reported no additional metastases in CLND specimens and a 5-year overall survival rate of 100% in patients with a SLN metastasis less than 0.1 mm.[3] Based on this study and 3 subsequent multicenter studies – two reporting 91% and the other 83% 5-year overall survival rates for such patients – the investigators concluded that these minimal metastases in SLNs are biologically different from larger metastases in that they do not progress, and thus these patients can be safely spared a CLND.[3–6] The appropriateness of this conclusion has been challenged because of the limited clinical follow-up in these studies, some overlapping cases, and possible lead-time bias. Additional limitations of these studies include the relatively small numbers of nodes that were examined per dissection specimen and the failure to examine multiple sections or immunohistochemistry (IHC) for pathologic examination of NSLNs in CLND specimens. As a consequence, the number of involved nodes may have been underestimated in these studies, which would undermine the basis for the authors' recommendations.

The aims of the present study were to firstly establish the frequency of NSLN involvement in patients with minimal SLN metastasis through detailed pathologic analysis, including using IHC, of all nodes in CLND specimens previously pathologically reported as negative, and secondly, to determine their clinical outcome.

Patients, materials, and methods

Patient selection

This study was conducted with institutional Human Ethics Review Committee approval. A total of 20 patients with SLN metastases with a maximum diameter of less than 0.1 mm and reported to be without positive lymph nodes in the CLND specimen managed between January 1991 and December 2013 were identified from the Melanoma Institute Australia research database. One patient had two eligible nodal fields. Patient and tumor characteristics are summarized in Table1. Of the 20 patients, 8 were male (40%) and 12 female (60%). The median age was 55

years (range 25–78 years). The median Breslow thickness of the primary tumors was 1.58 mm (range 0.40–6.00 mm), 7 (35%) were ulcerated, and the median mitotic rate was 3/mm^2 (range 0–21/mm^2). SLN biopsy was performed in the neck in 2 patients, the axilla in 6 patients, and the groin in 12 patients (with 1 patient having bilateral groin dissections). The median number of excised SLNs was 2 (range 1–5), and the median maximum diameter of the largest tumor deposit in the SLN was 0.07 mm (range 0.02–0.09 mm). The median number of nodes per CLND was 12 (range 6–69).

SLN biopsy and completion lymph node dissection

Dynamic and static lymphoscintigraphy using technetium-99m antimony trisulfide colloid was followed by SLN identification using blue dye and a hand-held gamma-ray detection probe. A SLN was defined as a lymph node receiving direct lymphatic drainage from the primary melanoma site. SLNs were cut into 3-mm thick slices and embedded in paraffin blocks. Four (five after 2008) consecutive sections were cut and stained with hematoxylin and eosin (H&E; first and last section) and IHC (S-100, HMB45, and after 2008 Melan-A). Lymph nodes from the CLND specimen had originally been sliced into 3-mm thick slices (or processed whole if 3 mm or less in thickness), embedded in paraffin, and stained with H&E only.

Pathological assessment

Formalin-fixed, paraffin-embedded tissue samples of all CLND lymph nodes were retrieved from the archival files of the Department of Tissue Pathology and Diagnostic Oncology at the Royal Prince Alfred Hospital, Sydney, Australia. Five consecutive 4-mm thick sections were cut from the original paraffin block(s) of each node and stained according to the SLN protocol. The first and last sections were stained with H&E and analyzed in each case to confirm the presence of lymph node tissue. The second, third, and fourth sections were stained with IHC for S-100, HMB45, and Melan-A, respectively. IHC was performed on a Dako Autostainer Plus (Dako, Glostrup, Denmark) using the Ultravision Quanto AP Detection System (TL-060-QAL, Thermo Scientific, USA) and visualized using the Permanent Fast Red Quanto Substrate System (TA-060-QAL, Thermo Scientific, USA). Following deparaffinization of the tissuesections, heat-induced epitope retrieval was applied for 20 min using high-pH Dako EnvisionFLEX target retrieval solution (Dako, USA). The sections were incubated with monoclonal mouse anti-Human Melanosome Clone HMB45 (1:100 dilution; Dako), monoclonal mouse anti-Human Melan-A, Clone A103(1:50 dilution; Dako), or polyclonal rabbit anti-HumanS100 A and B (1:400 dilution; Novocastra, Leica Biosystems, Germany), respectively, for 30 min followed by incubation with hematoxylin (Dako) for 5 min.

Histological assessment

Two investigators (L.H.J.H. and S.W.) independently evaluated all histology slides. Morphological and IHC characteristics were used to identify nodal melanoma metastases.[7] Cases were scored

as positive based on IHC staining and cell morphology. Cases in which there was diagnostic uncertainty were reviewed by R.V. and R.A.S.

Statistical analysis

The SPSS statistical package version 22 (SPSS Inc., Chicago, IL) was used for all statistical analyses. Disease-free survival and melanoma-specific survival were calculated from the time of SLN biopsy and were censored at the last contact date if there were no events. Survival analysis was performed using the Kaplan–Meier method.

Results

Detailed pathologic analysis of NSLN nodes in CLND specimens

Multiple H&E and IHC-stained sections of all 343 lymph nodes identified in the CLND specimens were histologically examined. In one patient a metastasis was identified in 1 of 11 lymph nodes (figure 2.1; table 1). This subcapsular sinus deposit had a maximum diameter of 0.18 mm. No metastases were identified in the multiple tissue sections of the other 342 lymph nodes. In three patients a capsular nevus was identified.

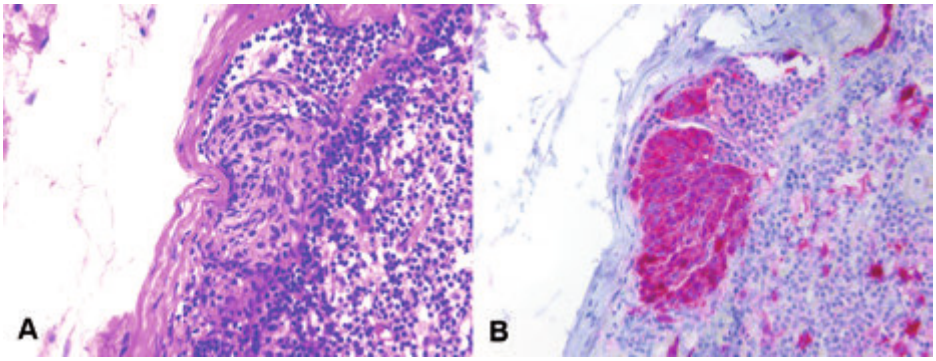

Figure 2.1 Metastatic melanoma deposit in the subcapsular sinus of the positive lymph node from a CLND specimen

Original magnification x400
A H&E stained section
B Section stained immunohistochemically for S-100 protein

Table 2.1 Patient and tumor characteristics

Case no.	Age	Sex	Primary melanoma					SLN biopsy site	No of SLNs positive/ removed	Maximum size SLN tumor deposit (mm)	No of nodes in CLND	Positive NSLNs on review/ presence of nevus	Recurrence	Status at last follow-up
			Anatomic site	Melanoma subtype	Breslow thickness (mm)	Mitotic rate (/mm^2)	Ulceration							
1	48	F	Trunk	SSM	1.05	2	No	Groin	1/1	0.06	22	0	No	Alive NSR
2	39	F	Trunk	NM	0.65	2	No	Axilla	1/1	0.05	7	0	No	Alive NSR
3	78	M	Trunk	NM	3.70	18	Yes	Axilla	1/2	0.06	20	0	Yes	Dead of melanoma
4	54	M	Trunk	NM	6.00	21	Yes	Axilla	1/5	0.07	27	0, nevus	Yes	Dead of melanoma
5	65	F	Lower limb	ALM	3.50	3	No	Groin	1/1	0.07	7	0	Yes	Dead of melanoma
6	72	M	Lower limb	ALM	3.10	0	No	Groin	1/2	0.09	7	0	Yes	Dead of melanoma
7	60	F	Upper limb	SSM	1.10	0	No	Axilla	1/4	0.09	20	0	No	Alive NSR
8	61	F	Lower limb	MBN	1.15	5	No	Groin	1/2	0.07	13	0	No	Alive NSR
9	34	F	Lower limb	Not known	1.10	3	Unknown	Groin	1/3	0.09	24	0	No	Alive NSR
10	34	F	Lower limb	SSM	0.95	2	No	Groin	1/3	0.05	11	1, nevus	No	Alive NSR
11	43	F	Lower limb	SSM	0.95	1	No	Groin	1/3	0.09	8	0	No	Alive NSR
12	49	F	Lower limb	NM	2.50	10	Yes	Groin	1/2	0.09	6	0	Yes	Dead of melanoma
13	66	F	Trunk	SSM	3.88	2	Yes	Axilla	1/2	0.05	16	0	No	Alive NSR
14	62	M	Lower limb	NM	0.40	0	Unknown	Groin	1/2	0.09	16	0	Yes	Alive NSR
15	45	M	Head and neck	NM	1.20	2	Unknown	Neck	1/3	0.05	69	0	No	Alive NSR
16	25	M	Trunk	SSM	1.00	2	No	Neck	1/4	0.05	17	0	No	Alive NSR
17	32	M	Lower limb	SSM	1.00	11	Yes	Groin	1/3	0.07	11	0	No	Alive NSR
18	76	F	Lower limb	NM	4.70	3	Unknown	Groin	1/3	0.02	8	0	No	Alive NSR
19	55	F	Trunk	SSM	2.20	15	Yes	Left groin	1/2	0.08	6	0	No	Alive NSR
								Right groin	1/2	0.07	6	0, nevus		
20	57	M	Upper limb	Not known	3.30	6	Yes	Axilla	1/2	0.04	7	0	No	Alive NSR

SLN sentinel node; *NSLN* nonsentinel node; *CLND* completion lymph node dissection; *M* male; *F* female; *ALM* acral lentiginous melanoma; *MBN* malignant blue nevus; *NM* nodular melanoma; *SSM* superficial spreading melanoma; *Alive NSR* alive, no sign of recurrence

Patient follow-up data

Median follow-up after SLN biopsy was 48 months (range 17–130 months). The patient with the NSLN metastasis has had no disease recurrence after 130 months (table 2.1). Of 20 patients, 6 (30%) developed a recurrence. In three patients (14 %) the first presentation of recurrence was local/in-transit. Of these three patients, 1 developed in-transit metastases after 1 year, distant metastases 2 years later, and he died 7 months thereafter. The second patient developed in-transit metastases 15 months after SLN biopsy, subsequent distant metastases within a month, and she died 2 months later. The third patient developed a local recurrence after 16 months, 4 months later in-transit disease, 2 years later distant metastases, and he died the subsequent month. The fourth patient developed a recurrence in the contralateral node field after 17 months and an in-transit metastasis after 23 months on the ipsilateral side. He has remained disease-free after excision. The fifth patient developed distant metastases after 26 months and died 20 months later. The sixth patient developed distant metastases after 17 months and died 2 months later. At the end of follow-up, 15 patients (75%) were alive without sign of melanoma recurrence and 5 patients (25%) had died of melanoma. Kaplan–Meier estimated 5-year melanoma-specific survival was 64% (figure 2.2) and estimated 5-year recurrence-free survival was 68%.

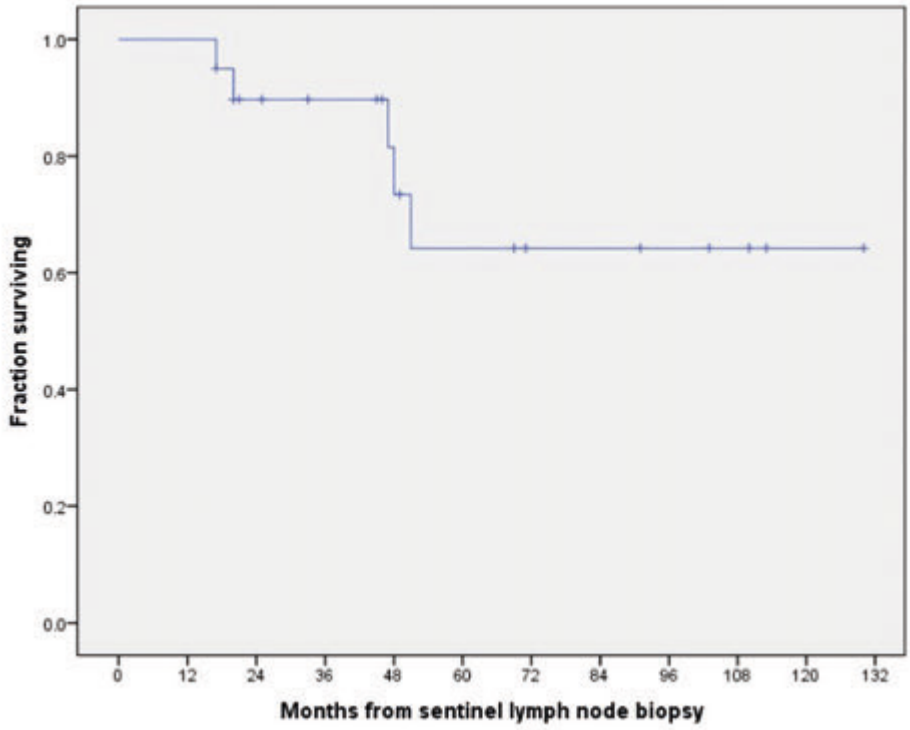

Figure 2.2 Kaplan-Meier estimated melanoma-specific survival

Discussion

Whether or not to perform a CLND in some SLN-positive melanoma patients is a subject of debate.[8–11] Most currently available data support the conclusion that all melanoma SLN metastases, however small, will ultimately progress to clinically relevant disease if the patient is followed for a sufficient period of time.[1] Ongoing clinical trials, such as the second Multicenter Selective Lymphadenectomy Trial (MSLT-II) and a European Organisation for Research and Treatment of Cancer study, will ultimately determine whether CLND is necessary in all patients with a positive SLN.[12,13] Pending the outcome of these trials, many investigators have attempted to identify predictors of both NSLN involvement in CLND specimens and patient outcome in SLN-positive patients. Following a review of the literature, we identified 31 predictive factors based on characteristics of the patient, the primary melanoma, or the SLN metastasis. The tumor burden in the SLN is clearly an important potential predictive factor, and multiple individual factors reflecting aspects of disease burden have been studied. While in large patient cohorts many of these factors have been predictive of both NSLN positivity and patient outcome, thus far none of these has proved to be accurate enough to predict CLND status in individual patients.[2] Concerning prediction of outcome, 1 study evaluated the natural course of the disease in 16 patients with a positive SLN with tumor penetrative depth £0.30 mm who were observed for more than 5 years and found no nodal recurrence.[11,14]

Studies assessing predictors of NSLN metastasis are frequently hampered by limitations in the pathologic assessment of CLND specimens including small numbers of examined NSLNs, minimal sampling of each NSLN, not using sensitive IHC pathological staining techniques, and lack of interobserver reproducibility for certain scoring systems. Despite the limited pathological assessment, the survival reported in these studies was between 80 and 100% for patients with minimal SLN tumor burden.[3–6] However, median follow-up was only 30–37 months, and much longer follow-up would be required to conclude that it is safe to omit CLND in these patients. The present study is the first to provide a thorough validation of the tumor status of NSLNs in patients with a minimally involved SLN. Using more detailed sampling and pathologic examination of all NSLNs from CLND specimens previously reported as negative, we found 1 NSLN metastasis in 1 of 20 patients. This highlights the fact that a metastasis in a NSLN may be missed because of a sampling error. Therefore, previous studies concluding patients with minimally involved SLNs do not require CLND as such specimens were reported pathologically as metastasis-free should be interpreted with caution, because these studies were relying on minimal pathologic examination of NSLNs in CLND specimens. The fact that more recent studies have demonstrated that with longer follow-up some patients with minimal SLN metastases will die of melanoma (including 25% of patients in this study), suggests that even these patients have a significant risk of disease progression.[15,16] This said, it is also known that even 10–15% of SLN negative patients will die of melanoma within 5–10 years.[1] CLND can

prevent further dissemination from involved lymph nodes but cannot change the clinical outcome of patients who already have distant metastases.

A possible limitation of our study is the extent of pathological assessment. Using our standard SLN pathologic examination protocol, only five extra sections were cut. For logistic reasons, we were unable to examine the SLN tissue in its entirety (which would require examination of up to 600 sections per tissue block). As our protocol already resulted in more than a thousand slides to assess, an even more extensive evaluation was beyond the capability of resources available to us.

Our study reveals that the prognosis of patients with a minimal SLN metastasis is not as good as is often assumed. Of twenty patients with a SLN metastasis with a diameter less than 0.1 mm and a negative CLND specimen, 6 patients (30%) developed a recurrence, although none of them recurred in the surgically treated nodal field. Our cohort had an estimated 5-year melanoma-specific survival of 64%, whereas Van Akkooi et al. and Van der Ploeg et al. reported 5-year survival rates between 83% and100% in overlapping patient cohorts.[3–6] An explanation for this discrepancy may be the length of follow-up or the sample size. The Van Akkooi and Van der Ploeg studies had median follow-up of 30–37 months, whereas in our study the median follow-up was 48 months. The excellent survival rates of Van Akkooi et al. and Van der Ploeg et al. might have been influenced by lead-time bias, which is a known confounder in the interpretation of survival data in clinical studies assessing minimally involved SLNs. Lead-time bias refers to the observation that the smaller the metastasis, the longer it is likely to take for the disease recurrence to be detected, as described by Scolyer et al.[9] It is known that in some patients melanoma metastases may be clinically undetected for more than 10 years after initial treatment.[1] Therefore, in patients with minimal volume SLN metastases, very long clinical follow-up is required to accurately evaluate survival data. This said, our study has a small sample size and a random effect cannot be excluded. The results of MSLT-II may shed further light on the clinical benefit of CLND in patients with minimally involved SLNs.[12]

A general argument often raised when considering the topic of whether or not a CLND should be performed is its complication rate. Several studies have reported complication rates of between 23 and 66% after CLND compared with rates between 5 and 14% after SLN biopsy alone.[16–20] Nevertheless, compared with patients with palpable nodal disease, a node dissection for a positive SLN often requires a less extensive operation and has been clearly shown to be associated with less morbidity, and a better quality of life, with no requirement for postoperative radiation therapy.[21,22,23]

In conclusion, melanoma patients with minimal-volume SLN metastases have a low risk of additional NSLN metastases in CLND specimens, but in this study they had a 64 % 5-year

melanoma-specific survival. Despite the small size of the SLN metastasis, these patients may harbor NSLN involvement not identified utilizing routine pathologic examination protocols. At Melanoma Institute Australia, CLND remains the standard treatment of patients with an involved sentinel node.

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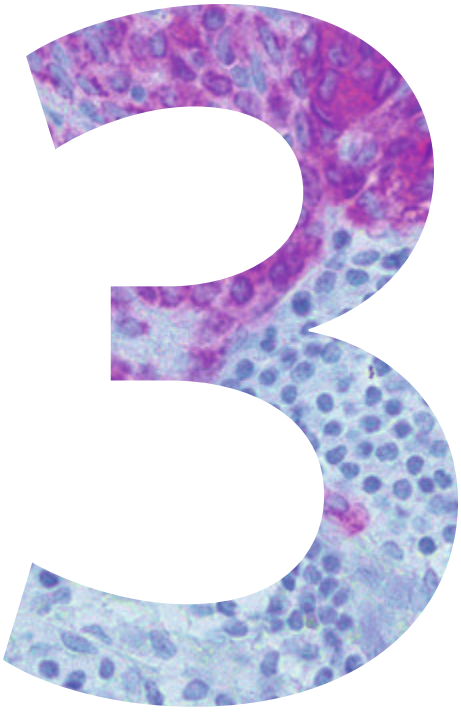
CHAPTER



Futility of imaging to stage melanoma patients with a positive sentinel lymph node

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Abstract

The use of staging imaging in melanoma patients with a positive sentinel lymph node (SLN) has been reported to be of limited value. Improved accuracy resulting from the development of time-of-flight positron emission tomography (PET) and ongoing image quality improvement of computed tomography (CT) may challenge this statement. Our retrospective study assessed the clinical value of routine staging CT and PET/CT imaging in a recent cohort of asymptomatic SLN-positive patients. Between January 2011 and April 2014, 143 patients with a positive SLN were routinely staged using CT of various parts of the body or whole-body PET/CT. Scores were assigned for level of certainty for regional or distant metastases and incidental second primary malignancies. Diagnostic test performance was assessed, as well as the number and nature of ensuing additional diagnostic actions. CT was performed in 102 of 143 (71%) patients and PET/CT in 41 (29%) patients. The use of PET/CT increased over the study period. Metastases were found in two of the 143 patients (true-positive yield 1.4%). Sensitivity, specificity and positive predictive value were 11, 73 and 4% for CT and 17, 57 and 6%, respectively, for PET/CT. None of the 143 patients had a change in AJCC stage.

Introduction

Conventional radiography, computed tomography (CT) and PET using fluorine-18-fluorodeoxyglucose are commonly used in the staging of patients with melanoma.[1–3] Although the utility of staging imaging has been established in patients with palpable lymph node disease and those with distant metastases, the accuracy of imaging in patients with earlier stages of the disease is controversial.[4,5] A low yield and a high rate of false-positive results have been shown in previous small studies.[6–8] Despite this evidence, international guidelines offer ambiguous recommendations.[9–11] This is particularly the case for patients with a positive sentinel lymph node (SLN).

The combination of PET and CT has improved accuracy as well as the development of time-of-flight PET.[12–15] Algorithms such as adaptive iterative dose reduction resulted in enhanced imaging quality of CT.[16] With these improved techniques, smaller metastases are visible, and this elicits the question of whether today staging imaging in early stages of melanoma might be useful.

At our institution, we observed that staging imaging with CT and PET/CT was frequently ordered in patients with an involved SLN. To assess the value of this approach, a retrospective study of a recent cohort of such patients was carried out. The objectives were to determine the yield, the rate of false-positive results, the positive predictive value, the impact on the AJCC/UICC staging of the disease and the number of additional diagnostic tests or actions that were required to clarify the imaging findings.

Patients and methods

Patients

In the Melanoma Institute Australia research database, 222 patients were identified who had a single primary melanoma and a positive SLN between January 2011 and April 2014. Staging imaging was defined as any CT of various areas of the body or PET/CT of the whole body within 90 days after sentinel lymph node biopsy. A total of 159 patients underwent staging imaging. No follow-up data were available for 16 patients, who were therefore excluded from the analysis. The remaining 143 patients comprised the study cohort. Patient characteristics are summarized in table 3.1. The study was approved by the Royal Prince Alfred Hospital Human Ethics Review Committee.

Table 3.1 Patient and tumor characteristics (*n* = 143)

Patient and tumor characteristics	
Sex	
Male	87 (61%)
Female	56 (39%)
Age (years)	
median (range)	57 (10-82)
Breslow thickness (mm)	
median (range)	2.4 (0.7-10.0)
Mitotic rate (/mm²)	
median (range)	6.5 (1-40)
Ulceration	
Present	62 (44%)
Absent	79 (55%)
Unknown	2 (1%)
Number of excised SLNs	
median (range)	3 (1-8)
Number of positive SLNs	
median (range)	1 (1-4)
Extracapsular spread	
Present	10 (7%)
Absent	132 (92%)
Unknown	1 (1%)
Maximum diameter of largest SLN tumor deposit (mm)	
median (range)	0.86 (0.02-12.00)
SLNB-positive site	
Total	153 (100%)
Neck	27 (18%)
Axilla	77 (50%)
Groin	44 (29%)
Others	5 (3%)

SLN sentinel lymph node

Imaging

CT or PET/CT was performed, either alone or combined with other imaging techniques such as magnetic resonance imaging (MRI) of the brain (figure 3.1). Because of the wide geographic distribution of patients visiting our institute and the multitude of imaging facilities, there were

no uniform protocols for the imaging techniques. The utility of and choice in type of staging imaging were left at the discretion of the treating physician.

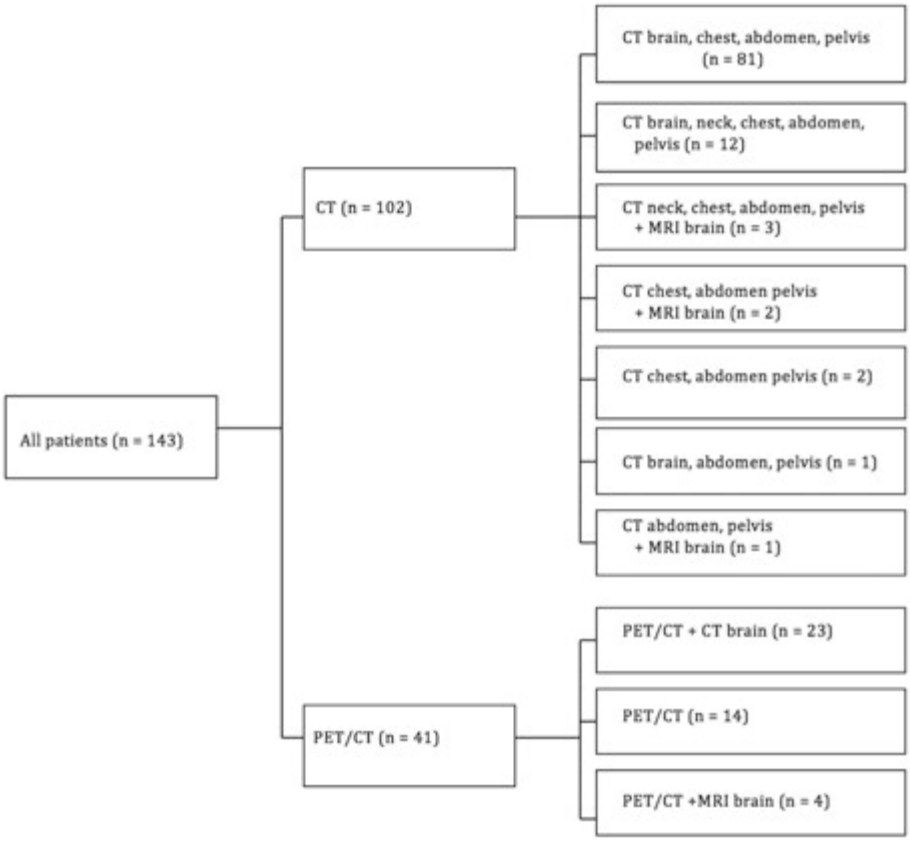

Figure 3.1 Flowchart of initial imaging modalities

CT computed tomography; *MRI* magnetic resonance imaging; *PET* positron emission tomography.

Interpretation of results

The imaging reports were retrieved from the patients' records. The reports were interpreted by two of the investigators (L.H. and R.R.) using a four-point scale. The findings were scored as follows: (i) diagnostic or suspicious for distant metastasis, (ii) diagnostic or suspicious for regional metastasis, (iii) equivocal for melanoma metastasis irrespective of site with further investigation indicated or (iv) negative for melanoma. In case of disagreement, the final score was decided upon by a third reader (O.N.). Reports were also scored as diagnostic/ suspicious, equivocal or negative for incidental second malignancies. The summary of the report entered

into the research database was assessed for seven patients whose original imaging report was not available.

Reference standard

To be able to perform the test statistics, scores 1–3 were considered to be indicative of melanoma. Pathological confirmation or clinical/radiological outcome after 6 months of follow-up was considered the gold standard.

Statistical analysis

Sensitivity, specificity and positive predictive values were calculated for CT and PET/CT. Statistical analyses were carried out using the SPSS statistical package v22 (IBM, Chicago, Illinois, USA).

Results

Use of CT and PET/CT

The median time interval between SLN biopsy and staging imaging was 17 days (range: 0–85 days). During the study period, a shift in imaging modality occurred from CT to PET/CT (figure 3.1).

Yield

The results of CT and PET/CT imaging are presented in table 3.2. Distant metastases were not detected, but imaging showed additional regional metastases in two of the 143 SLN-positive patients, resulting in a true-positive yield of 1.4%. One of these two patients initially presented with a primary melanoma of the right lateral upper arm with a Breslow thickness of 4.1 mm, ulceration and a mitotic rate of 5/mm^2. CT showed additional metastases in the axilla from which the SLN was taken; a diagnosis was confirmed upon completion node dissection. The second patient had a primary melanoma in the midline of his back with a Breslow thickness of 2.3 mm, no ulceration and a mitotic rate of 4/mm^2. He underwent bilateral SLN biopsy that showed a positive SLN in the left axilla. Despite two negative SLNs in the right axilla, subsequent PET/CT suggested a nodal metastasis in that nodal region. Bilateral axillary node dissection was performed and histopathology examination confirmed the nodal metastasis in the right axilla while no additional disease was found on the left side. Other primary malignancies PET/CT and CT detected two additional primary malignancies (1.4%). In one patient, CT showed splenomegaly that proved to be caused by primary myelofibrosis. In another patient, PET/CT showed increased uptake in the left groin as a result of follicular lymphoma (figure 3.2).

Table 3.2 Results of staging imaging in patients with a positive sentinal lymph node

	All metastases	Distant metastases	Regional metastases
CT (n = 102)			
True-positive	1	0	1
False-positive	25	21	8
True-negative	68	76	85
False-negative	8	5	8
PET/CT (n = 41)			
True-positive	1	0	1
False-positive	15	7	10
True-negative	20	31	26
False-negative	5	3	4
Total (n = 143)			
True-positive	2	0	2
False-positive	40	28	18
True-negative	88	107	111
False-negative	13	8	12

CT computed tomography; *PET* positron emission tomography.

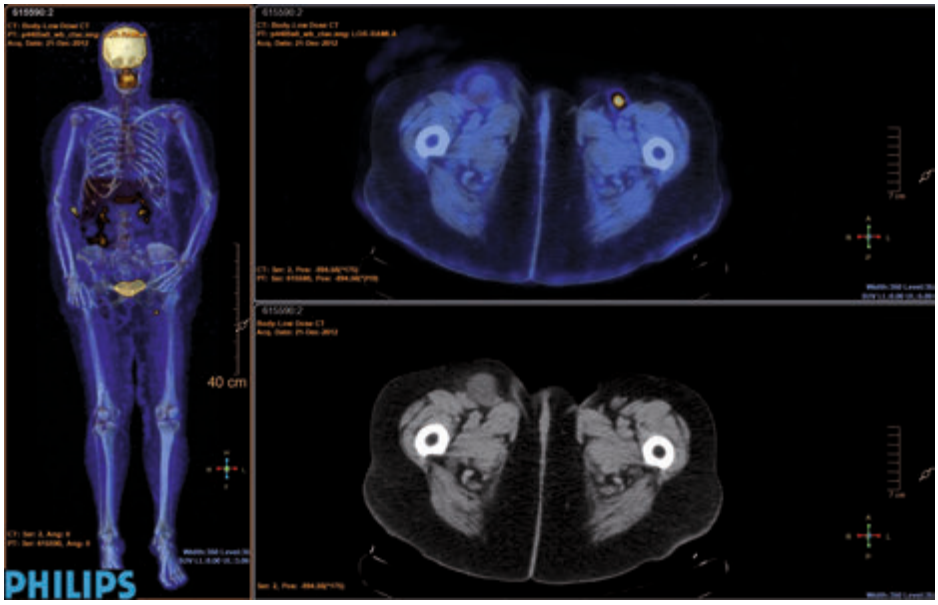

Figure 3.2 Example of a false-positive result and additional primary malignancy

The PET/CT image shows a possible metastasis in a left inguinal node and abnormal uptake in the left palate tonsil. The patient underwent tonsillectomy and excision of the groin lymph node (LN). The tonsil proved to be reactive lymphoid tissue and the groin LN contained a high-grade follicular lymphoma. Left panel: whole-body PET/CT image showing abnormal uptake in the left palate tonsil and left inguinal region. Right upper panel: transverse plane PET/CT image of the inguinal region showing abnormal fluorine-18-fluorodeoxyglucoseuptake in a left inguinal LN. Also visible is a postoperative seroma resulting from the sentinel LN biopsy in the right inguinal region. Right lower panel: corresponding CT image of the inguinal region. *CT* computed tomography. *PET* positron emission tomography.

Diagnostic test performance and additional investigations

Further disease was observed within 6 months in eight patients who underwent staging CT and five patients who underwent PET/CT (table 3.2). As a result, the sensitivity of CT was 11%, the specificity was 73% and the positive predictive value was 4%. For PET/CT, these percentages were 17, 57 and 6%, respectively. Twelve of the 25 patients with a false-positive CT and nine of the 15 patients with a false-positive PET/CT underwent 37 additional diagnostic tests, referrals and procedures before melanoma metastases were excluded (table 3.3). The investigations ranged from noninvasive diagnostic tests to substantial surgical procedures such as tonsillectomy and splenectomy.

Table 3.3 Description and number of additional tests, referrals and procedures to clarify CT and PET/CT findings

Additional investigations	Overall	CT	PET/CT
Total	37	21	16
Ultrasound	10	5	5
PET/CT	8	8	-
Referral to another specialist	6	4	2
Tissue biopsy	3	0	3
Blood test	2	1	1
Gastro/colonoscopy	2	1	1
CT	2	1	1
MRI	2	-	2
Surgical procedure under general anaesthesia[a]	2	1	1

CT computed tomography; *MRI* magnetic resonance imaging; *PET* positron emission tomography.
[a]Tonsillectomy, splenectomy.

Discussion

This study shows that, despite technical improvements, staging imaging in melanoma patients with an involved SLN using CT or PET/CT has a very low yield (1.4% regional metastases, 0% distant metastases). Our results confirm the outcomes of previous studies that showed 0–5% yields for distant metastases in patients with involved SLNs using a variety of imaging techniques.[6–8,17–21] As some 40% of the patients with a positive sentinel node die from their disease, they must have more metastases and these metastases are low volume and not identifiable by the current state-of-the art imaging technologies.

Until recently, early detection of distant metastases was not a high priority because there was usually no curative treatment. Now, effective systemic therapies can achieve long-term survival, if not cure, in a substantial proportion of such patients. It is our impression that these treatments are more effective in the presence of a low tumor load, making the early detection of distant disease a relevant issue.[22] It is possible that routine imaging would be more effective if performed after a longer time interval.

Two other primary malignancies (1.4%) were detected in our cohort. Aloia et al.[7] described the finding of occult second primary malignancies in 2.2% of their patients, Gold et al.[18] in 2.8%, Constantinidou et al.[8] in 3.3% and Meyers et al.[20] in 1.4%. These other malignancies were most often renal cell carcinomas detected by CT and thyroid malignancies detected by PET. These incidental findings are more frequent than the melanoma metastases for which the

studies were ordered. Although these findings may result in useful treatment, finding other abnormalities was not the primary purpose of imaging in these patients.

The frequent false-positive results (24% for CT and 37% for PET/CT) in our study resulted in 37 unnecessary additional investigations and procedures, including a splenectomy and a tonsillectomy – potentially morbid procedures. Several previously published studies have noted this phenomenon. The number of patients in whom additional investigations were required consistently exceeds the yield of melanoma metastases in this and other studies.[6–8] Miranda and colleagues found that 19% of their patients had a false-positive chest CT, 15% had a false-positive abdominal-pelvic CT and 6% had a false-positive brain CT or MRI. Their patients with a false-positive scan underwent 46 additional investigations and six procedures.[6] Aloia and colleagues noted 6% of patients to have a false-positive CT or MRI. All these patients underwent additional investigations before this became evident.[7] Constantinidou and colleagues reported 7% of patients to have a false-positive PET or PET/CT. All underwent additional investigations.[8] Although the percentages vary between studies, the number of patients in whom additional investigations were required consistently exceeds the yield. Furthermore, diagnostic tests and in particular a positive test result frequently cause considerable anxiety and psychological distress.[23–25]

Our estimated positive predictive values of 4% for CT and 6% for PET/CT imply that the chance of a positive result being melanoma is small. Patients with a positive sentinel node often request extra imaging. It is our experience that they understand that this is not in their interest when they are informed of the limited benefit and the frequent false-positive findings that lead to unnecessary additional testing.

The question of imaging to obtain a baseline is often raised. The present study does not provide a direct answer, but it is clear that a baseline would be useless in more than half of the patients because they will never develop a recurrence. One may even consider baseline imaging to be harmful in case of a false-positive finding in view of the sequelae discussed above. In the patients who do develop a recurrence later on, baseline imaging could result in false-positive results because of a transient condition such as an infected diverticulum in the colon or even a minor trauma that will no longer be present at the time of the recurrence. Again, a useless and potentially harmful additional work-up may be required. In terms of the more permanent false-positive findings such as a colonic polyp or an adenoma of the thyroid gland, it appears irrelevant whether these are elucidated sooner rather than later.

Our study has several limitations. Because of its retrospective nature, there was no uniformity in the indications for imaging. For instance, there may have been an under-representation of patients with minimal sentinel node involvement. Also, a variety of body sites were imaged. In

addition, the multiple radiology and nuclear medicine facilities used different imaging protocols and there may have been interobserver variability in the interpretation of the images. Because of the avidity of the cerebrum for fluorine-18-fluorodeoxyglucose, PET/CT has limited capacity for the detection of brain metastases. We prefer MRI when imaging the brain. We did not find any brain metastases in our series. Only Aloia et al.[7] reported finding brain metastases (in 0.7% of their patients). Finally, we limited the gold standard to clinical outcome at 6 months upon staging imaging. A shorter or a longer time period would influence the false-negative rate.

Notwithstanding the limitations of our study design, it seems reasonable to conclude that – despite technical improvements – CT and PET/CT still have a low yield and a high rate of false-positive results leading to frequent unnecessary additional tests and procedures. On the basis of the results of this study and other currently available evidence, we do not recommend routine staging imaging in patients with a tumor-positive SLN because its risks exceed its benefits.

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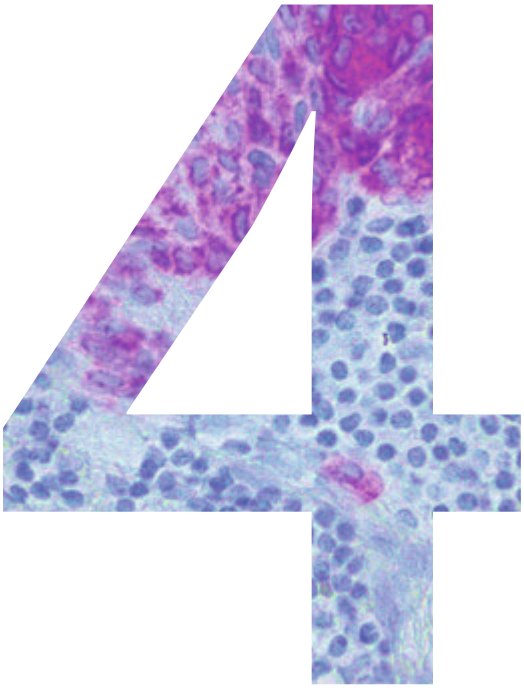
CHAPTER



Staging ^{18}F-FDG PET/CT influences the treatment plan in melanoma patients with satellite or in-transit metastases

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Abstract

Whole-body positron emission tomography/computed tomography (PET/CT) and brain magnetic resonance imaging (MRI) are commonly used to stage patients with palpable lymph node metastases from melanoma, but their role in patients with satellite and/or in-transit metastasis (S&ITM) is unclear. The aim of this study was to establish the diagnostic value of PET/CT and brain MRI in these patients, and to assess their influence on subsequent management decisions. In this prospective study, 25 melanoma patients with a first presentation of S&ITM who had no clinical evidence of palpable nodal or distant metastasis underwent whole-body ^{18}F-FDG PET/CT and brain MRI after a tentative pre-scan treatment plan had been made. Sensitivity and specificity of imaging were determined by pathological confirmation, clinical outcome and repeat PET/CT and MRI at 6 months. PET/CT led to a modification of the initial treatment plan in four patients (16%). All four were upstaged (AJCC stage eighth edition). PET/CT was false-positive in one patient, who had a Schwannoma in his trapezius muscle. A thyroid carcinoma was an incidental finding in another patient. The sensitivity of PET/CT was 58% and specificity 83%. In 6 months following the baseline PET/CT, further sites of in-transit or systemic disease were identified in 10 patients (40%). Brain MRI did not alter the treatment plan or change the disease stage in any patient. Whole-body PET/CT improved staging in melanoma patients with S&ITM and changed the originally-contemplated treatment plan in 16%. MRI of the brain appeared not to be useful.

Introduction

In cancer patients, the stage of their disease assists in the determination of an appropriate treatment plan, as well as providing prognostic information. Also, accurate knowledge of disease stage facilitates the exchange of information about patients and enables comparison of the outcome following different diagnostic and therapeutic strategies.

Because of the nature of their metabolism, melanoma cells require glucose to thrive. Melanoma cells have high glutamine receptor activity and high levels of intracellular hexokinase. For this reason, melanoma has high avidity for the glucose analogue 18F-fluorodeoxyglucose (FDG) that is used for positron emission tomography/computed tomography (PET/CT).[1] This makes PET/CT an ideal staging modality for the disease, except for the detection of brain Metastases. magnetic resonance imaging (MRI) has proved to be better for this purpose.[2,3] The value of whole-body PET/CT for the detection of metastatic melanoma has been well demonstrated in patients with distant disease and palpable lymph node metastases.[1,4,5] Unfortunately, PET/CT performance has proved disappointing in patients with a positive sentinel node because metastases are usually too small to be identified at this stage, and false-positive findings are frequent.[6,7] The value of PET/CT in patients with satellite and/or in-transit metastasis (S&ITM) has not been well studied.

The purposes of the current study were first to determine the implications of staging PET/CT and brain MRI for subsequent management of patients with S&ITM at the time of initial melanoma diagnosis or as a first recurrence, and secondly to establish the diagnostic accuracy of PET/CT and MRI in this population.

Materials and methods

Patients

The study was conducted with institutional ethics committee approval (HREC/13/RPAH/586). Between May 2014 and May 2015, 25 melanoma patients with a first presentation of S&ITM were prospectively enrolled. Patients were eligible if they had a first presentation of pathologically confirmed S&ITM without clinical evidence of metastases elsewhere, were at least 18 years of age and able to give informed consent, had an Eastern Cooperative Oncology Group performance status of 0, 1 or 2, and a life expectancy of at least 6 months. Patients were not eligible if they had a true local recurrence (in contact with a skin graft or scar of the primary melanoma excision) or if they were pregnant.

Microsatellites, satellites and in-transit metastases are thought to represent intralymphatic or angiotrophic tumor spread. Satellites were defined as clinically evident cutaneous and/or subcutaneous metastases within 2 cm of the primary melanoma and ITMs were defined as located between 2 cm from the primary melanoma and the first regional lymph node.[8] For microsatellites, the AJCC-UICC seventh edition definition was in use during the study period ('any discontinuous nest of metastatic cells more than 0.05 mm in diameter that are clearly separated by normal dermis from the main invasive component of melanoma by a distance of at least 0.3 mm').[9]

Patient and tumor characteristics are presented in table 4.1. Twelve patients had newly diagnosed melanomas and simultaneous S&ITM (cTNM stage III) and 13 developed S&ITM after a preceding primary melanoma diagnosis (rcTNM stage III). Eleven of the 25 patients had satellite metastases. Eight of these 11 patients had microsatellites identified histologically in the primary tumor excision specimen. Three patients had satellite metastases still present at time of enrolment. Fourteen patients had ITMs, of whom two had undergone partial or complete excision of their lesion(s). Before PET/CT and brain MRI results were obtained, a tentative treatment plan was drawn up and documented, on the assumption that no distant metastases would be demonstrated. The final treatment was compared with the pre-scan management plan. Patients were monitored for recurrence during the subsequent 6 months and at the end of this period physical examination, PET/CT and brain MRI were repeated.

Table 4.1

Patient and tumor characteristics	
Patients	
n	25
Median age	
Years (range)	64 (32-92)
Sex	
Male	12 (48%)
Female	13 (52%)
Primary melanoma site	
Head/neck	6 (24%)
Trunk	3 (12%)
Upper limb	3 (12%)
Lower limb	13 (52%)
Median Breslow thickness	
mm (range)	3 (0.4-26.0)
Median tumor mitotic rate	
/mm^2 (range)	6 (0-25)
Ulceration	
Yes	8 (32%)
No	17 (68%)
Median size of S&ITM	
mm (range)	5.5 (0.1-50.0)
Number of S&ITM	
1	13 (52%)
2-5	6 (24%)
>5	6 (24%)

^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography

A Phillips Ingenuity TOF 64 slice PET/CT scanner (Philips, Amsterdam, the Netherlands) was used for whole-body PET/CT imaging. Patients were fasted for at least 6 hours prior to intravenous administration of 3.5 MBq/kg of FDG. PET images were obtained approximately 60 minutes after radioisotope injection with six- to eight-bed positions and an acquisition time of three minutes per stop. Low-dose CT was performed using a 64 slice multi-detector CT from vertex to at least proximal thighs for attenuation correction and anatomical localization. PET/CT examinations were interpreted by a nuclear physician (L.E.). Lesions were scored as negative, equivocal – probably negative, equivocal – probably positive or positive for melanoma metastasis. Anatomical location, number of lesions and maximum standardized uptake values were recorded.

Magnetic resonance imaging

MRI of the brain was acquired with a dedicated head coil in a Siemens Avanto 1.5 Tesla scanner (Siemens, Erlangen, Germany). Sequences employed included sagittal T1, FLAIR axial, axial T2, T1 axial diffusion-weighted sequence and post-intravenous gadolinium VIBE axial T1 weighted imaging. Each patient was injected with 10 cc of IV gadolinium (Gadovist 1.0; Bayer AG, Leverkusen, Germany) and the number, location and size of each metastasis were documented.

Reference standard and statistical analysis

The primary outcome measure was the percentage of patients in whom PET/CT and brain MRI led to a change in disease management. We deemed a change of treatment in at least 10% of the patients clinically relevant. Secondary outcome measures were change of AJCC-UICC stage and performance parameters of the imaging. Pathological confirmation of suspicious/positive lesions with FDG uptake on PET/CT was pursued. If pathological confirmation was not possible, clinical outcome and imaging after 6 months were used as gold standards.

The sensitivity and specificity of PET/CT and brain MRI were determined by patient-based analysis. A true-positive finding surpassed a false-negative finding in the same patient. Scans were classified as true-positive if metastatic melanoma was suggested and confirmed, and as false-positive, if the suspected metastatic melanoma was confirmed to be something else. A lesion with FDG uptake unequivocally reported and confirmed to be a non-melanoma malignancy was classified as an incidental finding, not false-positive. Scans that were considered to be negative were classified as true-negative if the patient did not develop a recurrence during the 6 months following the baseline imaging. Scans were considered false-negative if the baseline scan failed to reveal the initial S&ITM that was still present or if evidence of any further metastasis was established during the 6 months of follow-up.

Disease progression was defined as detection of melanoma metastasis after baseline imaging and treatment, by the patient, the physician or through further imaging and preferably with subsequent pathological confirmation.

Results

The baseline PET/CT showed the known tumor sites in eight of the 15 patients (53%) in whom the S&ITM was still present at the time of the imaging, but no further S&ITM were visualized. In the other seven patients, the known S&ITM was not visible on the baseline PET/CT. Additional metastases were identified in four of the 25 patients in the study (16%). These were regional lymph node metastases in two patients, distant metastases in one patient, and simultaneous regional and distant metastases in the remaining patient (figure 4.1). PET/CT was false-positive in one of the 25 patients (4%), who had a Schwannoma in the right trapezius muscle. A thyroid carcinoma was an incidental finding in another patient.

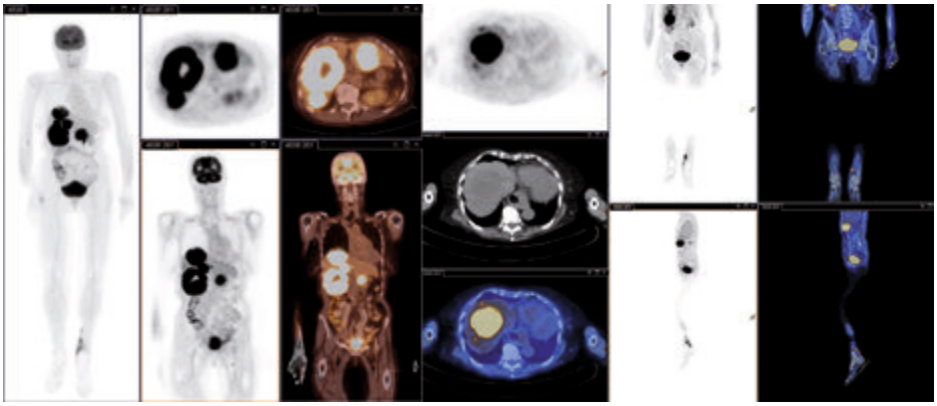

Figure 4.1 Whole-body FDG PET/CT patient 2 (Table 4.2)

Marked FDG avidity in the liver corresponding to numerous hypodense lesions throughout both lobes. Focal FDG avidity in the left os ilium, corresponding to a partially sclerotic lesion in the low dose CT scan. Mildly hypermetabolic 12 mm left inguinal lymph node. Extensive, contiguous, FDG avid soft tissue nodular thickening in the left leg medially.
CT computed tomography; *FDG* 18F-fluorodeoxyglucose *PET* positron emission tomography.

The primary aim of the study was to determine the implications of imaging for staging and subsequent management. PET/CT led to a change in the AJCC-UICC stage (eighth edition) of four of the 25 patients (16%). In all four patients (16%), PET/CT led to modification of the pre-imaging treatment plan (table 4.2).

Following the baseline PET/CT and treatment, further sites of disease became evident within 6 months in 10 of the 25 patients (40%) (table 4.3). In eight of these 10 patients, this was additional in-transit disease. Recurrences were detected prior to the 6-month PET/CT in nine of the 10 patients (90%). These recurrences were detected by the patient, by the physician or through

ultrasound follow-up. The PET/CT after 6 months revealed even more metastases in five of these nine patients (patients 1, 3, 4, 7 and 8).

Table 4.2 Characteristics of patients with a change in melanoma treatment plan

Patient no.	Age (y)	Sex	Primary tumor site	BT (mm)	TMR (/mm²)	Ulceration	S&ITM	First presentation or recurrence	Original treatment plan	Further metastases	Change in AJCC stage[a]	Change in treatment plan
1	76	F	Lower limb	1.2	0	No	Two ITMs	Recurrence	Excision of ITMs	Numerous additional ITMs, subcutaneous distant	IIIB → IV	Referred for immunotherapy
2	92	F	Lower limb	3.0	6	Yes	Multiple ITMs	Recurrence	T-VEC, ILI or systemic therapy for irresectable stage III melanoma	Regional LN, bone and liver	IIIC → IV	Referral to medical oncologist; died prior to appointment
3	76	F	Upper limb	26.0	22	Yes	Satellite	First presentation	WLE+SLNB	Regional LNs	IIIC → IIID	TLND right axilla
4	80	M	Head & neck	3.1	25	No	Microsatellite	First presentation	No further treatment (WLE already performed)	Regional LN	IIIB → IIIC	TLND right neck

AJCC American Joint Committee on Cancer; *BT* Breslow thickness; *F* female; *ILI* isolated limb infusion; *LN* lymph node; *M* male; *S&ITM* satellite and in-transit metastasis; *SLNB* sentinel lymph node biopsy; *TLND* therapeutic lymph node dissection; *TMR* tumor mitotic rate; *T-VEC* talimogene laherparepvec; *WLE* wide local excision.
[a]Eighth edition.

Table 4.3 Disease progression and PET/CT and brain MRI findings at 6 months

Patient no.	Age (y)	Sex	Primary tumor site	S&ITM	S&ITM location	Recurrence prior to six months imaging	Location of recurrence	Detection mode of recurrence	Treatment	Six months imaging findings	Treatment
1	84	M	R upper arm - cubital fossa	ITM	R upper arm	ITM	R upper arm	Patient	Excision	More ITMs	US nine lesions detected, excision of two palpable lesions
2[a]	64	M	L plantar foot	ITM	L dorsum foot	ITM	Inner thigh and left shin	Patient	Excision	-	-
3[b]	76	F	R posterior brachii region	Satellite	R posterior brachii region	-	-	-	-	LNs mediastinum + brain metastases	Pembrolizumab
4	66	M	R lateral shin	ITM	R proximal shin	ITM + regional LN	R anterior thigh, R knee, R groin	US surveillance, FNAC	Excision of ITM	Distant cutaneous metastasis and confirmation of more ITMs + regional LN	Pembrolizumab
5	53	F	R lateral thigh	ITM	R lateral thigh, medial to WLE scar	ITM	R lateral thigh	Patient (US and FNAC)	Excision	-	-
6	67	F	R lateral shin	Satellites & microsatellites	R lateral shin	ITMs	R anterior and lateral shin	Physician, US and shave biopsy	-	Confirmation of ITMs	Pembrolizumab
7	35	F	L lower calf	ITM	L upper calf below popliteal fossa	ITM	L calf	Patient (US and FNAC)	Excision	Liver & lung metastases	Pembrolizumab
8	45	M	L calf	ITM	L lateral shin, distal to WLE scar	ITM	L calf	Patient and US	Excision	More ITMs	Excision

Table 4.3 Disease progression and PET/CT and brain MRI findings at 6 months *(continued)*

Patient no.	Age (y)	Sex	Primary tumor site	S&ITM	S&ITM location	Recurrence prior to six months imaging	Location of recurrence	Detection mode of recurrence	Treatment	Six months imaging findings	Treatment
9	74	F	R lateral ankle	Microsatellite	R lateral ankle	ITMs + regional LNs	R shin and calf, R groin	Patient (US and FNAC)	Pembrolizumab	Confirmation of ITMs + regional LNs	Pembrolizumab
10	55	F	R plantar foot	Microsatellite	R plantar foot	regional LN	R groin	US and FNAC	Pembrolizumab	Confirmation of regional LNs	Pembrolizumab

F female; *FNAC* fine needle aspiration cytology; *L* left; *LN* lymph node; *M* male; *R* right; *S&ITM* satellite and in-transit metastasis; *US* ultrasound.

[a]Death non-melanoma prior to 6 months imaging.

[b]Patient no 3 in Table 4.2.

Including the S&ITM that were present initially but not apparent on the PET/CT, the baseline scans of 12 patients were false-negative. In four of these patients, the baseline scan was also true-positive. Because a true-positive finding trumped a false-negative finding in the same patient, eight scans were ultimately classified as false-negative. The performance of PET/CT in various categories is presented in tables 4.4 and 4.5.

Table 4.4 Contingency tables according to stage of disease and overall

	TP	FP	FN	TN
N-stage	11	0	8	6
S&ITM	8	0	9	8
LN	4	1	2	18
M-stage	2	1	3	19
Overall	11	1	8	5

FN false-negative; *FP* false-positive; *LN* lymph nodes; *S&ITM* satellites and in-transit metastasis; *TN* true-negative; *TP* true-positive.

Table 4.5 Performance of PET/CT

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
N-stage	58	100	100	43
S&ITM	47	100	100	47
LN	80	90	67	95
M-stage	40	95	67	86
Overall	58	83	92	39

LN lymph nodes; *NPV* negative predictive value; *PPV* positive predictive value; *S&ITM* satellites and in-transit metastasis.

Baseline brain MRI was normal in all patients and did not result in change of stage or treatment plan in any of them. The follow-up MRI at 6 months was not performed in three patients, because two had died and a third suffered a panic attack during the imaging. The brain MRI at 6 months revealed a previously unknown right superior frontal lobe metastasis in one patient, so in this case, the baseline scan was classified as false-negative.

All melanoma metastases found at baseline as well as during the 6 months of follow-up were treated, unless the patient was unfit for any treatment or the patient and physician decided upon close monitoring until further progress of disease. Two patients died during the 6 months observation period: one from distant metastatic disease and the other from an unrelated cause.

Discussion

As expected, the results of imaging melanoma patients who at first presentation with S&ITM, which were generally small, were less likely to reveal other metastatic disease than in patients with more bulky lesions. The 58% sensitivity of PET/CT found in this study is substantially less than the 87% previously reported in patients with palpable lymph node metastases.[1] It is likely that this is because the non-visualized metastases on staging PET/CT that became clinically evident later were below the spatial resolution of PET detection (approximately 3 mm), or due to rapidly progressive new metastases in the following 6 months. Despite this, upstaging and a change in the pre-scan management plan in 16% of patients imply a meaningful impact. The importance of changes in disease management has been affirmed in the recently published Cochrane review on staging imaging in melanoma.[10] In the present study, MRI of the brain never revealed a metastasis at baseline and its usefulness in this setting therefore appears dubious.

To our knowledge, this study is the first to report the impact of whole-body PET/CT and brain MRI staging on patients with a first presentation with S&ITM. Relevant aspects are its prospective design, the strict inclusion criteria for patients with S&ITM and the use of one PET/CT scanner for all patients. There are several possible limitations to discuss. We hypothesized that PET/CT and brain MRI should be able to detect metastases up to 6 months before they become evident otherwise. This time frame is, of course, arbitrary and the choice of a shorter period would have resulted in a lower false-negative rate.

We limited our study to patients with a first diagnosis of S&ITM. If we had included patients with recurrent in-transit metastases it is likely the sensitivity of imaging would have been greater, as the disease would be likely to be more advanced.

As the AJCC eighth edition cancer staging system provides a new staging classification, which includes a separate schema for patients who recur after their initial melanoma presentation (rTNM), our 25 patients may represent a heterogeneous group. However, to our knowledge this recurrent staging classification is not yet embedded in melanoma research, hence no conclusions can be drawn concerning the differences in sensitivity of imaging for the two scenarios. In terms of survival, Read et al.[11] reported an overall 5-year survival from time of ITM presentation of 47% in patients with ITMs as a first site of recurrence versus 43% in patients presenting with ITMs at primary diagnosis, suggesting the latter group has a somewhat worse prognosis.

We hypothesized that known S&ITM would be identified depending on their size, with a higher pick-up rate for larger S&ITM. Interestingly, only one of the seven patients in whom S&ITM was not detected on the baseline scan had lesions that were less than 3 mm in diameter. In the

other six patients, lesions from 3 to 7 mm (on histopathology) were missed on PET/CT. This suggests that cutaneous and subcutaneous metastases up to 7 mm in diameter might be difficult to detect on PET/CT.

Five previous studies have reported on change in treatment plan after primary staging with PET/CT. In a prospective study of 32 patients with stage III–IV melanoma being considered for surgical treatment, Bronstein et al.[12] found that the treatment plan in 12% of patients changed after treatment decisions based on PET/CT were compared with initial treatment decisions (based on conventional imaging with CT and MRI). Aukema et al. reported a change in treatment plan in 26 of 70 patients (37%) with palpable lymph node metastases.[1] In a retrospective cohort of 30 sentinel lymph node-positive patients, Constantinidou noted no change in treatment plans.[6] In a retrospective cohort, Arrangoiz et al.[13] reported a change in treatment plan in 18% of patients with T4 melanoma and no clinical evidence of metastatic disease. Their further analysis showed that 11% of the changes were due to identification of regional metastases and 7% were due to identification of distant metastases. Also, 45% of their patients had satellitosis, but no information on change in treatment in this subgroup was provided. In a retrospective study of 149 patients with stage IB–II melanoma, Barsky et al.[14] found that PET/CT never resulted in a change in treatment plan.

Two meta-analyses have addressed the value of PET/CT in primary staging of melanoma patients with AJCC-UICC (seventh edition) stages I–IV disease.[4,5] In the most recent of the two, Schröer-Günther et al. analyzed the diagnostic reliability of PET and PET/CT in prospective studies with a patient-based analysis published up to 2010.[5] Only studies with a clinical follow-up of >6 months were included. For PET/CT the sensitivity of all included studies ranged between 17 and 85% and the specificity ranged from 74 to 96%.

Studies evaluating the diagnostic accuracy of PET/CT specifically in stage III melanoma patients are scarce. Aukema et al. found a sensitivity of 87% and a specificity of 98% in the detection of distant disease in patients with palpable lymph node disease. Accuracy was 98%, positive predictive value (PPV) 96% and negative predictive value (NPV) 91%.[1]

In conclusion, the current study indicates that staging patients with S&ITM using PET/CT has a meaningful impact on patient management. There was upstaging of disease and a change in treatment as a result of whole-body PET/CT in 16% of the patients. The overall sensitivity was 58%, specificity 83%, PPV 92% and NPV 39%. MRI of the brain did not detect any metastases at baseline and might be omitted, particularly since modern-day PET/CT devices can provide a low radiation dose CT scan for screening the brain. Ten patients (40%) developed further metastasis within 6 months after the initial staging imaging and treatment. This suggests that

an appropriate interval for repeat imaging in this high-risk cohort may be 3 months rather than 6 months. A future study assessing this interval would be worthwhile.

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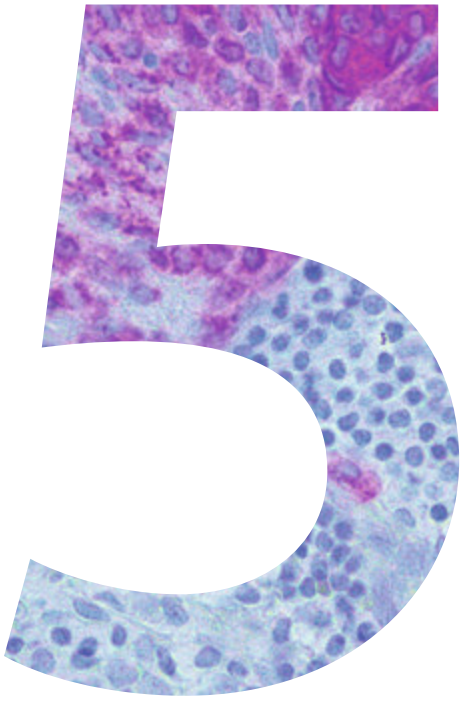
CHAPTER



Hypofractionated or conventionally fractionated adjuvant radiotherapy after regional lymph node dissection for high-risk stage III melanoma

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Abstract

Aims – Adjuvant radiotherapy can be beneficial after regional lymph node dissection for high-risk stage III melanoma, as it has been shown to reduce the risk of recurrence in the node field. However, the optimal fractionation schedule is unknown and both hypofractionated and conventionally fractionated adjuvant radiotherapy are used. The present study examined the oncological outcomes of these two approaches in patients treated in an era before effective systemic immunotherapy became available.

Materials and methods – This retrospective cohort study involved 335 patients with stage III melanoma who received adjuvant radiotherapy after therapeutic regional lymph node dissection for metastatic melanoma between 1990 and 2011. Information on tumor characteristics, radiotherapy doses and fractionation schedules and patient outcomes was retrieved from the institution's database and patients' medical records.

Results – Hypofractionated radiotherapy (median dose 33 Gy in six fractions over 3 weeks) was given to 95 patients (28%) and conventionally fractionated radiotherapy (median dose 48 Gy in 20 fractions over 4 weeks) to 240 patients (72%). Five-year lymph node field control rates were 86.0% (95% confidence interval 78.4-94.4%) for the hypofractionated group and 85.5% (95% confidence interval 80.5-90.7%) for the conventional fractionation group (P=0.87). There were no significant differences in recurrence-free survival (RFS) (41.7%, 95% confidence interval 32.5-53.5 versus 31.9%, 95% confidence interval 26.1-38.9; P=0.18) or overall survival (41.2%, 95% confidence interval 32.1-52.8 versus 45.0%, 95% confidence interval 38.7-52.4; P=0.77). On multivariate analysis, extranodal spread was associated with decreased RFS (P=0.04) and the number of resected lymph nodes containing metastatic melanoma was associated with decreased RFS (P=0.0006) and overall survival (P=0.01).

Conclusion – Lymph node field control rates, RFS and overall survival were similar after hypofractionated and conventionally fractionated adjuvant radiotherapy. The presence of extranodal spread and an increasing number of positive lymph nodes were predictive of an unfavourable outcome.

Introduction

The role of adjuvant radiotherapy following regional lymph node dissection for high-risk stage III melanoma was defined by a large randomised trial (ANZMTG 01.02/TROG02.01) that showed significant improvement in lymph node field control, although there was no impact on overall survival.[1] Based on this trial, consideration of adjuvant radiotherapy was recommended for patients in this clinical situation. However, its role is once again the subject of discussion, now that effective adjuvant systemic treatments are available.[2]

Another debate concerns the optimal fractionation schedule of adjuvant radiotherapy, if given. This has not been determined. Radiobiology studies show a large 'shoulder' on the in vitro melanoma cell survival curve.[3-5] This indicates a great ability of some melanoma cells to accumulate sublethal damage, suggesting that hypofractionation may be more effective and that conventional fractionation might have been a reason for the poor results that were originally reported.[4,5] In other words, rather than the total dose, larger fraction doses may be the key to achieving adequate responses to radiotherapy.[6,7] This concept led to the practice of using hypofractionation[8], which is often given as 30-33 Gy in five to six fractions, whereas the only randomised trial of adjuvant radiotherapy for resected stage III melanoma used the fractionation of 2.4Gy per fraction to a total dose of 48 Gy.[1] A prospective randomised trial of palliative radiotherapy in 126 patients with unresectable melanoma metastases comparing 32 Gy in four fractions and 50 Gy in 20 fractions showed no difference in the response rates.[9] Currently, there is no consensus and it has been suggested that the evidence for hypofractionation was potentially biased by small numbers, a wide range of tumor sizes and total doses, and short follow-up.[10]

The aim of the present study was to compare the oncological outcomes of hypofractionated and conventionally fractionated adjuvant radiotherapy following regional lymph node dissection for high-risk melanoma metastases in patients treated in an era before effective systemic therapy. The primary end point was node field recurrence (as a first recurrence). Secondary end points were recurrence-free survival (RFS) and overall survival. Risk factors associated with lymph node field recurrence (as a first recurrence), RFS and overall survival were also assessed.

Patients, Materials and Methods

Study Population and Treatment

Data for patients who had undergone regional lymph node dissection for a first lymph node metastasis followed by adjuvant radiotherapy between 1990 and 2011 were retrieved from our institution's database, which contains comprehensive prospectively collected data. Patients with recurrent nodal disease after previous node surgery or with distant metastasis at the time of radiotherapy and patients without adequate follow-up were excluded. All patients had given informed consent for their data to be collected and used for research purposes. The research protocol was approved by our institution's Research Committee.

The choice of fractionation schedule was at the discretion of each patient's treating radiation oncologist. Patients received either conventionally fractionated radiotherapy (usually 48-50.4 Gy in 20 fractions of 1.8-2.5 Gy, five times per week) or hypofractionated radiotherapy (33 Gy in six fractions of 5-6 Gy twice a week). Due to the wide geographical distribution of patients referred to the Melanoma Institute Australia (MIA), some patients in the study chose to receive radiotherapy at a local facility closer to their place of residence.

Recurrence at any site was defined by the detection of any clinical, histological or radiographic evidence of tumor. Node field recurrence was defined as soft-tissue or lymph node recurrence within the anatomical lymph node field.

Statistical Analysis

Patients' characteristics were summarised using standard descriptive statistics. Continuous variables were described by their median (range) and categorical variables by their frequency (proportion). The primary end point was lymph node field recurrence (as a first recurrence). This endpoint was consistent with the ANZMTG 01.02/TROG 02.01trial end point. Secondary end points were RFS and overall survival. Survival times were calculated from the first date of radiotherapy to the date of node field recurrence, recurrence (local, regional or distant), death due to melanoma or death from any cause. Patients without recurrence were censored at either their date of death or the last date known alive. Survival outcomes were described graphically using the Kaplan-Meier method and stratified by fractionation schedule (conventional versus hypofractionated). Univariate Cox regression was carried out to assess the risk factors associated with node field recurrence, RFS and overall survival. The investigated risk factors included fractionation schedule, known or unknown primary melanoma, RT treatment facility, presence or absence of extranodal spread, number of positive lymph nodes, size of largest positive lymph node and site of node field. Multivariate models were developed using stepwise backward selection on the initial models that included fractionation schedule and variables with a P-value < 20% from the univariate analysis. Ulceration was not included in the multivariate models

because this parameter was missing in 30% of cases, attributed to the large number of unknown primaries. P-value was based on Wald statistics to test the global effect of the covariate; $P < 0.05$ was considered significant. Acute skin toxicity data were scored using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) or Radiation Therapy Oncology Group (RTOG) toxicity criteria. Statistical analyses were carried out using SPSS version 26.0 (IBM Corporation, Armonk, NY, USA), SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R version 3.6.1 (R Core Team, Vienna, Austria).

Table 5.1 Baseline characteristics

Characteristics	**Hypofractionated radiotherapy (*n*=95)**	**Conventionally fractionated radiotherapy (*n*=240)**	***P*-value**
Age at nodal disease diagnosis (years)			
Median (range)	59.0 (23.0-90.0)	58.0 (19.0-88.0)	0.6296
Gender			
Female	18/95 (19%)	63/240 (26%)	0.1594
Male	77/95 (81%)	177/240 (74%)	
Breslow thickness (mm)			
Median (range)	2.3 (0.2-21.0)	2.1 (0.0-23.0)	0.2994
Location of the primary			
Head and neck	35/95 (37%)	57/240 (24%)	0.0748
Trunk	23/95 (24%)	72/240 (30%)	
Upper limb	4/95 (4%)	19/240 (8%)	
Lower limb	14/95 (15%)	52/240 (22%)	
Unknown primary	19/95 (20%)	40/240 (17%)	
Mitotic rate (59 unknown primary, 35 missing data)			
Absent	8/69 (12%)	16/172 (9%)	0.591
Present	61/69 (88%)	156/172 (91%)	
Ulceration (59 unknown primary, 41 missing data)			
No	42/66 (64%)	96/169 (57%)	0.339
Yes	24/66 (36%)	73/169 (43%)	
Type of surgery			
Therapeutic lymph node dissection	85/95 (89%)	219/240 (91%)	0.334
Completion lymph node dissection	8/95 (8%)	20/240 (8%)	
Elective lymph node dissection	2/95 (2%)	1/240 (0.4%)	
Node field			
Neck	45/95 (47%)	70/240 (29%)	0.005
Axilla	34/95 (36%)	103/240 (43%)	
Groin	16/95 (17%)	67/240 (28%)	

Table 5.1 Baseline characteristics *(continued)*

Characteristics	Hypofractionated radiotherapy (*n*=95)	Conventionally fractionated radiotherapy (*n*=240)	*P*-value
Number of nodes identified in specimen			
Total median (range)	24 (5-83)	24 (4-74)	0.171
Neck	36 (9-69)	34 (12-74)	
Axilla	24 (5-83)	24 (7-61)	
Groin	11 (8-25)	17 (4-38)	
Number of positive nodes			
Total median (range)	3 (1-83)	3 (1-51)	0.086
Neck	2 (1-44)	2 (1-26)	
Axilla	4 (1-83)	3 (1-51)	
Groin	3 (1-16)	4 (1-22)	
Presence of extranodal spread (five missing data)			
No	30/92 (33%)	106/238 (45%)	0.048
Yes	62/92 (67%)	132/238 (55%)	
Size of largest nodal deposit (mm)			
Median (range)	28.5 (5-120)	30 (5-120)	0.557
Radiotherapy treatment centre			
Melanoma Institute Australia	90/95 (95%)	136/240 (57%)	<0.001
Other	5/95 (5%)	104/240 (43%)	

Results

The inclusion criteria for the study were met by 335 patients (table 5.1). The indications for lymph node dissection were palpable metastatic disease in 304 patients (91%) positive sentinel nodes in 28 patients (8%) and an elective lymph node dissection with positive nodes in three patients (1%). Of the 137 patients with axillary nodal disease, 131 underwent level I-III axillary node dissection. Six patients had both a level I-III axillary dissection and various levels of cervical node dissection. The extent of the lymph node dissection varied in the 115 patients who had a cervical node dissection. In 28 patients, this was a level I-V+parotid (P) dissection, 19 patients had level II-V, 18 had level II-V+P, 17 had level I-V, 11 had level I-III+P, seven had level I-IV+P, three had level III-V and two had level I-III dissections. In solitary cases, the following levels were dissected: level I-IV+axillary I-III, level I-V, level II-III+P,level II-IV, level II-IV+P, level V, level V+P and P+suprahyoid region. In two patients the extent of the cervical dissection was not recorded. Of the 83 patients with inguinal node disease, 34 had an inguinal dissection and 49 had ilio-obturator-inguinal dissection.

Hypofractionated radiotherapy (median dose 33 Gy in six fractions over 3 weeks) was given to 95 patients (28%) and conventionally fractionated radiotherapy (median dose 48 Gy in 20 fractions over 4 weeks) to 240 patients (72%). Most of the baseline characteristics and burdens of nodal disease were well balanced between the two radiotherapy groups, except for node field site, presence of extranodal spread and radiotherapy treatment centre (Table 1). The median follow-up was 26 months (range 1 month - 21 years) and there was no difference between the hypo-fractionation group (25 months, range 2 months - 21 years) and the conventional fractionation group (26 months, range 1 month - 16 years). Patients treated at MIA had a slightly longer duration of follow-up than patients treated at other radiotherapy treatment centres (median 27 months versus 24 months).

In total, 226 patients (68%) received radiotherapy at MIA and the remaining 109 patients (33%) had their radiotherapy at other facilities. Of the patients treated at MIA, 58% received conventional fractionation, whereas 95% of those treated at other facilities did so. The median interval between surgery and the start of radiotherapy was 6 weeks (range 0-22 weeks). In five patients, radiotherapy treatment was ceased early; in four this was due to disease progression and one patient decided not to complete the treatment.

Adjuvant systemic treatment was given to 52 patients (16%); 39 of them received a vaccine or participated in an adjuvant vaccine therapy trial and 13 received interferon-α.[11-16] None of the patients in this series received modern adjuvant therapy with an immune checkpoint inhibitor or a BRAF/MEK inhibitor.

In the hypofractionation group, 12 of the 95 patients (13%) developed a first recurrence in or adjacent to their radiated field and in the conventional fractionation group this occurred in 29 of the 240 patients (12%; P=0.87; figure 5.1). Five-year lymph node field control rates were almost equal, being 86% (95% confidence interval 78.4-94.4%) in the hypofractionated group and 85.5% (95% confidence interval 80.5-90.7%) in the conventional fractionation group. Neither univariate analysis nor multivariate analysis revealed any factors predictive of node field recurrence (table 5.2).

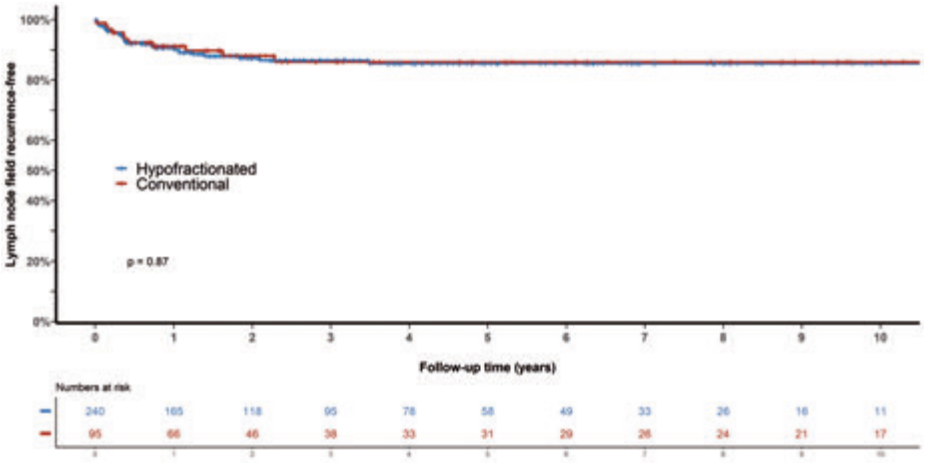

Figure 5.1 Lymph node field control: percentage of patients without lymph node field recurrence (as a first recurrence)

Most recurrences arose within the first 3 years (figure 5.2). Five-year RFS rates were 41.7% (95% confidence interval 32.5-53.5) in the hypofractionation group and 31.9% (95% confidence interval 26.1-38.9) in the conventional fractionation group (P=0.18). Multivariate analysis showed that the presence of extranodal extension (P=0.04) and an increasing number of positive lymph nodes (P=0.0006) were predictive of reduced RFS.

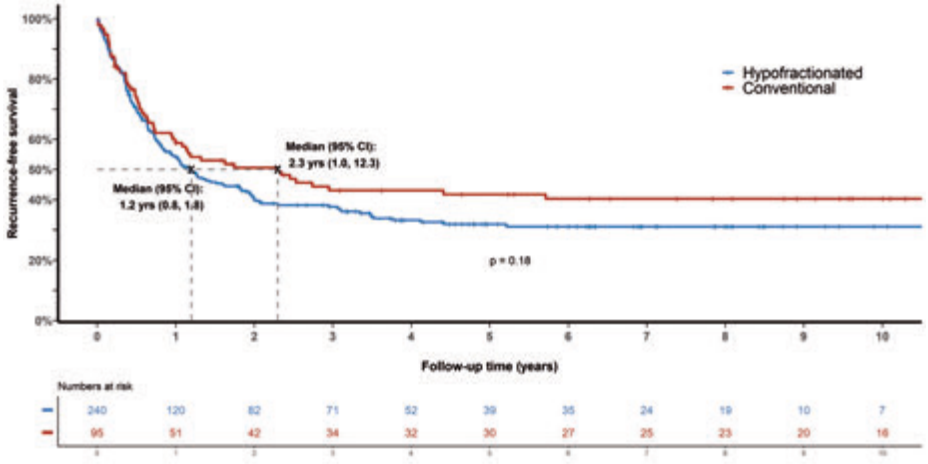

Figure 5.2 Recurrence-free survival

There was no significant difference in overall survival between the two groups (P=0.77; figure 5.3). The 5-year overall survival rates were 41.2% (95% confidence interval 32.1-52.8) in the

hypofractionation group and 45.0% (95% confidence interval 38.7-52.4) in the conventional fractionation group. An increasing number of positive lymph nodes was predictive of death on multivariate analysis (P=0.01; table 5.2).

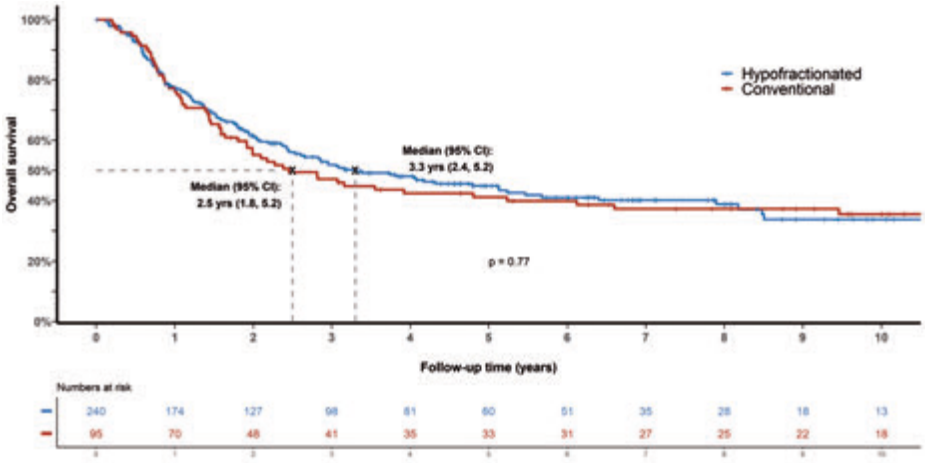

Figure 5.3 Overall survival

Acute skin toxicity data could be quantified into an NCI CTCAE or RTOG score in 233 of the 335 patients (70%). Grade 2 acute skin toxicity was documented in 24 patients (44%) in the hypofractionated group and in 108 patients (60%) in the conventional fractionation group. Grade 3 acute skin toxicity was documented in zero (0%) and 12 patients (7%) and grade 4 acute skin toxicity in five (9%) and 13 patients (7%), respectively. The following late toxicity data could be extracted from the patient files: atrophy, fibrosis and/or induration were noted in 28 patients (29%) in the hypofractionation group and in 51 patients (21%) in the conventional fractionation group. Lymphedema was documented in 32 patients (34%) and 106 patients (44%), respectively. The relationship to either lymph node dissection or radiotherapy treatment could not be distinguished. Long-term wound issues were documented in four patients (4%) and six patients (3%), respectively. Osteoradionecrosis occurred in one patient who was treated with conventional fractionation.

Table 5.2 Univariate/multivariate Cox regression analysis

Variables	*n*	Lymph node fieldrecurrence				Recurrence-free survival				Overall survival			
		Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
		HR (95% CI)	P-value*	HR (95% CI)	P-value*	HR (95% CI)	P-value*	HR (95% CI)	P-value*	HR (95% CI)	P-value*	HR (95% CI)	P-value*
Radiotherapy type													
Hypofractionated radiotherapy	95	1		1		1		1		1		1	
Conventional radiotherapy	240	1.06 (0.53-2.12)	0.87	1.27 (0.45-3.54)	0.64	1.24 (0.91-1.69)	0.18	0.77 (0.56-1.06)	0.11	0.94 (0.69-1.29)	0.70	1.04 (0.75-1.45)	0.80
Unknown primary													
No	277	1				1				1			
Yes	58	0.34 (0.11-1.11)	0.07			0.80 (0.55-1.16)	0.24			0.79 (0.53-1.17)	0.23		
Institution													
Other institution	109	1		1		1				1			
Melanoma Institute Australia	226	0.57 (0.31-1.07)	0.08	1.69 (0.79-3.64)	0.18	0.90 (0.67-1.21)	0.48			1.12 (0.81-1.54)	0.49		
Presence of extranodal spread													
No	136	1				1		1		1		1	
Yes	194	1.30 (0.69-2.45)	0.42			1.38 (1.04-1.83)	0.03	1.35 (1.01-1.80)	0.04	1.42 (1.05-1.92)	0.02	1.35 (0.99-1.83)	0.06

Table 5.2 Univariate/multivariate Cox regression analysis *(continued)*

Variables	*n*	Lymph node fieldrecurrence				Recurrence-free survival				Overall survival			
		Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
		HR (95% CI)	P-value*	HR (95% CI)	P-value*	HR (95% CI)	P-value*	HR (95% CI)	P-value*	HR (95% CI)	P-value*	HR (95% CI)	P-value*
Number of positive lymph nodes													
0-1	99	1				1		1		1		1	
2-3	116	1.70 (0.72-4.02)	0.18			1.36 (0.94-1.95)	0.0002	1.39 (0.96-2.01)	0.0006	1.38 (0.94-2.02)	0.0007	1.35 (0.91-2.00)	0.01
>	120	2.20 (0.95-5.08)				2.05 (1.44-2.90)		2.00 (1.39-2.82)		2.02 (1.40-2.91)		1.80 (1.22-2.67)	
Node field													
Axilla	131	1		1		1				1		1	
Groin	83	0.94 (0.43-2.09)	0.97	0.94 (0.39-2.14)	0.77	1.29 (0.92-1.80)	0.05			1.10 (0.77-1.57)	0.07	1.00 (0.69-1.42)	0.38
Neck	121	0.91 (0.45-1.85)		0.74 (0.30-1.82)		0.84 (0.61-1.16)				0.73 (0.52-1.02)		0.79 (0.55-1.12)	

CI confidence interval; *HR* hazard ratio.

*Based on Wald statistics to test the global effect of the covariate.

Discussion

In the only completed randomised trial of adjuvant radiotherapy following regional node dissection for high-risk stage III melanoma, 48 Gy in 20 fractions was shown to significantly improve node field control after regional lymph node dissection, but there was no overall survival benefit.[1] The present study showed that the risk of node field recurrence, RFS and overall survival with hypofractionated and conventionally fractionated adjuvant radiotherapy were similar. There was no indication of increased toxicity in the hypofractionation group. With the availability of effective adjuvant systemic therapy, the role of adjuvant radiotherapy for resected high-risk stage III melanoma needs to be re-evaluated. Adjuvant radiotherapy is less frequently recommended, whereas adjuvant systemic therapies with immune checkpoint inhibitors or targeted therapy have become standard. Recent studies suggest an immunogenic effect when combining radiotherapy with concurrent checkpoint inhibition, especially with the use of hypofractionated radiotherapy.[17-19] This development warrants further investigation of radiotherapy fractionation in patients with melanoma. In addition to the potential immunogenic effect, a hypofractionated schedule would also mean six treatment visits instead of 20, which would be easier for patients and reduce demand on healthcare resources. During the recent COVID-19 pandemic, the use of hypofractionation in cancer patients has been successfully expanded to minimise treatment time.[20]

Finally, adjuvant radiotherapy remains an important option for patients who are not eligible for adjuvant systemic therapy (such as those patients with a history of significant autoimmune disease or transplantation).

Our results correspond with those of three previous studies that have compared hypofractionated and conventionally fractionated radiotherapy in patients with melanoma. In the only prospective study, the RTOG 83-05 trial directly compared 4 x 8 Gy in 62 patients with 20 x 2.5 Gy in 64 patients.[9] However, the radiotherapy was not used as adjuvant therapy, but its purpose was palliation for macroscopic disease. There was no difference in the measurable response rate between the two arms, but toxicity was slightly higher in the hypofractionation group. Chang et al.21 retrospectively assessed locoregional control in patients treated with nodal radiotherapy with curative intent. They compared 5 x 6 Gy in 41 patients with 30 x 2 Gy in 14 patients and found no difference in node field control, melanoma-specific survival or overall survival. In the combined group, 5-year node field control was 87%, melanoma-specific survival 57% and overall survival 46%, after a median follow-up of 4.4 years. The authors reported minimal toxicity in the hypofractionation group. Patient selection, however, was different from selection for our study, as 52% of patients were treated after recurrence of disease, whereas our cohort comprised solely patients with first nodal disease presentation. Also, 87% of their patients had undergone a cervical lymph node dissection, which was the case in only 48% of

our patients. In the most recently reported study, Mendenhall et al.[22] compared 42 patients treated with hypofractionated radiotherapy and 40 patients who underwent conventionally fractionated radiotherapy in the adjuvant setting because they were considered to beat high risk of locoregional recurrence. In 78% of patients, the nodal disease was located in the head and neck region. Most (62%) patients were treated for recurrent disease after initial surgery; the others were clinically disease-free after initial surgery. In the latter group, four patients (5%) did not have sentinel lymph node biopsy or lymph node dissection, so their nodal status at the time of radiotherapy was unknown. There was no significant difference in 5-year node field control when comparing the fractionation schedules. In the combined group of 82 patients, node field control was 82%, melanoma-specific survival 56% and overall survival 43% after a median follow-up of 3 years. Toxicity was modest in the combined group.

We assessed the risk factors associated with node field recurrence, RFS and overall survival. Extranodal spread was associated with worse RFS. An increasing number of positive lymph nodes was associated with worse RFS and overall survival. This is in line with the findings from other studies.[23-28]

The main strength of the present study was the size of the cohort, to our knowledge the largest comparing fractionation schedules in a strictly adjuvant setting after regional lymph node dissection for metastatic melanoma. One of the risk factors we had planned to assess was the size of the metastatic lymph nodes, but incompleteness of the available data prevented a meaningful analysis. As 91% of patients had undergone an initial therapeutic lymph node dissection for palpable disease, the sizes of positive lymph nodes were unlikely to have been documented by pathologists as thoroughly as in patients undergoing sentinel lymph node biopsy. Follow-up was somewhat shorter in the group of patients receiving radiotherapy at other institutions, which may have influenced the assessment of outcomes for this subgroup.

The retrospective nature of our study limited reliable assessment of acute and late toxicity; therefore, our toxicity data should be interpreted with caution. Hypofractionated radiotherapy is potentially associated with more late toxicity, such as fibrosis and lymphedema. However, this suggestion is based on the results of older retrospective studies using outdated radiotherapy techniques, and hypofractionation has not been prospectively compared with conventional fractionation in the adjuvant setting inpatients with melanoma.[8,27,29] In another adjuvant radiotherapy setting, whole-breast irradiation after breast-conserving surgery has been studied prospectively and long-term follow-up data have shown no difference in late toxicity effects between hypofractionated and conventionally fractionated radiotherapy schedules.[30] Furthermore, advances in radiotherapy treatment planning and the use of image guidance and motion management allow for better sparing of normal structures and, hence, toxicity is

probably reduced. We emphasise the importance of prospectively collected toxicity data to further determine the role of hypofractionated radiotherapy.

Conclusions

This study confirms that adjuvant hypofractionated radiotherapy to the node field is an option after lymph node dissection in patients with high-risk nodal melanoma metastases, with outcomes similar to those achieved by conventionally fractionated radiotherapy. There were no significant differences in the risk of node field recurrence (P=0.87), RFS (P=0.2) or overall survival (P=0.77). In both groups, on multivariate analysis, extranodal spread was associated with decreased RFS (P=0.04) and the number of metastatic lymph nodes was associated with decreased RFS and overall survival (P=0.0006, P=0.01, respectively). Future studies should investigate the benefit of radiotherapy in patients with high-risk nodal disease receiving contemporary adjuvant systemic therapy.

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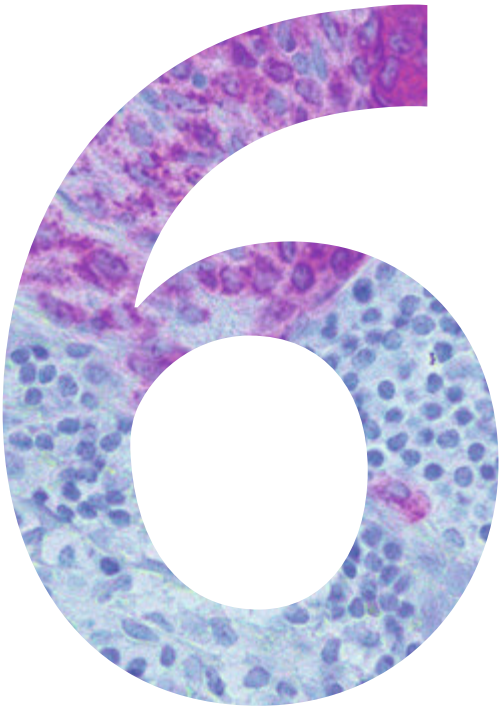
CHAPTER



Adjuvant radiotherapy after salvage surgery for melanoma recurrence in a node field following a previous lymph node dissection

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Abstract

Background and Objectives – Adjuvant radiotherapy (RT) can be given to melanoma patients following salvage surgery for node field recurrence after a previous regional node dissection, but the value of this treatment strategy is poorly documented. This study evaluated long-term node field control and survival of patients treated in this way in an era before effective adjuvant systemic therapy became available.

Methods – Data for 76 patients treated between 1990 and 2011 were extracted from an institutional database. Baseline patient characteristics, treatment details and oncological outcomes were analysed.

Results – Adjuvant RT with conventional fractionation (median dose 48 Gy in20 fractions) was given to 43 patients (57%) and hypofractionated RT (median dose33 Gy in 6 fractions) to 33 patients (43%). The 5-year node field control rate was 70%, 5-year recurrence-free survival 17%, 5-year melanoma-specific survival 26% and 5-year overall survival 25%.

Conclusions – Salvage surgery with adjuvant RT achieved node field control in 70% of melanoma patients with node field recurrence following a prior node dissection. However, disease progression at distant sites was common and survival outcomes were poor. Prospective data will be required to assess outcomes for contemporary combinations of surgery, adjuvant RT and systemic therapy.

Introduction

The optimal combination and sequencing of surgery, radiotherapy (RT) and systemic therapy in melanoma patients with lymph node metastases are the subjects of ongoing investigation. RT is a long-established adjuvant treatment option after lymph node dissection for patients with high-risk stage III melanoma.[1–6] Published regional control rates after therapeutic lymph node dissection range from 40% to 85%, whereas control rates of 81%–95% have been reported with the addition of RT.[7] Despite this improvement in node field control, adjuvant RT does not provide a melanoma-specific survival (MSS) benefit or an overall survival (OS) benefit because of the high incidence of distant metastases in this cohort of patients.[6] Effective adjuvant systemic therapy is now available and is increasingly given in lieu of adjuvant RT.[8,9] Based on a subgroup analysis of their landmark randomized trial, undertaken in an era when potentially effective adjuvant systemic therapy was not available, Henderson et al. suggested that adjuvant RT after salvage surgery for recurrence after lymph node dissection might also be of benefit in these high-risk patients, but this has not been adequately studied.[10] Nor has the combined effect of both adjuvant RT and systemic therapy in this clinical situation been assessed.

The present study examined the outcome of further (salvage) surgery followed by adjuvant RT in patients who developed isolated node field recurrence after a previous lymph node dissection for stage III melanoma, but who did not receive adjuvant systemic therapy with a checkpoint inhibitor or with agents targeting the MAP kinase pathway. The primary study aim was to assess the frequency of further node field recurrence, and secondary aims were to determine relapse-free survival (RFS), MSS and OS. Our hypothesis was that adjuvant RT in this setting would result in node field control comparable to that achieved by immediate adjuvant RT after an initial therapeutic node dissection. A further objective of the study was to provide baseline data that would allow the efficacy of adjuvant systemic therapy in this setting to be assessed.

Patients and methods

Patients

Patients were eligible for the study if they had developed node field recurrence after a previous lymph node dissection for microscopic or macroscopic (clinically apparent) metastatic melanoma, had no evidence of disease at any other site and had undergone further surgery followed by adjuvant RT. Patients treated between 1990 and 2011 were identified from the Melanoma Institute Australia (MIA) database, which contains comprehensive prospectively collected data. The study cohort included longer follow-up of 29 patients treated between 1990 and 1998, some of whom have been reportedpreviously.[1] Patients with an initial negative

elective lymph node dissection, those with distant metastasis (beyond the regional node field) at the time of their further surgery and those without complete follow-up data were excluded. All patients had given informed consent for their data to be collected and used for research purposes. The study protocol was approved by the MIA Research Committee, and ethics approval was obtained from the Sydney Local Health District Ethics Office (protocols X15-031 and 2019/ETH06854).

RT

The RT fractionation schedule for each patient was at the discretion of the treating radiation oncologist. Conventionally fractionated RT was defined as 1.8–2.5 Gy per fraction, usually a total dose of 48 Gy in 20 fractions, 5 fractions per week over 4 weeks. Hypofractionated RT was defined as 5–6 Gy per fraction, usually a total dose of 33 Gy in six fractions, two fractions per week over 3 weeks. Due to the wide geographic distribution of patients who had surgery at MIA, some chose to receive RT at a local facility closer to their place of residence.

Follow-up

Recurrence at any site was defined as the detection of any clinical, histological or radiological evidence of melanoma. Node field recurrence was defined as lymph node recurrence or soft tissue recurrence within the anatomical lymph node field.

Statistical analysis

Patient characteristics were summarized using standard non-parametric descriptive statistics, given the moderate cohort sample size. Continuous variables were described by their median (range) and categorical variables by their frequency (proportion). The study endpoints were node field recurrence (as a first recurrence), RFS, MSS and OS. Survival times were calculated from the first date of RT to the date of node field recurrence, local, regional or distant recurrence, death due to melanoma or death from any cause, respectively. Patients without recurrence were censored at either their date of death or the last date that they were known to be alive. Survival outcomes were described graphically using the Kaplan–Meier method. Statistical analyses were performed using SPSS version 26.0 (IBM Corporation) and R version 3.6.1 (R Core Team). A two-sided p-value of <0.05 was considered statistically significant.

Results

A total of 76 patients with a median age of 60 years received RT after resection of one or more melanoma recurrences in a previously dissected lymph node field (see table 6.1). The indications for the initial lymph node dissection were macroscopic nodal metastatic disease in 46 patients (61%), completion lymph node dissection after a positive sentinel lymph node biopsy in 26 patients (34%) and elective lymph node dissection, with involved nodes identified, in the remaining 4 patients (5%).

The extent of the initial node dissection and disease burden is documented in table 6.1. The initial operation was a full level I–III axillary dissection in 32 of the 37 patients (87%) with axillary nodal disease. An inguinal dissection was performed in 9 of the 20 patients (45%) with groin node disease, and iliac–obturator–inguinal dissection in the other 11 (55%). The extent of the operation varied in the 19 patients with disease in cervical nodes. Seven patients (37%) had a level II–V dissection, five patients (21%) had a level I–V dissection, three patients (16%) had a level I–V plus parotid dissection and the remaining patients had various extents of neck dissection.

Table 6.1 Baseline patient characteristics and treatment details

Patient characteristics (*n* = 76)	
Median age at the time of RT	
Years (range)	60 (31-89)
Gender	
Male	47 (62%)
Female	29 (38%)
Primary site	
Head and neck	16 (21%)
Trunk	33 (43%)
Upper limb	9 (12%)
Lower limb	15 (20%)
Unknown primary	3 (4%)
Median Breslow thickness of the primary	
mm (range)	2.2 (0.5-13.0)
Ulceration	
Yes	20 (26%)
No	45 (59%)
Unknown	11 (15%)
Patients with multiple primaries	9 (12%)

Table 6.1 Baseline patient characteristics and treatment details *(continued)*

Patient characteristics (*n* = 76)	
Indication for the initial node dissection	
Therapeutic lymph node dissection	46 (61%)
Completion of lymph node dissection	26 (34%)
Elective lymph node dissection	4 (5%)
Node field	
Axilla	37 (49%)
Groin	20 (26%)
Neck	19 (25%)
Initial node dissection	
Median number of excised / positive nodes (range)	20 (5-86) / 2 (1-31)
Axilla	19 (5-51) / 1 (1-13)
Neck	35 (11-86) / 1 (1-31)
Groin	15 (5-27) / 2 (1-11)
Involved surgical margin	1 (1%)
Extracapsular extension	24 (32%)
Surgery for the node field recurrence	
Median number of excised / positive nodes (range)[a]	0 (0-42) / 0 (0-12)
Axilla	0 (0-42) / 0 (0-10)
Neck	1 (0-16) / 0 (0-6)
Groin (inguinal and pelvic)	0 (0-22) / 0 (0-12)
Involved surgical margin	19 (25%)
Extracapsular extension	18 (24%)

RT radiotherapy.
[a]Some patients had surgical excision of soft tissue recurrence, therefore no nodes were excised.

Salvage surgery

The median time to diagnosis of node field recurrence after initial node dissection was 8 months (range 23 days to 17 years). Twenty-nine patients (38%) had a nodal recurrence and 47 patients (62%) had an apparently nonnodal soft tissue recurrence within the anatomical node field. The salvage surgery in all these patients was performed with the objective of obtaining complete macroscopic clearance of the recurrent disease in the node field. It involved local excision of the scar and/or soft tissue recurrence (*n*= 40), local excision of an involved node (*n*= 10) or a full redo lymph node dissection (*n*= 26), as considered appropriate for the individual patient to achieve macroscopic clearance. However, involved surgical margins were reported in 25% of patients, and there was extranodal spread in 14 of the 29 patients with nodal recurrence (48%). The median time between salvage surgery and the commencement of RT was 40 days

(range 9–113 days). Thirteen patients (17%) underwent more than one surgical procedure to remove node field recurrences before receiving adjuvant RT to the node field. The median time between surgical procedures when there was more than one procedure was 3 months (range 1–13 months).

RT dosage and fractionation

Conventional fractionated RT (median dose 48 Gy over 4 weeks) was given to 43 patients (57%) and hypofractionated RT (median dose 33 Gy over 3 weeks) to 33 patients (43%). In total, 66 patients (87%) received their RT at MIA, and the remaining 10 patients (13%) at other facilities. Equal numbers of the patients treated at MIA received hypofractionated RT and conventionally fractionated RT. Patients treated at other facilities all received conventional RT fractionation. No patient failed to complete their planned course of RT due to toxicity.

Other adjuvant treatments

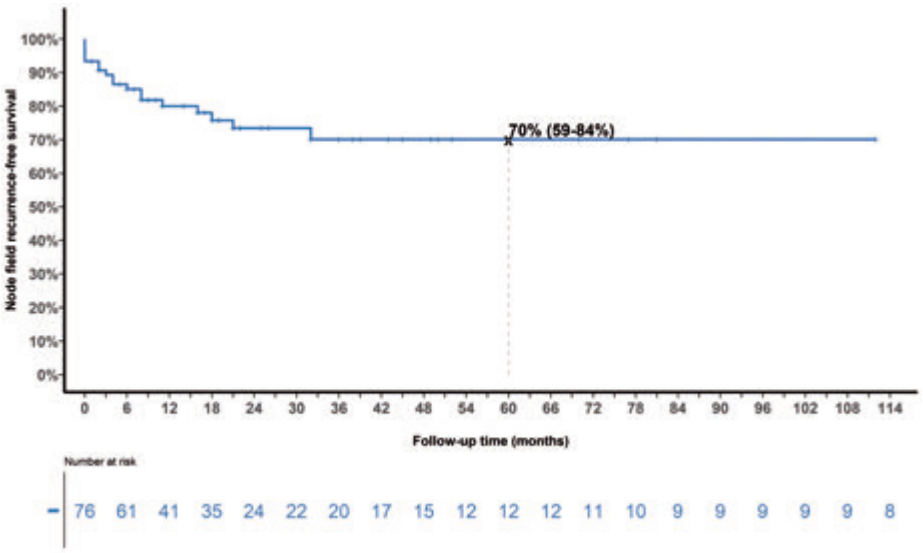
No patients in this series received adjuvant systemic therapy with an immune checkpoint inhibitor or a BRAF/MEK inhibitor. However, other forms of adjuvant systemic treatment were provided to 34 patients (45%). Adjuvant interferon-α was given to 4 patients, while 32 patients received either adjuvant vaccine therapy or participated in the treatment arm of an adjuvant vaccine therapy trial.[11–16]

Treatment outcome

The primary endpoint was node field recurrence (as a first recurrence). During RT, 13 of the 76 patients (17%) developed a melanoma recurrence (three in-transit metastasis, nine further node field recurrence and one distant metastasis). RT was terminated early in two patients (one with node field recurrence and one with distant metastasis). After RT, another nine patients developed a node field recurrence, three of whom also had concurrent local recurrence or distant metastasis). This resulted in a total of 18 patients (24%) with node field recurrence as a first recurrence at the time of the last follow-up (figure 6.1A). Node field recurrence as a first recurrence occurred in 15.6% of the patients treated with hypofractionated RT and in 29.5% of those treated with conventional RT (P=0.16). The node field recurrences were in the irradiated field in 12 patients (67%) and just outside the irradiated field but within the anatomical node field in 6 patients (33%). Overall, the 5-year node field control rate was 70% (95% confidence interval [CI]: 59%–84%; figure 6.1A). At the time of the last follow-up, 13 patients (17%) remained disease-free, and 60 of the 76 patients (79%) had developed distant metastatic disease. The 5-year RFS was 17% (95% CI: 10%–29%; figure 6.1B). The 5-year MSS was 26% (95% CI: 18%–39%, Figure 6.1C). At the last follow-up, 58 patients had died (37 of the 40 patients who had local excision of the recurrence, 7 of the 10 patients who had excision of involved nodes and 14 of the 26 patients who had a full redo node dissection). Most of the death occurred during the first 24 months. The 5-year OS was 25% (95% CI: 17%–38%; figure 6.1D). The median follow-up

duration for the entire group was 19 months (range 1–246 months) in the total group. The median follow-up duration for the group that remained disease-free was 50 months (range 10–246 months).

A Node field control



B Recurrence-free survival

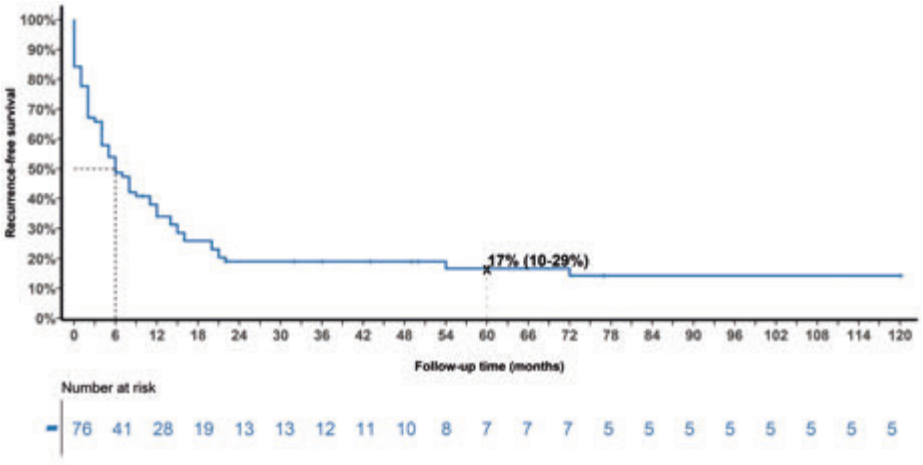
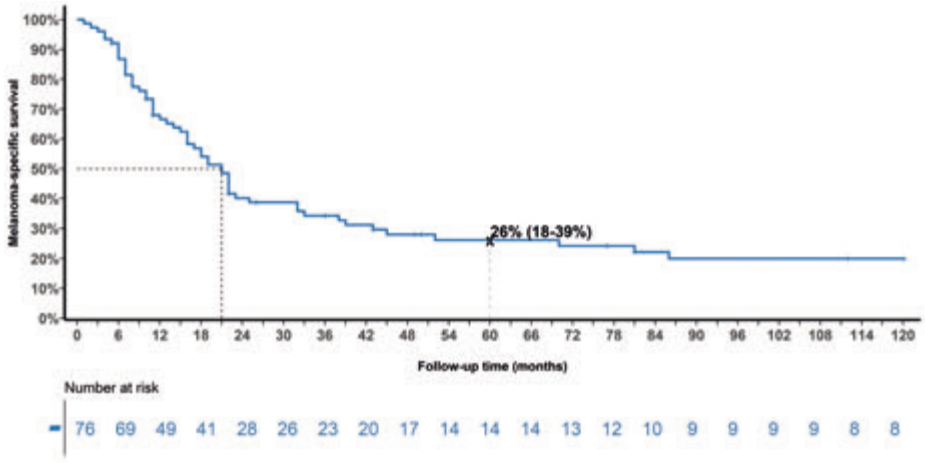


Figure 6.1 Kaplan–Meier curves for adjuvant radiotherapy after surgical treatment of node field recurrence following a prior node dissection for stage III melanoma ($n = 76$)

C Melanoma-specific survival



D Overall survival

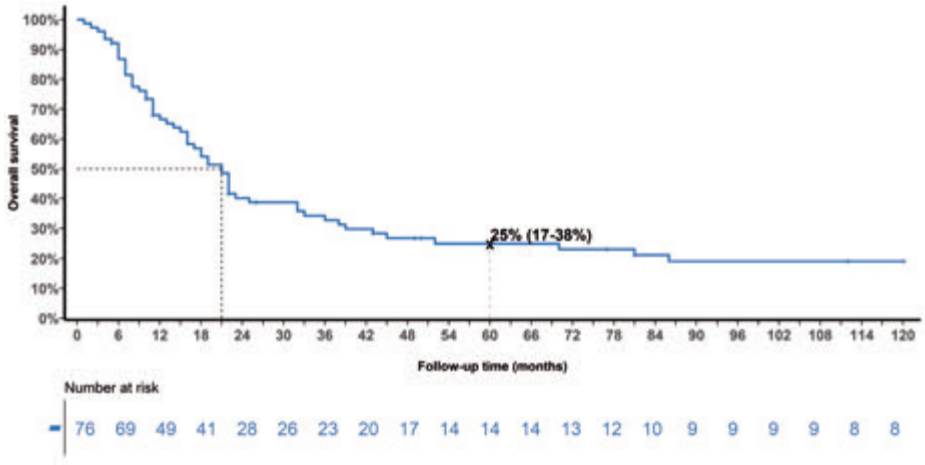


Figure 6.1 Kaplan–Meier curves for adjuvant radiotherapy after surgical treatment of node field recurrence following a prior node dissection for stage III melanoma ($n = 76$) *(continued)*

Discussion

The efficacy of adjuvant nodal RT for recurrent melanoma after a previous therapeutic regional lymph node dissection has not been extensively described. Despite the recurrence and the presence of high-risk features such as a positive margin in 25% of patients and extranodal spread in almost half of the patients with nodal recurrence, the 5-year node field control rate in the present study was 70% after further surgery and adjuvant RT. However, the 5-year OS was poor (25%).

The only prospectively collected data examining this question have been from a small subgroup of patients in the Australia and New Zealand Melanoma Trials Group (ANZMTG 01.02)/Trans-Tasman Radiation Oncology Group (TROG 02.01) randomized trial comparing immediate RT with observation after nodal dissection.[10] In the observation group of this trial, 26 patients developed an isolated node field recurrence, with a median time to recurrence of 7 months (interquartile range: 4–12). Twenty of them (77%) were treated with further surgery and RT. The other six patients received either surgery alone, RT alone or no treatment. Overall long-term node field control was achieved in 23 of them (88%).[10] The 5-year OS of this cohort was 34%, but the CIs were wide (95% CI: 18%–63%) due to the small patient numbers. Several retrospective studies of adjuvant RT have included patients with recurrence in a previously dissected lymph node field and reported node field control rates in this group of patients ranging from 85% to 96%.[1,4,17] However, the numbers of patients in these analyses were again small, which limits the reliability of the reported outcomes. In an earlier MIA study by Stevens et al., 35 patients received hypofractionated RT after resection of recurrent disease in a previously dissected lymph node field, while 107 patients received hypofractionated RT after initial surgery for metastatic lymph node disease.[1] The node field control rate for the two groups combined was 89% (median follow-up 30 months) and the MSS (38%) did not significantly differ between the two groups. Conill et al. reported a cohort of 77 patients treated mainly with hypofractionated RT.[17] For the 27 patients with recurrent nodal disease, the node field control rate was 96.3%; however, median follow-up was not stated. Beadle et al. presented results for 200 melanoma patients receiving hypofractionated RT after axillary lymph node dissection (median follow-up 59 months).[4] For the 37 patients with recurrent nodal disease, the node field control rate was 85%. The choice between conventionally fractionated RT and hypofractionated RT in the present study was at the discretion of the treating radiation oncologists based on institutional preference and patient choice. We have recently published an analysis of the effects of adjuvant RT fractionation on outcomes in 335 patients after resection of high-risk stage 3 melanoma.[18] There were no significant differences in node field control, RFS or OS between hypofractionated and convention-ally fractionated adjuvant RT.

The best way to assess the efficacy of adjuvant RT in patients with recurrent nodal disease would be to compare their results with those of patients who underwent salvage surgery but who did not have RT. Based on the currently available literature no valid comparison can be made since node field control rates inpatients who recurred and had further surgery but did not receive adjuvant RT have not been reported. We sought to address this question by analysing data from our own institution but could identify only eight patients who did not receive adjuvant RT after a second surgical procedure for node field recurrence, too few for meaningful analysis. An important finding in our study was that 33% of the subsequent recurrences after RT occurred in areas of anatomical lymph node fields as defined in the ANZMTG 01.02/TROG 02.01trial protocol that had not been actually irradiated. This suggests that even better control rates might have been achieved if RT planning had ensured that the entire anatomical field was treated in every patient. In the ANZMTG 01.02/TROG 02.01 trial, the anatomical boundaries of the cervical, axillary and inguinal lymph node fields were very precisely specified and coverage of the entire anatomical field was mandated in the trial protocol.[10] The importance of quality control in RT in achieving optimal outcomes is well documented. In a large head and neck cancer trial, an independent central review of the quality of the RT showed that a noncompliant RT plan was associated with significantly worse 2-year OS, with more patients developing locoregional failure.[19] Similarly, in a randomized trial of preoperative RT for retroperitoneal sarcoma, a noncompliant RT plan was associated with worse abdominal RFS and a trend towards worse OS.[20]

The poor overall RFS of 17% in our study points to the need for effective systemic treatment. Today, checkpoint inhibitors and targeted therapies are available as adjuvant treatments, and these agents have been shown to improve the RFS of patients with resected nodal melanoma recurrences.[8,9,21] Patients with a first presentation of stage III disease are increasingly being treated with neoadjuvant systemic therapy, which has been reported to produce a complete pathological response in 40% ofthem.[22] Those with a complete pathological response to neoadjuvant systemic therapy had an excellent 2-year RFS of 89% and an OS of 95%. Given the high risk of disease relapse inpatients with node field recurrence after a previous node dissection, there is thus a likely benefit of neoadjuvant systemic therapy as well.[22–24] Therefore, most melanoma clinicians would currently recommend neoadjuvant and/or adjuvant systemic therapy and consider RT for patients with high-risk disease, those who develop isolated node field recurrence despite systemic therapy or those with a poor pathological response to neoadjuvant therapy. This is consistent with the recommendation for RT in the most recent National Comprehensive Cancer Network guidelines.[25] In the setting of isolated node field recurrence after adjuvant immunotherapy, preliminary data reported in an abstract indicate that adjuvant RT after further surgery substantially reduced the risk of further nodal recurrence (from 36% to 8%).[26] Our results from the era before modern systemic therapy became available can serve as a baseline to assess the efficacy of systemic therapy in this setting.

There is increasing preclinical as well as clinical evidence that combining RT with checkpoint inhibitors may increase the immune response and further improve long-term control of metastaticdisease.[27] It is, therefore, likely that node field control will be improved further by the combined use of both RT and checkpoint inhibition as adjuvant therapies. This could be a particularly useful strategy for those patients who have had a poor pathological response to neoadjuvant systemic therapy.

In addition to its retrospective nature, the present study has several limitations. The follow-up of some patients was incomplete, mainly for those who had their RT at other facilities. A possible confounder was the administration of other adjuvant treatments; however, the efficacy of the systemic agents that were used (interferon-α, vaccinia melanoma cell lysate, CancerVax and dendritic cell vaccines) is likely to have been negligible, based on reportedresults.[11–16] Comparing our node field control rate after salvage surgery and adjuvant RT with a cohort treated with salvage surgery without RT was not feasible using our institutional database because the number of patients who had salvage surgery without RT was too small to draw any reliable conclusions. Detailed toxicity data were also not available.

Conclusions

Salvage surgery with adjuvant RT achieved node field control in 70% of patients with node field recurrence following prior node dissection for stage III melanoma. When further recurrence did occur, it was in a nonirradiated area of the anatomically defined node field in 33% of cases, explaining the importance of RT quality control. The overall prognosis was poor due to the subsequent development of distant metastatic disease.

Prospective studies examining the role of adjuvant RT in the setting of adjuvant systemic therapy with checkpoint inhibitors or targeted therapy and sequencing options with surgery will be required to determine the value of each modality in these patients. The results from this study serve as a baseline to assess the efficacy of modern systemic therapies. Until then, adjuvant RT remains a valid option after salvage surgery for an isolated node field recurrence after a previous lymph node dissection for stage III melanoma.

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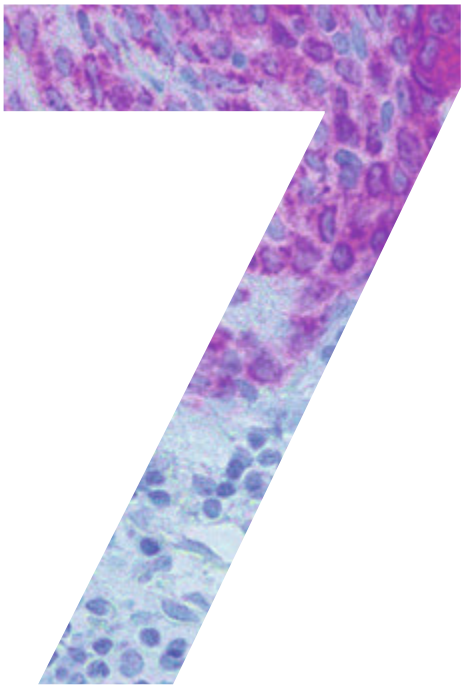
CHAPTER



General discussion and future perspectives

This thesis considers aspects of staging and treatment of selected subgroups of patients with stage III melanoma. Its chapters discuss the pathological assessment of non-sentinel lymph nodes (NSLN), the usefulness of staging imaging, the fractionation and timing of adjuvant radiotherapy, and recent and future developments in these areas. A substantial amount of time has elapsed between the publication of articles that are recorded in the second and sixth chapters of this thesis. During this time novel systemic therapy options have become available for several subgroups of patients with stage III melanoma. Additionally, publication of the results of two large prospective trials assessing the value of completion lymph node dissection (CLND) in patients with a positive sentinel lymph node (SLN) resulted in virtual abandonment of this procedure.

The origin of the research is another important aspect of this thesis. All research was performed using Australian research data. Climate differences may play a role in etiology and may impact the biology of the disease. Cultural differences may play a role as well. An Australian melanoma patient faces different challenges compared to a Dutch melanoma patient. Healthcare access may not be equal for melanoma patients in Australia due to long travel distances. Limited access to private health insurance might result in longer waiting times for non-urgent procedures. Fortunately, urgent patients will be prioritised and thus being dependent on public health insurance will not cause significant cancer treatment delay in daily practice. In the Netherlands, mandatory basic healthcare insurance with income-dependent rebate replaced the system of public versus private healthcare insurance in 2006 to improve equality of healthcare access. However, increasing out-of-pocket costs and limited contracting of health care providers by insurance companies might limit healthcare access in the Netherlands as well. That said, a considerable effort has been made to provide access to regional melanoma treatment centres for all patients and travel distances are short compared to Australia. These differences in healthcare access may impact certain management decisions.

Pathological assessment of non-sentinel lymph nodes

Chapter 2 describes the findings of detailed pathological examination of all residual lymph nodes (NSLNs) in completion lymph node dissection specimens of 20 patients with a minimal (<0.1mm) SLN metastasis and their outcome. Only one patient was found to have an additional NSLN metastasis (5%). Unfortunately, 30% of the patients developed recurrences. The estimated 5-year melanoma-specific survival (MSS) was 64%. This chapter was published before the results of the German Dermatologic Cooperative Oncology Group Selective Lymphadenectomy Trial (DeCOG-SLT) and the second Multicenter Selective Lymphadenectomy Trial (MSLT-II) became available.[1,2] Neither study found a significant improvement in MSS from CLND compared to node field observation with regular ultrasound examination. This finding radically changed management, and most SLN-positive patients are now spared CLND.[3] Instead, the node field

is monitored with physical examination and regular high-resolution ultrasound to identify any nodal recurrence at an early stage.

With a steadily increasing proportion of SLN-positive patients receiving adjuvant systemic therapy, cross-sectional whole-body imaging appears to be replacing ultrasound during follow up.[4,5] It would be interesting to compare the diagnostic value of ultrasound surveillance of the node field to cross-sectional imaging in a prospective trial to determine which is better. However, as both ultrasound and whole-body imaging require sophisticated equipment and specific expertise, the real-world feasibility of adequate surveillance is a challenge, especially in countries without such expertise and/or easy access to healthcare. In the United States for instance, factors like the number of melanoma patients seen in a centre, insurance coverage and travel distance appear to impact treatment decisions in SLN-positive patients.[6] Hence, for patients without access to state-of-the-art management, CLND may remain a reasonable option.

Based on the findings of the two MSLT studies, one may conclude that SLNB is not only a staging procedure but also a therapeutic procedure, improving the survival rate in selected patients and improving regional control, as 75% of the SLN-positive patients had no other involved lymph nodes. Interestingly, in MSLT-II, the pathologic status of NSLNs was a significant prognostic factor (hazard ratio for death, 1.78; P=0.005) in the dissection group whereas the number of involved SLNs was not prognostic.[2] Patients with NSLN metastases appear to have a worse prognosis compared to patients with only SLN metastases (5-year MSS 50% vs. 78%).[7] Although long-term outcomes are unknown, adjuvant systemic therapy is currently recommended for AJCC stage IIIB, C, and D or stage IIIA with SLN tumor deposits >1 mm in diameter.[8] Withholding adjuvant therapy if the SLN metastasis is less than 1 mm in diameter may occasionally result in undertreatment of the patient, but in this group of patients the benefits might not outweigh the risk of serious toxicity from adjuvant treatment.

Another subject of current interest is the sequencing of surgical and immunological treatment in patients with stage III melanoma. Preoperative "neoadjuvant" systemic therapy appears to evoke a stronger immunological response than postoperative adjuvant therapy.[9] In theory, a good tumor response to neoadjuvant systemic therapy could result in less extensive surgery and improved long-term outcomes. The Optimal Neo-adjuvant Combination Scheme of Ipilimumab and Nivolumab trial (OpACIN-neo) identified a tolerable dosage that could be used in other, larger trials.[10] In the subsequent PRADO cohort study, therapeutic LND was omitted in patients with a major pathological response in the index lymph node, resulting in a 2-year recurrence-free survival (RFS) of 93% and a 2-year distant RFS of 98%.[11] Several phase II trials of neoadjuvant systemic therapy are in progress at the time of writing. The results of the OpACIN-neo trial and the PRADO study are the basis for the NADINA trial, an international, randomized

phase III trial comparing the efficacy of neoadjuvant ipilimumab plus nivolumab with adjuvant nivolumab in patients with macroscopic stage III melanoma.[12]

Staging imaging

Staging with ^{18}F-fluoro-deoxyglucose (FDG) whole-body positron emission tomography / computed tomography (PET/CT) plus magnetic resonance imaging (MRI) of the brain has proven to be useful in melanoma patients with palpable lymph node metastases.[13] This generated the question of its value in patients with a positive SLN, who typically have a smaller tumor burden. Not every patient with melanoma benefits from imaging to screen for the presence of more disease. The philosophy "If it doesn't help it won't hurt" does not apply to patients with microscopic lymph node disease. First of all, it would be useless in more than half the patients with a positive SLN because they will never develop a recurrence. Actually, it would be *harmful* because of the typical radiation dose of 17.6 mSv for a whole-body PET/CT. To place this risk in context, the radiation dose from a chest x-ray amounts to just 0.1 mSv.[14]

Chapter 3 demonstrates that staging SLN-positive patients with CT or whole-body PET/CT is of limited use as metastases were found in only two of the 143 patients (true-positive yield 1.4%) and resulted in none of the patients having a change in AJCC stage. Moreover, when staging patients with microscopic lymph node disease, the 43% false-positive rate instigated 37 additional investigations that later proved to be unnecessary in 21 patients (15%). Some of these sequelae could have been harmful. Also, these false-positive results and the ensuing (waiting period for) further investigations and their results may cause unnecessary anxiety and psychological distress.[15-17] We often hear "This may well be true, but we need a baseline and patients demand more information. Patients want their metastases to be detected and if they have no further disease, they want to be reassured." And that is indeed the information that we doctors want to provide. But can we do so with confidence? The 83% false-negative rate that we found means 83% of those who will be found to have a recurrence within the subsequent six months have a normal initial scan and are told that they are fine. They are reassured, but they should not have been reassured. Our false positive rate of 43% means that 43% of the patients who are really fine and will not recur within six months in fact have a positive scan suggestive of a recurrence. They are not reassured, whereas they should have been reassured. The ultimate reason for staging should be to improve the survival rates. We emphasize that the studies reported in this thesis only assessed staging imaging, not surveillance imaging. Nevertheless, even surveillance imaging does not seem to impact survival rates.[18]

Since the publication of Chapter 3 the treatment options for stage III melanoma patients have expanded. Adjuvant systemic therapy is now being offered to patients with AJCC stage IIIB, C, and D or stage IIIA with SLN tumor deposits >1 mm in diameter. In SLN-positive patients receiving adjuvant systemic therapy a baseline PET/CT is routinely performed, not purely

for staging, but to compare with subsequent follow-up imaging during adjuvant systemic treatment. False-positive results in these early scans still pose a challenge. Furthermore, the cost of these scans and the ensuing investigations needs to be considered in this era of ever-increasing healthcare expenditure. The Netherlands has developed appropriate healthcare projects in which unnecessary investigations or treatments are discouraged. Accordingly, the findings in Chapter 3 suggest reconsidering current imaging guidelines, especially for stage IIIA melanoma patients with SLN tumor deposits < 1mm. Given these results, the question of whether stage II patients would also benefit from adjuvant therapy has become the next subject of interest. Randomized phase 3 clinical trials to assess this are in progress (KEYNOTE-716 and COLUMBUS-AD).[19]

Chapter 4 shows the benefit of staging with whole-body FDG PET/CT in patients with satellite or in-transit metastases, revealing a change in the originally contemplated treatment plan in 16%. In all these patients MRI of the brain was normal, and appeared to be non-contributory, although the sample size of 25 patients in our prospective study was limited. Compared to patients with microscopic lymph node disease who have a MSS of 72%, patients with satellite or in-transit disease were found to have a greater risk of developing distant metastases and have a considerable worse 5-year MSS (53% in the case of satellite metastases and 43% in the case of in-transit metastases).[20-22] In patients with satellite or in-transit disease, staging imaging could be used to select the appropriate treatment, although there is a limit to the minimum size of a lesion to render it detectable (4-5 mm for conventional PET).[23] An interesting development has been the total-body PET/CT.[24,25] Instead of scanning a 20cm slice of the body at a time, this new PET/CT technique is able to scan the whole body (up to 2m) all at once. The long axial field of view allows for higher quality images and reduced scan time. Also, the increased sensitivity of this technique may be used to reduce the injected radiopharmaceutical dose without jeopardizing the quality of the images.[26] Total body PET/CT also allows dynamic imaging, which provides the opportunity to study the kinetics of novel positron-emitting radiopharmaceuticals and the body's response to such new agents.[27]

Adjuvant nodal radiotherapy

Adjuvant radiotherapy has been shown to reduce the risk of recurrence in a lymph node field following LND for melanoma metastases.[28] As the optimal fractionation schedule is unknown, both hypofractionated and conventionally-fractionated schedules are used.[29] Chapter 5 describes a retrospective study in which two fractionation regimens of adjuvant radiotherapy were compared in a cohort of 335 melanoma patients after regional LND for lymph node metastases. Oncological outcomes were similar in the two groups. In the hypofractionated group the 5-year lymph node field control rate was 86.0% (95% confidence interval (CI) 78.4-94.4%) and in the conventional fractionation group 85.5% (95% CI 80.5-90.7%) (P=0.87). RFS (41.7% vs. 31.9%, P=0.18) and overall survival (OS) (41.2% vs. 45.0%, P=0.77) were not significantly

different. Multivariate analysis showed that extra-nodal spread was associated with decreased RFS (P=0.04). The number of resected metastatic lymph nodes was inversely associated with RFS (P=0.0006) and OS (P=0.01).

So, in this largest study to date, a hypofractionated schedule was as effective as a conventionally-fractionated schedule. One could dispute the relevance of this finding, as the data are from the era prior to the availability of the effective systemic therapy that many of these patients would nowadays receive. However, the use of radiotherapy appears to be re-emerging. Recent studies suggest a benefit from sequencing radiotherapy and immunotherapy, notably with hypofractionated schedules, and this matter warrants further investigation.[30-32] Radiotherapy could possibly intensify the antitumor immune response in a number of ways. Firstly, radiotherapy could enhance the release of tumor-specific antigens, which elicit a T cell-mediated immune response. Secondly, radiotherapy may increase the expression of MHC-I receptors, facilitating antigen presentation. Thirdly, other cell surface receptors that promote lymphocyte-mediated cytotoxicity may be activated. And lastly, radiation can alter the tumor microenvironment through effects on endothelium and vascular permeability, attracting leucocytes and T cells.[31] Hence, radiotherapy could not only act as a local treatment but also elicit and stimulate systemic effects, the "abscopal" effect. The results of recent preclinical and clinical studies support this hypothesis.[33] The precise mechanisms through which radiation induces a T cell-mediated immune response are complex but seem to be dose-dependent with an optimal dose around 8 Gy per fraction and a more durable effect with multiple fractions.[34] With larger doses per fraction the immune response vanishes, which suggest a delicate balance between immunogenic and immunosuppressive effects of radiotherapy.[35]

An additional benefit of the routine use of hypofractionation would be a reduction of the number of treatment visits, which would be more patient friendly and might reduce healthcare costs. During the COVID-19 pandemic hypofractionation was successfully implemented in some treatment facilities to reduce virus-contact time.[36]

Another issue that we aimed to clarify is the role of adjuvant radiotherapy after salvage surgery for melanoma recurrence in a node field following a previous LND. In this scenario one might expect an aggressive tumor biology and thus a different effect of radiotherapy. Data documenting the value of adjuvant radiotherapy in this scenario are sparse. Chapter 6 describes the outcome for 76 patients who received adjuvant radiotherapy after excision of node field recurrences following a previous LND. Conventional fractionation (median dose 48 Gy in 20 fractions) was given to 43 patients (57%) and hypofractionated radiotherapy (median dose 33 Gy in 6 fractions) to 33 patients (43%). The 5-year node field control rate for the whole group was 70%, 5-year RFS 17%, 5-year MSS 26% and 5-year OS 25%. Unfortunately, our database did not contain a sufficient number of patients treated for a node field recurrence

without radiotherapy for a meaningful comparison. It would be valuable to combine data from different institutional databases to determine outcome in this group. It would also be interesting to assess the role of adjuvant radiotherapy in this clinical scenario in the current era of effective adjuvant systemic therapy. Preliminary data (reported in an abstract) for 71 patients with isolated node field recurrence after adjuvant immunotherapy demonstrated a reduction in the risk of further locoregional recurrence from 36% to 8% (P=0.012) after adjuvant radiotherapy following salvage surgery.[37] The results presented in Chapter 6 are from an era when there was no effective systemic therapy and therefore could serve as a baseline to assess the efficacy of modern systemic therapy in the same setting. Furthermore, the results highlight the importance of radiotherapy quality control, as further recurrence occurred in 33% of cases in a non-irradiated area of the anatomically-defined node field.

Future perspectives

Basic scientists are unravelling various aspects of melanoma at the molecular level. This could lead to a major shift in the treatment strategy for patients with stage III melanoma. Gene expression profiling could be a promising addition to SLNB for prediction of recurrence or distant metastasis in stage I-III melanoma patients.[38] Now that we have evidence that adjuvant treatment benefits a substantial proportion of patients with advanced stage III melanoma, the next step is to assess its value in earlier stages of the disease. This is currently being examined. Interim results from the KEYNOTE-716 trial suggest that high-risk stage IIB/C patients benefit from adjuvant pembrolizumab following resection of the primary tumor.[39] Artificial intelligence is being studied in initial melanoma diagnosis, but could also play a part in aiding the performance of staging imaging.[40]

Another interesting step will be to further explore the usefulness of systemic therapy before an operation for high-risk stage II, stage III and IV melanoma. This "neoadjuvant" systemic therapy may provide an opportunity to test the tumor's sensitivity upfront and select an appropriate systemic therapy with greater confidence.[41,42,43]

And although several immunomodulatory effects of radiation are being elucidated, optimal sequencing of radiotherapy and immunotherapy as well as selection of the optimal target site(s) for radiation need to be determined in future prospective trials.[44]

A further recent exciting development has been the discovery of a third class of immune checkpoint inhibitors (LAG-3 inhibitors), allowing for novel combination therapies.[45-48] So, on many different fronts, we are making progress towards the goal of "zero deaths from melanoma".

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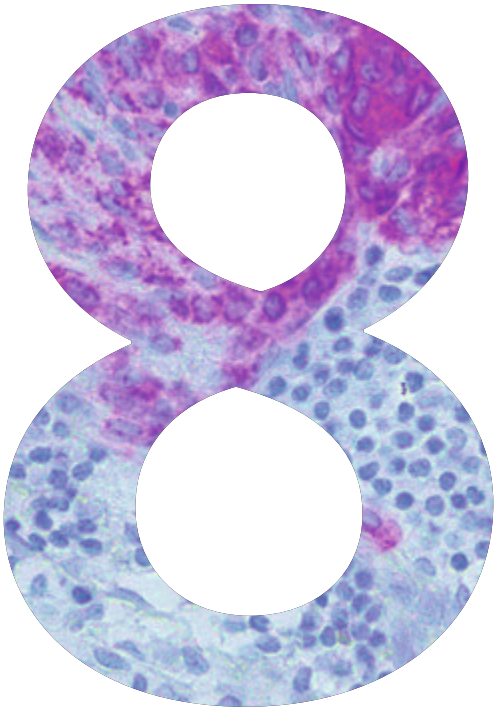
CHAPTER



Summary

Samenvatting

Summary

Patients with stage III melanoma have a 10-year melanoma-specific survival (MSS) that ranges from 24% to 88%, depending on whether they have satellite, in-transit and/or lymph node metastasis. This thesis discusses the pathological assessment of non-sentinel lymph nodes (NSLN) in patients with minimal SLN involvement, the usefulness of staging imaging and the fractionation and timing of adjuvant radiotherapy in patients with selected subgroups of stage III melanoma. All research data were collected from and in collaboration with Melanoma Institute Australia.

Patients with a minimal (<0.1mm) metastasis in the SLN are rarely found to have metastases in NSLNs and therefore it has been suggested that completion lymph node dissection (CLND) could be omitted in these patients. Due to the large number of nodes in a CLND specimen, routine pathological assessment of NSLNs is usually limited to bisectional staining of each node with hematoxylin and eosin (H&E). This limited sampling without the sensitive immunohistochemical staining could underestimate the number of positive nodes. **Chapter 2** describes the more thorough examination of NSLNs retrieved from 21 tumor-negative CLND specimens from 20 patients with a minimal SLN metastasis using the standard SLN protocol. Five additional sections from each node were cut and stained sequentially with H&E, S-100, HMB45, Melan-A, and H&E. A metastasis was found in only one of the 343 examined NSLNs, located in the subcapsular sinus. Clinical follow-up data were also collected. Thirty percent of the patients developed a recurrence. The estimated 5-year MSS was 64%. Although the risk of additional nodal involvement is low, the prognosis of patients with a minimal SLN metastasis is worse than is often assumed. Chapter 2 was published prior to publication of the results of two prospective trials comparing CLND and nodal observation. As neither study showed a significant survival difference, most SLN–positive patients are now spared CLND and their lymph node fields are monitored with physical examination and high-resolution ultrasound for the early detection of nodal recurrence.

Chapters 3 and 4 discuss the value of staging imaging. In patients with a microscopic lymph node metastasis the use of positron emission tomography (PET) or computed tomography (CT) has been reported to be of limited value because of a low yield and a high rate of false-positive results. Technical improvements and the combination of PET with CT might have changed this. **Chapter 3** assesses the diagnostic test performance of either CT or PET/CT and the number and nature of additional tests that were requested for 143 patients with a positive SLN. Sensitivity of PET/CT was 11%, specificity 73% and the positive predictive value 4%. For CT the sensitivity was 17%, the specificity 57% and the positive predictive value 6%. Further metastases were found in only two of the 143 patients (true-positive yield 1.4%), but this did not change their AJCC stage as the metastases were regional in both patients. Furthermore, 21 patients (15%) with

a false-positive result were subjected to 37 additional investigations, referrals or procedures that proved unnecessary in hindsight. Since the publication of chapter 3 the treatment options for stage III melanoma patients have broadened as adjuvant systemic therapy is now being offered to patients with a positive SLN with SLN tumor deposits >1 mm and a baseline PET/CT is often obtained in these patients. Until imaging techniques are able to more reliably identify smaller metastases, we urge caution with routine cross-sectional staging imaging in melanoma patients with a positive SLN.

Chapter 4 describes the role of whole-body PET/CT and magnetic resonance imaging (MRI) of the brain in patients with satellite and/or in-transit metastasis (ITM). In this prospective study containing 25 patients, PET/CT led to a change in treatment plan in four patients (16%) and all four were upstaged. The sensitivity of PET/CT was 58% and specificity 83%. Brain MRI did not change the treatment plan or stage in any of the patients. Our study indicates that PET/CT staging of patients with satellite and/or in-transit metastasis has a meaningful impact on patient management.

Adjuvant radiotherapy has been shown to reduce the risk of melanoma recurrence in the lymph node field after regional lymph node dissection. However, the timing and the optimal fractionation schedule have been subjects of debate. Patients included in the following cohort studies were treated in an era before effective systemic immunotherapy became available. **Chapter 5** assesses the oncological outcomes in 335 patients treated either with hypofractionation (95 patients, median dose 33 Gy in six fractions over 3 weeks) or conventional fractionation (240 patients, median dose 48 Gy in 20 fractions over 4 weeks). The outcomes were similar. Five-year lymph node field control rates were 86.0% for the hypofractionation group and 85.5% for the conventional fractionation group (P=0.87), recurrence-free survival (RFS) was 41.7% versus 31.9% (P=0.18) and overall survival (OS) was 41.2% versus 45.0% (P=0.77). Extranodal spread and the number of resected lymph nodes containing metastatic melanoma were predictive of an unfavorable outcome.

Same site recurrence might indicate more aggressive tumor biology. Adjuvant radiotherapy can be given to melanoma patients following salvage surgery for node field recurrence after a previous regional node dissection, but the value of this treatment strategy is poorly documented. **Chapter 6** describes oncological outcomes in 76 patients treated with adjuvant nodal radiotherapy following one or more regional lymph node field recurrences after LND. Conventional fractionated radiotherapy (median dose 48 Gy in 20 fractions) was given to 43 patients (57%) and hypofractionated radiotherapy (median dose 33 Gy in 6 fractions) to 33 patients (43%). The 5-year node field control rate was 70%, 5-year RFS 17%, 5-year MSS 26% and 5-year OS 25%. Salvage surgery plus adjuvant radiotherapy achieved reasonable node field control, but unfortunately the prognosis was poor due to progression of disease to distant sites.

The results of the study can serve as a baseline for prospective studies assessing the sequencing of surgery, radiotherapy and modern systemic therapies. Another finding was that in 33% of cases further recurrences occurred in a non-irradiated area of the anatomically-defined node field, emphasizing the importance of radiotherapy quality control.

Samenvatting

Patiënten met stadium III melanoom hebben een heterogene 10-jaars melanoom-specifieke overleving (MSS) van 24-88%, afhankelijk van de aan- of afwezigheid van satelliet-, in-transit- en/of lymfekliermetastasen. Dit proefschrift bespreekt de pathologische beoordeling van niet-schildwachtklieren (NSLN) in patiënten met een minimale metastase in de schildwachtklier, het nut van stadiëring met behulp van beeldvorming en de fractionering en timing van adjuvante radiotherapie bij patiënten binnen geselecteerde subgroepen van melanoom stadium III. Alle onderzoeksdata werd verzameld in Australië bij het Melanoma Institute Australia.

Patiënten met een minimale metastase in de schildwachtklier (SLN) - een klier die rechtstreekse drainage ontvangt vanuit de primaire tumor - lijken zelden metastasen in de NSLN's te hebben en daarom is er gesuggereerd dat complete lymfeklierdissectie (CLND) mogelijk niet nodig is bij deze patiënten. Vanwege het grote aantal lymfeklieren in een CLND-weefselpreparaat, wordt de routinematige pathologische beoordeling van NSLN's gewoonlijk beperkt tot één bi-sectionele kleuring met hematoxyline en eosine (H&E). Deze beperkte beoordeling zonder gebruik van gevoelige immunohistochemische kleuringen zou het aantal positieve lymfeklieren kunnen onderschatten. **Hoofdstuk 2** beschrijft een grondiger onderzoek van de NSLN's van 21 tumor-negatieve CLND-weefselpreparaten van 20 patiënten met een minimale (<0,1 mm) SLN-metastase met behulp van het SLN-protocol. Vijf bijkomende coupes werden gesneden van iedere klier en gekleurd met opeenvolgend H&E, S-100, HMB45, Melan-A en H&E. In slechts één van de 343 onderzochte NSLN's werd een metastase gevonden, gelokaliseerd in de subcapsulaire sinus. Daarnaast werden klinische follow-up gegevens verzameld. Bij dertig procent van de patiënten keerde het melanoom terug. De geschatte 5-jaars MSS was 64%. Hoewel het risico op bijkomende kliermetastasering laag is, is de prognose van patiënten met een minimale SLN-metastase niet zo goed als vaak wordt aangenomen.

Hoofdstuk 2 werd gepubliceerd in afwachting van de resultaten van twee prospectieve onderzoeken waarin CLND en klierobservatie met elkaar werd vergeleken. Aangezien geen van de studies een significant verschil in overleving aantoonde, wordt bij de meeste SLN-positieve patiënten geen klierdissectie meer gedaan en worden hun lymfeklieren nu met regelmatig lichamelijk onderzoek en echografie in de gaten gehouden om terugkeer van het melanoom zo snel mogelijk te ontdekken.

In **hoofdstuk 3 en 4** wordt de waarde van beeldvorming onderzocht bij het bepalen van het tumorstadium. Bij patiënten met microscopische lymfekliermetastasen is bekend dat het inzetten van een stadiërende positron-emissie-tomografie (PET) of computer tomografie (CT) van beperkte waarde is vanwege een lage opbrengst en een hoog aantal fout-positieve resultaten. Deze cijfers zijn wellicht veranderd door de technische verbeteringen en de

combinatie van PET met CT. **Hoofdstuk 3** beoordeelt de diagnostische testprestaties van een stadiërende CT of PET/CT en daarnaast het aantal en de aard van aanvullende testen die werden aangevraagd voor 143 patiënten met een positieve SLN. De sensitiviteit van PET/CT was 11%, de specificiteit 73% en de positief voorspellende waarde 4%. Voor CT was de sensitiviteit 17%, de specificiteit 57% en de positief voorspellende waarde 6%. Bijkomende metastasering werd bij slechts twee van de 143 patiënten (echt-positieve opbrengst 1,4%) vastgesteld, maar dit veranderde hun tumorstadium niet aangezien de metastasen bij beide patiënten in een regionaal kliergebied zaten. Daarnaast werden 21 patiënten (15%) met een fout-positieve uitslag onderworpen aan 37 aanvullende onderzoeken, verwijzingen of ingrepen die achteraf niet nodig bleken.

Sinds de publicatie van hoofdstuk 3 zijn de behandelingsmogelijkheden voor stadium III melanoompatiënten uitgebreid. Adjuvante systemische therapie wordt nu aangeboden aan patiënten met een positieve SLN met SLN tumorafmetingen van >1 mm en daarbij wordt vaak een baseline PET/CT gedaan. Totdat beeldvormingstechnieken kleinere metastasen betrouwbaarder kunnen identificeren, dringen we erop aan voorzichtig te zijn met routinematige stadiëringsbeeldvorming bij melanoompatiënten met een positieve SLN.

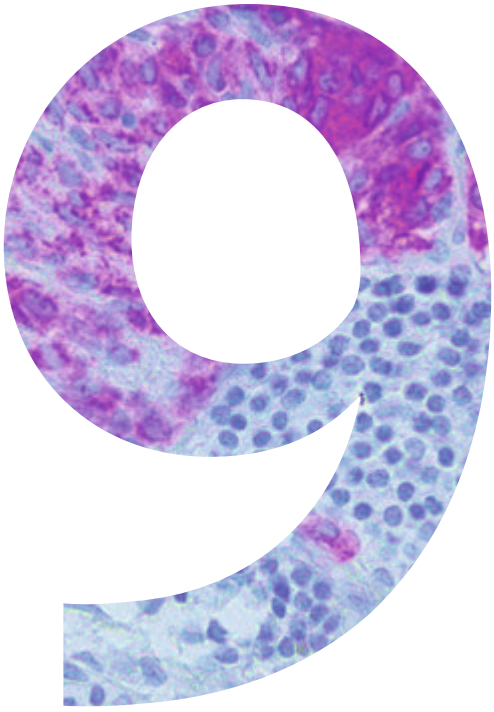
Hoofdstuk 4 onderzoekt de rol van een stadiërende PET/CT van het hele lichaam en magnetische resonantie-beeldvorming (MRI) van de hersenen bij patiënten met satelliet- en/of in-transitmetastasen (ITM). In deze prospectieve studie met 25 patiënten leidde een stadiërende PET/CT tot een verandering in het behandelplan bij vier patiënten (16%) en bij alle vier patiënten werd een hoger tumorstadium vastgesteld. De sensitiviteit van PET/CT was 58% en specificiteit 83%. Een MRI van de hersenen veranderde bij geen van de patiënten het behandelplan of het stadium. Deze studie geeft aan dat een stadiërende PET/CT bij patiënten met satelliet- en/of in-transit-metastasen een betekenisvolle impact kan hebben op het behandelplan.

Adjuvante radiotherapie vermindert het risico op terugkeer van het melanoom in het lymfeklierveld na regionale lymfeklierdissectie. De timing en het optimale fractioneringsschema - de stralingsdosis per behandelsessie - zijn echter nog steeds onderwerp van discussie. De patiënten in de cohortstudies in dit proefschrift werden behandeld in een tijd voordat effectieve systemische immunotherapie beschikbaar kwam. **Hoofdstuk 5** rapporteert de oncologische uitkomsten van 335 patiënten die werden behandeld met hypofractionering (95 patiënten, mediane dosis 33 Gy in zes fracties gedurende 3 weken) of conventionele fractionering (240 patiënten, mediane dosis 48 Gy in 20 fracties gedurende 4 weken). De uitkomsten waren vergelijkbaar. De 5-jaarsoverleving met tumorcontrole in het lymfekliergebied was 86,0% in de hypofractioneringsgroep en 85,5% in de conventionele fractioneringsgroep (P=0,87), het 5-jaars ziektevrij interval was 41,7% versus 31,9% (P=0,18) en de 5-jaars totale overleving was 41,2% versus 45,0% (P=0,77). Aanwezigheid van tumorcellen buiten het kapsel van de lymfeklier

(extranodal spread) en het aantal verwijderde lymfeklieren met gemetastaseerd melanoom voorspelden een ongunstig resultaat.

Terugkeer van het melanoom op dezelfde plaats zou een uitdrukking kunnen zijn van een meer agressieve tumorbiologie. Adjuvante radiotherapie kan worden gegeven aan melanoompatiënten na operatieve verwijdering van teruggekeerd melanoom in hetzelfde kliergebied (salvage chirurgie) na een eerdere regionale klierdissectie, maar de waarde van deze behandelingsstrategie is slecht gedocumenteerd. **Hoofdstuk 6** beschrijft de oncologische uitkomsten van 76 patiënten behandeld met adjuvante radiotherapie op het kliergebied na één of meer regionale lymfekliergebied-recidieven na lymfeklierdissectie. Conventionele gefractioneerde radiotherapie (mediane dosis 48 Gy in 20 fracties) werd gegeven aan 43 patiënten (57%) en gehypofractioneerde radiotherapie (mediane dosis 33 Gy in 6 fracties) aan 33 patiënten (43%). De 5-jaarsoverleving met tumorcontrole in het lymfekliergebied was 70%, 5-jaars ziektevrij interval 17%, 5-jaars melanoom specifieke overleving 26% en 5-jaars totale overleving 25%. Met salvage chirurgie plus adjuvante radiotherapie wordt een redelijke controle in het lymfekliergebied bereikt, maar helaas hebben patiënten een slechte prognose als gevolg van progressie door uitzaaiingen op afstand. De resultaten van onze studie kunnen dienen als basis voor prospectieve studies die de volgorde van chirurgie, radiotherapie en moderne systemische therapieën onderzoeken. Daarnaast kwam het melanoom in 33% van de gevallen terug in een niet-bestraald gebied van het anatomisch gedefinieerde lymfekliergebied, wat het belang van een goede radiotherapie-kwaliteitscontrole benadrukt.

APPENDICES



List of publications

Acknowledgements / Dankwoord

Curriculum Vitae

List of publications

Holtkamp LH, Wang S, Wilmott JS, Madore J, Vilain R, Thompson JF, Nieweg OE, Scolyer RA. Detailed pathological examination of completion node dissection specimens and outcome in melanoma patients with minimal (<0.1 mm) sentinel lymph node metastases. Ann Surg Oncol. 2015 Sep;22(9):2972-7

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Naïef als ik was, dacht ik niet direct aan ernstige dingen toen ik begin 2016 heel moe was en een vergrote lymfeklier in mijn oksel vond. De diagnose luidde een T-cellymfoom. Na een week bleek gelukkig maar bizar de eerste beoordeling foutpositief en was mijn leven helemaal niet afgelopen. Wel bleek ik een zeer zeldzame aandoening (Kikuchi) te hebben die er onder de microscoop erg op leek maar die goedaardig was. Deze ervaring heeft mij geleerd wat het betekent om een fout-positieve diagnose te krijgen. Het duurde een paar jaar voordat ik weer vertrouwen in mijn lichaam had. En een traag herstel van de Kikuchi en een Thoracic Outlet Syndroom erbij zorgden voor veel frustraties en vertraging van opleiding en onderzoek. Dank aan iedereen die in mij bleef geloven. Ik weet dat ik niet bepaald gezellig en vrolijk was in die periode en waardeer het als je daar doorheen kon kijken.

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Curriculum Vitae

Wieke Holtkamp was born in Apeldoorn on November 20, 1984. She obtained her high school diploma at Sprengeloo in 2003. From a very young age Wieke had a desire to become a medical doctor. Studying medicine however, was made complicated by the 'numerus fixus' in the Netherlands. This 'lottery system' prevented her from studying medicine there and as such she moved abroad to Leuven, Belgium. She obtained her primary medical degree 'cum laude' (with distinction) at the KU Leuven in 2012. She subsequently returned to Apeldoorn to work as a Principal House Officer in General Surgery at Gelre Hospital. In 2013 she was given the opportunity to complete a 12-month research fellowship at the Melanoma Institute Australia in Sydney. Wieke was provided with a Grant from the Melanoma Sarcoma Groningen Foundation and was supported by het Vreedefonds. She worked under the guidance of Prof. Dr. H.J. Hoekstra and Prof. Dr. O.E. Nieweg. With the support of Prof. J.F.T. Thompson she was able to extend her stay for another six months. Afterwards, she returned to the Netherlands and commenced work as a Principal House Officer in General Surgery at Isala in Zwolle. In 2016, after a lengthy deliberation, Wieke decided to switch specialties and was accepted into the General Practice (GP) training program. However, due to unexpected illness, her GP training and PhD research were delayed. Wieke persisted in her desire to complete both through her dedication and determination. Since 2020, she has been working as a GP in various General Practices in Overijssel and Drenthe, combining clinical work with projects in primary care innovation.

Wieke Holtkamp werd geboren op 20 november 1984 te Apeldoorn. Daar behaalde ze in 2003 op Sprengeloo haar gymnasiumdiploma. Al heel jong wist ze dat ze dokter wilde worden en de studie geneeskunde was de volgende stap. Helaas werd ze drie keer uitgeloot, waardoor ze moest uitwijken naar Leuven, België. Daar behaalde ze in 2012 cum laude haar artsenbul aan de Katholieke Universiteit Leuven, waarna ze terugkeerde naar Apeldoorn om als ANIOS chirurgie aan de slag te gaan bij het Gelre Ziekenhuis. In 2013 kreeg ze de kans om onder leiding van prof. dr. H.J. Hoekstra en prof. dr. O.E. Nieweg een jaar lang onderzoek te doen aan het Melanoma Institute Australia te Sydney, Australië. Ze werd ondersteund met een beurs van de Stichting Melanoma Sarcoma Groningen en een renteloze lening van het Vreedefonds. Haar verblijf werd dankzij prof. J.F.T. Thompson verlengd tot anderhalf jaar. Daarna keerde ze terug naar Nederland en kon toen verder als ANIOS chirurgie bij het Isala in Zwolle. Na lang wikken en wegen besloot ze in 2016 de generalist in haar de vrijheid te geven en werd ze aangenomen voor de opleiding Huisartsgeneeskunde. Wegens ziekte liepen zowel de opleiding als het promotietraject vertraging op, maar dit uitstel betekende gelukkig geen afstel. Sinds 2020 werkt ze als huisarts in verschillende praktijken in Overijssel en Drenthe. Daarbij combineert ze praktijkwaarneming met projecten op het gebied van innovatie in de eerstelijnszorg.