Combining surgery and systemic therapy in metastatic melanoma

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Combining surgery and systemic therapy in metastatic melanoma

Combineren van chirurgie en systeemtherapie in gemetastaseerd melanoom

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Safety is in the numbers

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Chapter 1

General introduction and thesis outline

1

GENERAL INTRODUCTION

Melanoma is a malignant tumor originating from melanocytes. The most common type is cutaneous melanoma, located in the skin. The incidence of cutaneous melanoma has been increasing over the past decades, with a global incidence of 324.635 in 2020.[1] The age-standardized rates in the Netherlands were 27.0 per 100.000 in 2020, which is the fourth highest in the world, with Australia and New-Zeeland having the highest rates.[2] Melanoma is an aggressive disease and prognosis is strongly dependent on the stage of the disease, which depends on Breslow thickness and ulceration status (T-stage); presence of in-transit and/or nodal metastases (N-stage); or distant metastases (M-stage). Tumor staging is described according to the American Joint Committee on Cancer (AJCC) classification: majority of patients present with localized disease (stage I and II), but a proportion of patients either present with, or progress to stage III (regional metastases) or stage IV (distant metastases) disease.[3,4]

Treatment of primary melanoma

Surgery is the cornerstone of treatment in localized disease. After diagnosis of primary melanoma, wide local excision (WLE) is recommended to resect potential microsatellites. The clinical safety margin of the WLE depends on the Breslow thickness: 0.5 cm in melanoma in situ, 1 cm in melanomas ≤2 mm, and 2 cm in melanomas >2 mm.[5] Additionally, in stage T1b and higher, guidelines recommend to combine WLE with a sentinel lymph node biopsy (SLNB).[6,7] If tumor cells are found in the first draining lymph node, i.e. a positive SLNB, patients are classified as having stage III melanoma.

Surgery in stage III melanoma

As described in the previous paragraph, SLNB is recommended for T1b melanomas and above. The important prognostic value of this procedure was described by Morton et al., in the MSLT-1 trial.

In this trial, patients with primary melanoma were randomly assigned to either WLE with nodal observation, or WLE with SLNB. Patients with a negative SLNB showed a significantly better melanoma-specific survival (85%) than patients with a positive SLNB (62%). In patients with a positive SLNB a completion lymph node dissection (CLND) was performed. No survival differences were seen between the nodal observation and SLNB (± CLND) groups.[8] This led to a successive trial: the MSLT-2, which randomized patients with positive SLNB between nodal observation and CLND.[9] Since both this trial and the similar DeCOG-SLT trial failed to show a survival benefit, CLND is no longer recommended by guidelines in patients with a positive SLNB.[10]

Although lymph node dissections are no longer performed in this patient population, this is still the main treatment in patients with clinical/imaging detected (macroscopic) nodal metastases. In this situation, the procedure is described as a therapeutic lymph node dissection (TLND).[5,11]

Despite the surgical efforts described above, recurrences and progression to stage IV disease are common in patients with stage III melanoma after surgery alone.

Systemic therapy for melanoma

Over the past decades, drastic developments have taken place in the systemic treatment of melanoma patients. Both immune checkpoint inhibitors (ICI) and targeted therapy (TT) have improved the prognosis of patients with advanced melanoma drastically. Inhibitors targeting immune checkpoints PD-1 or CTLA-4 can be applied as either monotherapy or combination therapy and have shown durable responses.[12-18] Targeted therapy directed at tyrosine-kinases BRAF and MEK is available to treat patients with BRAF V600-mutated melanoma, approximately 50% of patients, and shows rapid and high response rates, but has shown to induce resistance.[19-21] These therapies were first introduced in stage IV melanoma patients and showed clear survival benefits compared to chemotherapy or best-supportive care. Due to these encouraging results and the observation that a substantial proportion of patients with stage III melanoma treated with surgery alone develop a recurrence in due course, the indications for these new systemic therapies have broadened.

Due to the high risk of recurrence after surgery in stage III melanoma, adjuvant therapies have been a topic of interest in melanoma research over the past decades. Interferon-α-2b (IFN) was the first adjuvant treatment approved by the Food and Drug Administration, because it had shown recurrence free (RFS) and overall (OS) survival benefits in the ECOG 1684 trial, with a limited sample size.[22] However, in Europe IFN has never been approved due to conflicting results of the subsequent EORTC 18592 and EORTC 18991 trials, which did not show an OS benefit.[23-25]

The development of both TT and ICI have brought new options for the adjuvant treatment of stage III melanoma patients. High-dose anti-CTLA-4 ipilimumab (10 mg/kg) was the first to show a RFS and OS benefit, however, at the cost of very high toxicity rates.[26,27] Both anti-PD-1 agents nivolumab and pembrolizumab have shown encouraging results in improving RFS compared to ipilimumab or placebo, respectively, but have yet to show an OS benefit.[28-31] The BRAF/MEK inhibitor combination dabrafenib and trametinib has also shown an RFS benefit compared to placebo.[32]

This has caused the standard of care to shift from WLE + SLNB, followed by a CLND in case of a positive SN to currently no longer performing adjuvant surgery, but rather offering adjuvant systemic therapy to these patients.

As both TT and ICI have shown to improve recurrence free survival in patients with resected stage III melanoma, the following topic of interest is optimally selecting patients who benefit the most from these therapies.

The success of adjuvant therapy has shown the value of combining surgery and systemic therapy. This collaboration will be explored further in other patient populations: using

systemic therapy to pave the road to surgery in unresectable advanced melanoma, surgery after initial treatment with systemic therapy in metastatic melanoma, or neoadjuvant therapy in patients with macroscopic lymph node metastases to prevent recurrences.

OUTLINE OF THIS THESIS

The aim of this thesis is to create insight in combining surgery and systemic therapy to enhance melanoma treatment. In **chapter 2** and **chapter 3**, results of the REDUCTOR trial are discussed, which investigated the use of a short-term induction with BRAF/MEK inhibition in unresectable locally advanced melanoma. Chapters 4 and 5 focus on adjuvant therapy in stage III melanoma: **chapter 4** is a review on current adjuvant trials and **chapter 5** describes a biomarker study to select patients who will benefit from adjuvant therapy. The following chapters 6 and 7 are population-based studies, using data from the Dutch Melanoma Treatment Registry (DMTR). **Chapter 6** describes the appliance of surgery after a response to systemic therapy in advanced melanoma. **Chapter 7** focusses on the retrospective value of SLNB once patients have progressed to advanced disease.

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PART I

Neoadjuvant targeted therapy in unresectable locally advanced melanoma

Chapter 2

Neoadjuvant cytoreductive treatment with BRAF/MEK inhibition of prior unresectable regionally advanced melanoma to allow complete surgical resection, REDUCTOR: a prospective, single arm, open-label phase II trial

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ABSTRACT

Objective: To evaluate the potency of short-term neoadjuvant cytoreductive therapy with dabrafenib plus trametinib (BRAF and MEK inhibitor) to allow for radical surgical resection in patients with unresectable locally advanced melanoma.

Summary background data: Approximately 5% of stage III melanoma patients presents with unresectable locally advanced disease, making standard of care with resection followed by adjuvant systemic therapy impossible. Although neoadjuvant targeted therapy has shown promising results in resectable stage III melanoma, its potency to enable surgical resection in patients with primarily unresectable locally advanced stage III melanoma is still unclear.

Methods: In this prospective, single arm, phase II trial, patients with unresectable BRAF-mutated locally advanced stage IIIC or oligometastatic stage IV melanoma were included. After 8 weeks of treatment with dabrafenib and trametinib, evaluation by PET/CT and physical examination were used to assess sufficient downsizing of the tumor to enable resection. The primary objective was the percentage of patients who achieved a radical (R0) resection.

Results: Between August 2014 and March 2019, 21 patients were included. Planned inclusion of 25 patients was not reached due to slow accrual and changing treatment landscape. Despite this, the predefined endpoint was successfully met. In 18/21 (86%) patients a resection was performed, of which 17 were R0 resections. At a median follow-up of 50 months (IQR 37.7-57.1 months), median recurrence free survival was 9.9 months (95% CI 7.52-not reached) in patients undergoing surgery.

Conclusions: This prospective, single arm, open-label phase II trial, shows neoadjuvant dabrafenib plus trametinib as a potent cytoreductive treatment, allowing radical resection of metastases in 17/21 (81%) patients with prior unresectable locally advanced melanoma.

INTRODUCTION

Historically, the standard of care for macroscopic regional metastatic melanoma has been complete surgical resection of lymph node metastases. However, approximately five percent of patients presents with unresectable locally advanced disease with very bulky lymph node metastases or in-transit metastases, making a complete (R0) resection unfeasible. These patients are currently given the same treatment as patients with stage IV disease and subsequently have a similar prognosis.[1]

The systemic treatment of patients with unresectable stage III and IV melanoma has evolved drastically over the past decade with the development of both immune checkpoint inhibitors (ICIs) and targeted therapy. In metastatic melanoma, targeting the mitogen-activated protein kinase (MAPK)-pathway with BRAF plus MEK inhibitors (BRAF/MEKi) has proven to be successful in patients with BRAF-mutated melanoma.[2-5] Compared to ICI, treatment with BRAF/MEKi shows higher response rates (up to 68% versus up to 59% with the combination ICI of nivolumab and ipilimumab), but most patients acquire resistance in due course (5-year progression free survival (PFS) of 19% versus 36%).[4-14]

Indications for these systemic therapies are broadening, since treatment in the adjuvant setting has recently become the standard of care for patients with resectable stage III melanoma, who have a substantial risk of recurrence after resection.[1, 15, 16] Both adjuvant nivolumab or pembrolizumab, and adjuvant BRAF/MEKi were shown to improve the recurrence free survival (RFS) in this patient population.[17-19] This treatment regimen is not feasible in patients with unresectable locally advanced melanoma, however, systemic therapy could possibly enable a complete resection when given in a neoadjuvant, cytoreductive setting. ICI and BRAF/MEKi have recently been demonstrated to downsize resectable stage III melanoma substantially, with very impressive major pathological responses, including complete remissions in only a very short period of time (6-12 weeks).[20, 21] Yet no data are available for patients with primarily unresectable stage IIIC disease. For such patients with BRAF V600 mutated melanoma, the BRAF/MEKi combination may be suitable to enable enough downsizing to allow R0 resection, given the high response rate and short time to response.

Similar to unresectable stage IIIC melanoma, systemic therapy is the first choice of treatment in most stage IV melanoma patients. However, in patients with oligometastatic stage IV melanoma (≤3 metastatic lesions), surgical resection could be a potential curative approach for a minority of patients.[22, 23] Nowadays, these patients are also eligible for adjuvant systemic therapy with nivolumab.[17] Neoadjuvant treatment in these patients may also reduce the development of new metastatic lesions from undetected micrometastatic disease.[1]

The aim of this prospective, single arm, open-label phase II study is to evaluate the potency of short-term neoadjuvant cytoreductive therapy with dabrafenib plus trametinib (BRAF and MEK inhibitor respectively) to allow for radical surgical resection in patients with unresectable and BRAF-mutated, locally advanced stage III or oligometastatic stage IV melanoma.

PATIENTS AND METHODS

Study design and population

This study was designed as a prospective, single arm, phase II trial including 25 patients when at least four of the first 14 included patients would respond to the neoadjuvant treatment. The study was approved by the ethics committee of the Netherlands Cancer Institute (NKI, EudraCT number 2013-002616-28) and all participants provided written informed consent. The trial was conducted according to the principles of the Declaration of Helsinki and the Medical Research Involving Human Subjects Act (WMO). Patients were enrolled and treated at the Departments of Medical and Surgical Oncology at NKI (Amsterdam, The Netherlands).

Patients with unresectable BRAF-mutated stage IIIC melanoma or stage IV melanoma with ≤3 metastases, in which surgery alone was deemed not to be a feasible treatment option, were eligible for this trial. As official criteria for unresectability have not yet been defined, it is therefore considered as the result of a multisurgeon decision during multidisciplinary melanoma meetings. Staging was performed according to the 7th edition of the melanoma American Joint Committee on Cancer staging manual (AJCC 7th).[24] Pathologic confirmation of cutaneous melanoma harboring a BRAF V600E or -K mutation was required. Also, patients had to be treatment naïve for this disease stage and the intended operation should be considered to offer a chance of curation or substantial palliation. Other main inclusion criteria were: patients of ≥18 years of age; WHO performance status of 0 or 1; evaluable lesions on CT, MRI or PET/CT; and adequate organ functions. Main exclusion criteria were presence of central nervous system metastases; major surgery, radiotherapy or systemic therapy in the four weeks prior to inclusion; pregnancy or lactation; known Human Immunodeficiency Virus (HIV), Hepatitis B or C infection; and cardiac abnormalities.

Treatment and procedures

All patients underwent 18F-Fluorodeoxyglucose (18F-FDG) PET/CT and MRI of the brain as baseline measurements. Neoadjuvant treatment consisted of dabrafenib 150 mg twice daily (BID) and trametinib 2 mg once daily (QD) for a period of eight weeks. Evaluation was performed with physical examination by the surgeon after 4 and 8 weeks and with PET/CT after 2 and 8 weeks of treatment. If the tumor had been downsized sufficiently (as defined by a multidisciplinary board) and no new lesions had occurred, the operation was performed within the next 2-3 weeks. In this period the dabrafenib and trametinib were continued. If the tumor was still deemed unresectable, treatment with BRAF/MEKi was continued until disease progression or intolerable toxicity.

Outcomes

The primary endpoint of this study was the percentage of patients for whom a radical (R0) resection was achieved, defined as tumor free margins of the resection specimen, confirmed by an expert pathologist. Pathologic responses were assessed using the consensus guidelines of the International Neoadjuvant Melanoma Consortium (INMC), using the percentage of tumor bed occupied by viable tumor cells. [25] Secondary endpoints were RFS, PFS and overall survival (OS). RFS was calculated in patients undergoing a resection, defined as the time between surgery and time of first disease progression (PD). In all patients, PFS was determined as the interval between the date of first administration of BRAF/MEKi and the time of PD or time of death due to any cause. The time of PD is defined as the date of radiological PD scored on imaging data according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.[26] OS is defined as the time between the date of first administration of BRAF/MEKi and date of death due to any cause. Patients not experiencing an event will be censored at the day of last contact. Metabolic response rates were evaluated on 18F-FDG PET/CT according to response criteria of the European Organization for Research and Treatment of Cancer (EORTC), and PET Response Criteria in Solid Tumors (PERCIST).[27, 28] Radiologic response rates were evaluated on (low dose) CT following RECIST 1.1.

Additionally, adverse events (AE) were evaluated by the investigators during the study according to the National Cancer Institute's Terminology Criteria for Adverse Events version 4.03 (NCI-CTCAE v.4.03). New AEs were registered from the moment of signing informed consent until 30 days after the last study intervention and all existing AEs were followed-up until recovery or baseline levels. Only clinically significant laboratory abnormalities were reported (grade ≥3).

Statistical analyses

The hypothesis is that if downsizing by dabrafenib and trametinib treatment results in an R0 resection in 45% or more patients, the treatment modality is considered effective (alternative hypothesis). An R0 resection in 20% or less patients is considered futile (null hypothesis). The sample size was calculated using the Simon optimal two-stage method using an alpha of 0.1 and beta of 10% (power 90%).[29] The first stage would include 14 patients and the trial continued to the second stage, with 25 patients enrolled in total, if an R0 resection was achieved in at least four patients. Statistical analyses were performed using R version 3.5.1. Descriptive statistics were used to describe baseline patient and tumor characteristics; adverse events; responses; and recurrences. Survival endpoints (RFS, PFS, and OS) were analyzed using Kaplan-Meier estimates and medians were presented with 95% confidence intervals (CI).

RESULTS

Patient and treatment characteristics

Between August 2014 and March 2019 a total of 21 patients were included in this study. After inclusion of the first 14 patients, sufficient downsizing of the tumor was seen in 10 patients and the trial proceeded. However, inclusion was ceased before reaching the predefined total of 25 patients due to slow accrual rate, changing treatment landscape in the running time of the trial and results that could not fail to meet the predefined endpoints anymore, as further elucidated in the discussion section. Baseline characteristics of the 21 included patients are summarized in table 1. All but one patient (95%) had unresectable stage IIIC disease at the time of inclusion. A large proportion (43%) of the included patients had a melanoma of unknown primary (MUP).

All patients completed treatment with neoadjuvant BRAF/MEKi for eight weeks, although in six patients a short interruption was necessary due to toxicity (median four days (range 1 –18 days).

Table 1. Baseline characteristics

Characteristic	n	%
Age, years		
Median	53	
Range	25-76	
Sex		
Female	10	48.0
Male	11	52.0
WHO performance status		
0	20	95.2
1	1	4.8
2	0	0.0
3	0	0.0
Disease stage (AJCC 7th edition)		
IIIC	20	95.2
IV	1	4.8
Location primary melanoma		
Extremity	7	33.3
Trunk	5	23.8
Head and neck	0	0.0
Unknown primary	9	42.9
Type		
Superficial spreading	5	23.8
Nodular	3	14.3
Acral lentiginous	2	9.5
Lentigo maligna	0	0.0
Desmoplastic	0	0.0
Unknown primary	9	42.9

Characteristic	n	%
Unknown	2	9.5
Breslow thickness		
≤1.0 mm	0	0.0
1.01-2.0 mm	6	28.6
2.01-4.0 mm	2	9.5
>4.0 mm	2	9.5
Unknown primary	9	42.9
Unknown	2	9.5
Ulceration		
Yes	2	9.5
No	10	47.6
Unknown primary	9	42.9
Site locoregional metastases		
Axillary LN	10	47.6
Axillary and cervical LN	5	23.8
Inguinal LN	0	0.0
Iliac LN	3	14.3
Inguinal and iliac LN	3	14.3
BRAF-mutation		
V600E	20	95.2
V600K	1	4.8
LDH		
≤ULN	11	52.4
>ULN	9	42.9
Unknown	1	4.8

LDH, lactatedehydrogenase; LN, lymph node, ULN, upper limit of normal.

Clinical responses

After eight weeks of treatment, two patients had experienced PD as detected on PET/CT, both of whom developed distant metastases during the neoadjuvant treatment period. In one of these patients, an axillary lymph node dissection (LND) was performed for palliative reasons and to obtain local control, as the initial lymph node metastases had responded and became resectable, despite the development of new distant lesions. Sufficient downsizing of the tumor was seen in the remaining 19 patients and all of these proceeded to surgery. Despite significant downsizing, in one patient the tumor was still deemed unresectable during surgery due to encasement of the iliac artery and vein. Therefore, in 18/21 patients (86%) a complete macroscopic resection could be performed.

Table 2 shows radiologic and metabolic responses after eight weeks. According to RECIST, most patients (76.2%) had a partial response (PR) on CT and one patient had a radiologic complete response (CR). Metabolic responses according to EORTC and PERCIST were comparable in all patients but one, predominantly revealing a partial metabolic response (PMR, 61.9% PERCIST). This one patient with a different EORTC and PERCIST response showed

stable disease (SD) according to PERCIST, but PMR when using EORTC. Four patients (19.0%) had a complete metabolic response (CMR).

Table 2. Response and surgery

Response	N	%
Radiologic response		
Complete response	1	4.8%
Partial response	16	76.2%
Stable disease	1	4.8%
Progressive disease	2	9.5%
Missing*	1	4.8%
Metabolic response EORTC criteria		
Complete metabolic response	4	19.0%
Partial metabolic response	14	66.7%
Stable metabolic disease	0	0.0%
Progressive metabolic disease	2	9.5%
Missing*	1	4.8%
Metabolic response PERCIST		
Complete metabolic response	4	19.0%
Partial metabolic response	13	61.9%
Stable metabolic disease	1	4.8%
Progressive metabolic disease	2	9.5%
Missing*	1	4.8%
Pathologic response		
Pathologic complete response	6	28.6%
Pathologic near complete response	3	14.3%
Pathologic partial response	4	19.0%
Pathologic non response	5	23.8%
N.A.	3	14.3%
Radical excision		
R0	17	81.0%
R1	1	4.8%
R2	0	0.0%
N.A.	3	14.3%

* In one patient lesions did not meet RECIST 1.1 criteria, additionally PERCIST and EORTC could not be assessed in this patient due to an incompatible FDG-PET at baseline. N.A., not applicable; R0, radical resection; R1, tumor positive microscopic resection margins; R2, macroscopic residual tumor

Surgical and pathological outcomes

Since the vast majority of patients in this trial had locally advanced stage III melanoma, surgery primarily consisted of a LND. In 13 (72%) patients an axillary LND was performed, in four patients this procedure was combined with a resection of either supraclavicular or cervical lymph nodes. Five (28%) patients underwent an extensive superficial (inguinal-femoral) and deep (iliac-obturatory) groin LND. In the patient with stage IV disease at inclusion, an iliac LND was combined with resection of a spermatic cord metastasis and in a second procedure resection of a lung metastasis was performed.

Of the 18 patients undergoing a resection, the expert pathologist classified 17 as an R0 resection. In one patient the resection margin was not tumor-free and a re-excision was performed. In this second specimen no vital melanoma cells could be found. Pathologic responses were evaluated in the 18 patients undergoing a per protocol resection: six patients had a pathologic CR (pCR); three a near pCR; four had a pathologic PR (pPR) and pathologic non response (pNR) was seen in five patients. In the patient that underwent a palliative axillary LND despite development of distant metastases, a pPR was still seen in the resection specimen and tumor margins were free. However, due to the development of distant metastases during neoadjuvant treatment, this procedure is not classified as a per protocol resection nor an R0 resection. Neither radiologic response on CT nor metabolic response on PET could accurately predict pathologic response, which is shown in table 3.

Table 3. Radiologic and metabolic versus pathologic response

Pathology	pCR	pnCR	pPR	pNR	N.A.
Imaging					
Radiologic response					
CR	0	0	0	1	0
PR	6	3	4	2	1
SD	0	0	0	1	0
PD	0	0	0	0	2
Metabolic response EORTC criteria					
CMR	1	0	0	3	0
PMR	5	3	4	1	1
SMD	0	0	0	0	0
PMD	0	0	0	0	2
Metabolic response PERCIST					
CMR	1	0	0	3	0
PMR	5	3	3	1	1
SMD	0	0	1	0	0
PMD	0	0	0	0	2

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; CMR, complete metabolic response; PMR, partial metabolic response; SMD, stable metabolic disease; PMD, progressive metabolic disease; pCR, pathologic complete response; pPR, pathologic partial response; nPCR, pathologic near complete response; pNR, pathologic non response. * In one patient lesions did not meet RECIST 1.1 criteria, additionally PERCIST and EORTC could not be assessed in this patient due to an incompatible FDG-PET at baseline.

Per protocol, no adjuvant therapy was given, except for adjuvant radiotherapy in two patients. One patient traveled abroad to receive adjuvant systemic therapy (one year of PD-1 blockade), since this was not part of the study treatment nor standard and reimbursed care at that time in the Netherlands.

Adverse events

Most patients experienced some form of toxicity during neoadjuvant systemic treatment with BRAF/MEKi: only two (10%) patients experienced no treatment-related AEs. In the majority of patients the worst toxicity was grade 1 (11 patients, 52%), in four (19%) patients grade 2 and four (19%) patients experienced grade 3 toxicity. The most commonly reported AEs related to dabrafenib plus trametinib were fever (48%), chills (19%), fatigue (19%), nausea (19%) and myalgia (14%).

The majority of patients that underwent surgery (16/20, 80%) experienced a surgical complication, consisting mainly of the development of seroma (55%) and wound infections (30%). Three patients had grade 1 seroma, five patients grade 2 and three patients experienced grade 3 seroma for which elective radiologic or surgical intervention was indicated. Grade 2 wound infection was seen in two patients and four patients endured a grade 3 wound infection requiring intravenous antibiotics and hospital admittance.

Survival outcomes

At a median follow-up of 50 months (IQR 37.7-57.1 months), the median RFS in the 18 patients undergoing surgery was 9.9 months (95% CI 7.52-not reached). Median PFS in all 21 patients was 12.4 months (95% CI 8.67 – not reached). The patient who had received adjuvant systemic therapy abroad, outside of the study protocol, was censored for PFS and RFS at time of commencement of adjuvant systemic treatment. Median OS was not reached. The 1-year OS was 100% and 2-year OS 85% (95% CI 70.0-100.0). RFS, PFS and OS are shown in figure 1a, b, and c respectively.

Figure 1a. Recurrence free survival

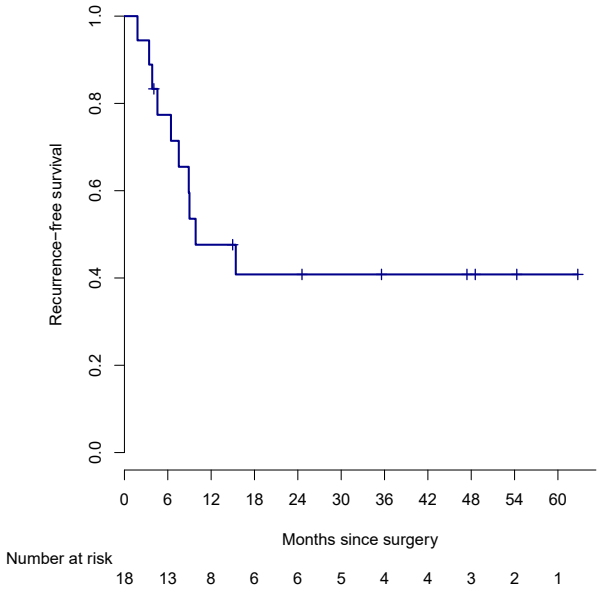


Figure 1b. Progression free survival

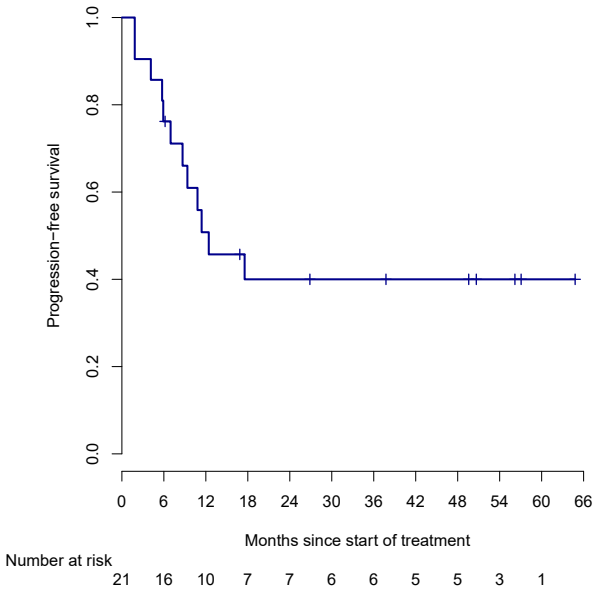
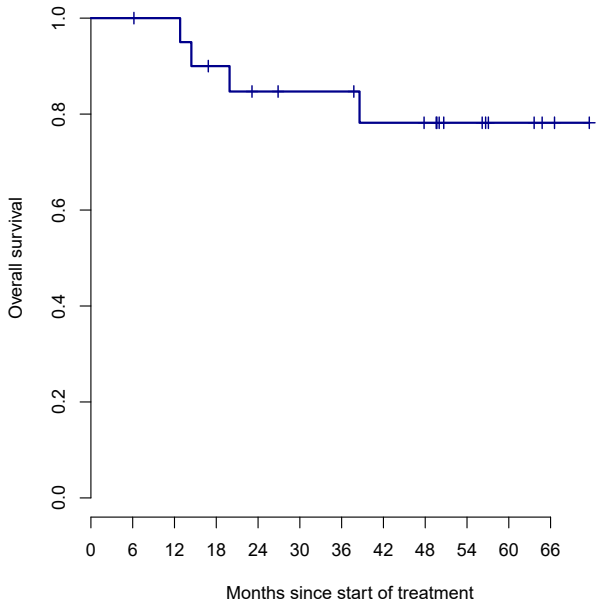


Figure 1c. Overall survival



Recurrences

Recurrences were seen in half of the patients that underwent surgery (9/18). Most patients developed distant recurrences (6/9), versus three patients with locoregional recurrences as a first presentation of relapse. The three patients with locoregional recurrences were treated with surgery, but all developed distant metastases and were treated with systemic therapy at later time points. Of patients with a pCR, five (83%) did not relapse, but one (17%) did develop a recurrence. Four out of five patients with a pNR relapsed. All three patients that could not undergo a resection, due to either PD during study treatment or insufficient response, were treated with ICI, resulting in a CR in these patients.

DISCUSSION

In this prospective, single arm, open-label phase II trial, neoadjuvant treatment with dabrafenib and trametinib has shown to be a potent cytoreductive treatment, allowing radical resection of metastases in 17/21 (81%) patients with prior unresectable locally advanced melanoma. Encouraging RFS and OS are also seen in these patients, even compared to patients with resectable stage IIID melanoma.[30]

Several trials have reported neoadjuvant treatment with either targeted therapy or ICI in stage III melanoma. Two studies reported treatment with neoadjuvant BRAF/ MEKi in resectable BRAF-mutated stage III melanoma. Both Amaria et al.[31] and Long et al.[20] treated patients with neoadjuvant (8 and 12 weeks respectively) and adjuvant (44 and 40 weeks respectively) BRAF/ MEKi for one year in total. Patients presented with a MUP in 14% (standard of care arm) and 29% (neoadjuvant arm) of cases in the trial by Amaria et al. The site of the primary melanoma was not described by Long et al. In our current trial, a relatively large proportion of patients (43%) presented with a MUP. This could be due to the fact that they were unaware of their melanoma and that patients were not seen in a standard follow-up program, resulting in later clinical presentation. In our current trial, the neoadjuvant treatment was given for a relatively short time compared to the trials by Amaria et al and Long et al. The cytoreductive effect of BRAF/MEK inhibitors usually occurs quickly after initiation of treatment, thus it was hypothesized that 8 weeks neoadjuvant treatment should suffice to achieve enough tumor downsizing to enable resection. A later response after this time was deemed unlikely. In the trial by Amaria et al., the RFS in patients that received this treatment was 19.7 months (95% CI 16.2-not reached), versus 2.9 months (95% CI 1.7-not reached, HR 0.016) in the standard of care arm of this trial. In the study by Long et al., the RFS was 23.3 months (95% CI 17.7-not reached). In both trials high response rates could be observed, with 85% of patients achieving a radiological response scored per RECIST 1.1 and 58% of patients achieving a pCR in de trial by Amaria et al. In the trial by Long et al., 86% of patients achieved a radiological response prior to surgery and in all patients a pathological response was seen in the resection specimen (49% pCR, 51% non-complete pathological response). These striking differences in

(complete) response rates (RR) compared to the current trial can potentially be explained by the different patient populations included in these trials. These other two trials only included patients with resectable stage IIIB-C disease, whereas our current trial only included patients with unresectable disease, presently considered as stage IIID disease following AJCC 8th edition. The differences in RFS may in turn be explained by the absence of adjuvant treatment after resection in our current trial, this in contrast to the trials by Amaria et al and Long et al.

Of the patients undergoing surgical resection, 17% presented with locoregional disease as site of first recurrence in our present trial. This is comparable to earlier reported locoregional recurrence rates in patients with resectable IIIC melanoma undergoing resection without (neo) adjuvant systemic therapy, as represented by placebo arms of recent adjuvant randomized trials.[32–34] However, the patients in our trial would not been able to undergo surgery, without cytoreductive treatment.

In our study, both radiologic and metabolic response prior to surgery were not able to predict pathologic response. This was also shown by Eroglu et al. in a retrospective study in a comparable patient population.[32] Of nine patients with a (near) pCR, only one patient had shown a corresponding metabolic CR and no patients had a radiologic CR; the remainder of these pCR patients had shown a radiologic and metabolic PR. The poor performance of radiologic response as a predictor of pCR might be explained by fibrotic tissue visible as a remaining lesion on CT, although no viable tumor cells are present. Tan et al. described that FDG-PET may be superior to CT in showing a CR in metastatic melanoma patients treated with ICI.[33] However, in our cohort this distinction was not as clear. Both EORTC and PERCIST criteria were designed to measure response to chemotherapy, and perhaps this cannot be directly translated to treatment with targeted therapy.

Besides neoadjuvant targeted therapy, different schemes of neoadjuvant anti-PD-1 or the combination ICI with nivolumab and ipilimumab have been reported in patients with resectable palpable stage III melanoma as well, as described by Blank et al.[34], Rozeman et al.[21], Amaria et al.[35], and Huang et al.[36] In combination ICI studies, very high pathologic RR were seen of 73-80% and to date, pathologic response seemed predictive of RFS, as none of the patients who achieved a pathologic response relapsed in the OpACIN-neo study.[21, 34, 35]

As yet, no direct comparison between (neo)adjuvant targeted therapy and ICI has been performed in resectable stage III melanoma. Although response rates are high in the neoadjuvant setting for ICI, it is uncertain whether this can be translated to the population of patients with unresectable stage III melanoma. Both BRAF/ MEKi and ipilimumab/nivolumab have shown rapid responses and high objective RR in stage IV melanoma.[4, 11] In our current trial we have shown that 18/21 patients had a radiological and metabolic response after only 8 weeks of dabrafenib/trametinib treatment and radical resection could be achieved in 17/21 patients. The majority of patients did not develop distant metastases during this treatment period, despite being at very high risk for metastatic disease and with a median follow-up of 50 months 43%

was still disease free. Whether similar or better results could be obtained for unresectable stage III disease with short term neoadjuvant ICI is currently unknown.

Due to the rapid developments in the systemic treatment of stage IV melanoma and the oftentimes quickly progressive nature of stage IV disease, only one patient with stage IV disease was included in this study. Currently, the standard of care in these patients is first line systemic treatment with PD-1 blockade or combined ipilimumab/nivolumab and surgery is only considered in select cases of residual disease or oligoprogression.[1]

This single arm, open-label phase II trial is, to our knowledge, the first prospective trial treating unresectable locally advanced melanoma patients with neoadjuvant cytoreductive targeted therapy, of which the results look very promising. A limitation of this trial however, is the small patient cohort. This is partly due to the specific patient population, as most patients have either limited locoregional disease only or bulky locoregional metastases combined with multiple distant metastases. Also, during the course of this trial, BRAF/MEKi became available for unresectable stage III melanoma patients outside of clinical trials, reducing the number of referrals from other sites. Due to the slow accrual rate, the decision was made to cease the trial prematurely to retain its clinical relevance. An unplanned interim analysis of the, up until then, 21 included patients showed that predefined endpoints had already been exceeded. Moreover, since the neoadjuvant treatment with BRAF/MEKi was effective in most patients and adjuvant treatment had become standard of care in resected stage III melanoma during the course of this study, many patients inquired about the possibility of receiving adjuvant treatment after resection. Due to the combination of the slow accrual rate, results of the unplanned interim analysis and the changing treatment landscape with adjuvant systemic therapy as the new standard of care, the decision was made to cease inclusion and report the outcomes of this study.

Dabrafenib plus trametinib has shown to be a potent neoadjuvant cytoreductive treatment in this select population of BRAF-mutated unresectable locally advanced melanoma patients. Patients with no recurrence remained disease-free for a prolonged period of time. However, when recurrences were seen, this usually occurred quickly after surgery. This may present a window of opportunity for adjuvant therapy with ICI, possibly in combination with BRAF/MEKi, in order to achieve improved and more durable RFS. Further clinical trials are needed to explore the additional benefit of such adjuvant therapy after previous neoadjuvant BRAF/MEKi.

CONCLUSION

In this prospective single arm, open-label phase II trial, neoadjuvant dabrafenib and trametinib has shown to be a potent cytoreductive treatment, allowing radical resection of metastases in 17/21 (81%) patients with prior unresectable locally advanced melanoma. If relapses occurred, this was usually quickly (within months) after surgery. This could present an opportunity for tailored adjuvant therapy.

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Chapter 3

[18F]-FDG PET/CT during neoadjuvant targeted therapy in prior unresectable stage III melanoma patients: can (early) metabolic imaging predict histopathologic response or recurrence?

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ABSTRACT

Purpose: The aim of this study was to investigate whether 18F-FDG PET/CT can predict histopathological response or recurrence in BRAF-mutated unresectable locally advanced stage III melanoma treated with neoadjuvant BRAF/MEK inhibition followed by resection and the value of PET in detecting early recurrence after resection.

Patients and Methods: Twenty BRAF-mutated, unresectable stage III melanoma patients received BRAF/MEK inhibitors before surgery. 18F-FDG PET/CT was performed at baseline and 2 and 8 weeks after initiation of therapy. After resection, PET/CT was performed at specific time points during 5 years of follow-up. Pathological response was assessed on the dissection specimen. Response monitoring was measured with SUVmax, SUVpeak, MATV, and TLG and according to EORTC and PERCIST criteria.

Results: Pathological response was assessed in 18 patients. Nine patients (50%) had a pathologic complete or near-complete response, and 9 (50%) had a pathologic partial or no response. EORTC or PERCIST response measurements did not correspond with pathologic outcome. SUVmax, SUVpeak, MATV, and TLG at all time points and absolute or percentage change among the 3 initial time points did not differ between the groups. During follow-up, 8 of 17 patients with R0 resection developed a recurrence, 6 recurrences were detected with imaging only, 4 of which with PET/CT in less than 6 months after surgery. PET parameters before surgery did not predict recurrence.

Conclusions: Baseline 18F-FDG PET or PET response in previous unresectable stage III melanoma patients seems not useful to predict pathologic response after neoadjuvant BRAF/MEK inhibitors treatment. However, PET/CT seems valuable in detecting recurrence early after R0 resection.

INTRODUCTION

In stage III/IV BRAF-mutated melanoma patients, targeted therapy with BRAF inhibitors has shown to induce significant levels of tumor shrinkage in the majority of patients, even shortly after treatment initiation. When combined with MEK inhibitors, even higher objective response rates of approximately 65% have been reported.[1–4]

These impressive therapy responses have led to an increased interest in BRAF/MEK inhibitors as neoadjuvant systemic therapy, especially in borderline resectable or unresectable stage III or oligometastatic melanoma. Several studies have demonstrated encouraging results that sufficient downsizing of the tumor enables radical resection of previous unresectable locally advanced or oligometastatic disease.[5–7] Despite these encouraging data, however, recurrences after radical surgery still occur.

The ability to predict recurrence before surgery could help to personalize patient monitoring after surgery. Furthermore, recurrent disease is preferably detected at an early stage. Indeed, at this phase, the tumor is either still resectable or the tumor burden is still low, resulting in better efficacy of therapies and targeted therapies, which could benefit patients' survival.

In melanoma patients treated with neoadjuvant BRAF/MEK inhibition, the degree of pathologic response depends on the response pattern found in the histologic specimen and varies from pathologic complete response (pCR) to partial pathologic response (pPR) or pathologic nonresponse (pNR).[8] These histopathologic response patterns seem a predictive biomarker for relapse-free survival (RFS) in these patients, where the extent and histopathologic features of the response after neoadjuvant BRAF/MEK inhibitors correlate with RFS.[9] In the recently published NeoCombi trial, in which 35 BRAF-mutated stage IIIB or IIIC (AJCC seventh edition) melanoma patients received neoadjuvant and adjuvant dabrafenib and trametinib, pCR was associated with an improved prognosis compared with patients without a pCR.[10] Similar results were shown in the study of Eroglu et al[11] in which patients with a pCR had a significantly improved RFS and overall survival compared with patients with residual viable tumor.

Metabolic imaging with 18F-FDG PET/CT is a noninvasive imaging technique that shows a homogeneous and rapid decrease of glucose metabolism in melanoma metastases treated with BRAF-targeted therapy.[12] Because these metabolic alterations occur even shortly after the initiation of therapy, 18F-FDG PET/CT imaging might be predictive for pathologic response. Furthermore, because metabolic alterations often are the first sign of recurrent disease, 18F-FDG PET/CT could be of value to detect early recurrence. With this study, we aim to investigate whether 18F-FDG PET/CT before and during treatment with BRAF/MEK inhibitors dabrafenib and trametinib in high-risk unresectable stage III melanoma can predict histopathologic response or recurrence. Second, we aim to investigate the additive value of PET to detect early recurrence after surgery.

PATIENTS AND METHODS

Patients

This study was designed as a side study of the reductor trial (EudraCT no. 2013-002616-28), which was a prospective, single-arm, phase 2 study to investigate the ability to achieve R0 resection after neoadjuvant treatment with BRAF/MEK inhibitors in patients with prior inoperable BRAF-mutated stage III or oligometastatic stage IV cutaneous melanoma.[7] Patients were treated with neoadjuvant dabrafenib 150 mg twice daily and trametinib 2 mg once daily for a period of 8 weeks. In case of sufficient downsizing of the tumor as defined by a multidisciplinary board and without occurrence of new lesions, surgical resection was planned within 2 to 3 weeks. Dabrafenib and trametinib were continued until surgery. Patients were assigned to 2 groups based on histopathological outcome: (1) pCR or near-complete response (near-CR), and (2) pPR or pNR. Patients with R0 resection and no evidence of disease elsewhere were monitored for recurrence.

18F-FDG PET/CT

All patients underwent 18F-FDG PET/CT at baseline as part of the staging procedure, 2 weeks after starting neoadjuvant therapy (early), and at 8 weeks (late), the latter being within 2 to 3 weeks before surgical resection. 18F-FDG was administered intravenously with an activity of 180 to 240 MBq (4.9–6.5 mCi) depending on the body mass index, after fasting for 6 hours and 400 mL of fluid intake. Approximately 60 minutes after administration, low-dose CT images (40 mAs, 140 keV, 2–5 mm slices) without IV contrast were obtained for attenuation correction and anatomic correlation, followed by whole-body PET acquisitions with an acquisition time of 1 to 3 minutes per bed position. Patients were scanned on a cross-calibrated Phillips Gemini TF 16 or Phillips Gemini TF big-bore PET/CT scanner (Philips, Cleveland, OH). The 18F-FDG PET/CT images were reviewed by experienced nuclear medicine physicians using a commercially available software package (OsiriX MD, Pixmeo Sarl, version 12.0.1). Semiquantitative PET/CT analysis was performed according to the EORTC and PERCIST criteria.13,14 The total tumor burden was delineated by a threshold of 50%SUVmax, 41%SUVmax, SUVmax2.5, and SUVmax4.0. Each voxel included in at least 3 delineations was taken into account for further analysis.[14,15] Quantitative PET/CT analysis of the total tumor burden was performed using Accurate (v06-07-2019, developed by professor R. Boellaard et al). Data were normalized for patient body weight and injected dose. Tumor SUVmax, SUVmean, and SUVpeak; metabolic activity tumor volume (MATV); and total lesion glycolysis (TLG; defined as SUVmean × MATV) of the total tumor burden were measured. Metabolic response was calculated by measuring early and late absolute and percentage changes in relation to baseline.

Surgery and histopathologic response analysis

Surgery was performed by experienced melanoma surgeons and consisted of lymph node dissection of the involved metastatic area. Histopathological response assessment in the resected specimen was performed by an experienced melanoma pathologist. Histopathologic response was graded according to guidelines published by Tetzlaff et al[8]: pCR in case of complete absence of viable tumor cells, nearCR if 0% to ≤10% of viable tumor cells were observed, pPR when >10% to ≤50% are occupied by viable tumor cells, and pNR when >50% viable tumor cells were observed. pCR/nearCR and pPR/pNR were analyzed as 2 separate groups for the purpose of this current study.

Follow-up

Recurrence-free survival after surgery was assessed every 3 months by physical examination and imaging with 18F-FDG PET/CT for 2 years, then every 6 months for 2 years, and once in year 5. PET/CT was considered true-positive when patients had a recurrence, which was either confirmed with pathology or sequential imaging with contrast-enhanced CT (ceCT) or MRI.

Statistical analysis

Baseline patient and tumor characteristics were recorded and summarized; quantitative values are expressed as mean (range). For analysis of baseline PET characteristics and quantified PET parameters, the Shapiro-Wilk test was used to test normality of distribution between pCR/nearCR and pPR/pNR groups, and between the recurrence and nonrecurrence groups. The independent t test was used in case values were normally distributed; the nonparametric Mann-Whitney U test was used when values were not normally distributed. A P value smaller than or equal to 0.05 was considered statistically significant. Quantified PET parameters were described as median and interquartile range (IQR). Sensitivity, specificity, positive predictive value, and negative predictive value of 18F-FDG PET/CT were calculated. Statistical analysis was performed by using SPSS (IBM v.22.0, Armonk, NY). Relapse-free survival was defined as the time interval between the date of surgical resection and the date that recurrence was identified by imaging or clinical examination during the follow-up period.

RESULTS

Study population

From August 2014 to March 2019, 21 patients with unresectable locally advanced stage IIIC (AJCC seventh edition) melanoma were included in the reductor trial.[7] In 1 patient, treatment response was not monitored with PET/CT for unknown reasons, 1 patient did not proceed to surgery due to progressive disease, and in 1 patient, resection turned out unfeasible intraoperatively due to encasement of vital structures. Therefore, pathology response was assessed in 18 dissection specimens. The site of the regional metastases dissected was axillary (n = 9), axillary and cervical (n = 5), iliac (n = 2), or inguinal and iliac (n = 2). In 1 patient, the

resected specimen revealed tumor-positive microscopic resection margins (R1). Recurrence was evaluated in the remaining 17 patients with R0 resection and no evidence of disease elsewhere in the body. The median follow-up after surgery was 36 months (range, 10–61 months). Patient demographics are listed in Table 1.

Table 1. Patient Demographics and Imaging Characteristics

	Total	pCR/nearCR	pPR/NR	P-value
Number of patients	18	9	9	
Male/female	11/7	4/5	7/2	
Age in years (mean, (range))	52 (25-76)	56 (39-76)	47 (25-73)	
Stage IIIC AJCC 7th edition	18	9	9	
Location primary				
Extremity	6	4	2	
Trunk	3	1	2	
Unknown primary	9	4	5	
Breslow thickness				
≤1.0 mm	0	0	0	
>1.0–2.0 mm	5	4	1	
>2.0–4.0 mm	1	0	1	
>4.0 mm	1	0	1	
Unknown primary	9	4	5	
Unknown	2	1	1	
Ulceration				
Yes	2	1	1	
No	7	4	3	
Unknown	9	4	5	
Site locoregional metastases				
Axillary LN	9	3	6	
Axillary with cervical LN	5	4	1	
Inguinal with iliac LN	2	1	1	
Iliac LN	2	1	1	
No. of lymph nodes resected				
0-10	2	1	1	
11-20	5	2	3	
21-30	5	3	2	
>30	5	3	2	
NA	1	0	1	
Size largest lnn metastasis				
≤6.0 mm	2	0	2	
>6.0-8.0 mm	10	7	3	
>8.0 mm	5	2	3	
unknown	1	0	1	
Timeframe in days (mean, (range)):				
PET_{bl} to Start therapy	20.17 (2-49)	24.44 (3-49)	15.89 (2-28)	0.206[$]
Start therapy to PET_{2weeks}	15.24 (12-19)	15.67 (12-19)	14.75 (13-18)	0.420[$]
Start therapy to PET_{8weeks}	56.56 (49-67)	55.33 (49-61)	57.78 (53-67)	0.208[$]
PET_{8weeks} to surgery	9.44 (2-18)	9.56 (3-18)	9.33 (2-17)	0.935[$]

	Total	pCR/nearCR	pPR/NR	P-value
R0 resection and NED elsewhere, n=17, mo (median (range))				
Time of follow-up	36 (10-61)			
Time to recurrence (n=8)	6 (3-9)			

pCR/nearCR pathologic Complete Response/ near Complete Response, pPR/NR pathologic Partial Response/ No Response, $ Independent T-test, NED No Evidence of Disease, mo months.

Metabolic response evaluation

Baseline and late 18F-FDG PET/CTs at 8 weeks were performed in all patients, and the early 18F-FDG PET/CT at 2 weeks was performed in 17/18 patients (94%). The median time between baseline 18F-FDG PET/CT and start neoadjuvant therapy was 20 days (range, 2–49 days), and between start neoadjuvant therapy and early 18F-FDG PET/CT and late 18F-FDG PET/CT 15 days (range, 12–19 days) and 57 days (range, 49–67 days), respectively. The median time between late 18F-FDG PET/CT and surgery was 9 days (range, 2–18 days) (Table 1).

Semiquantitative analysis according to EORTC criteria classified 4/18 patients (22%) as complete metabolic responders, 12/18 (67%) as partial metabolic responders (PMR), and 1 patient (6%) as progressive metabolic disease based on the presence of new lesions. These outcomes were the same when classifying according to PERCIST, except for 1 patient who was classified as stable metabolic disease with PERCIST instead of PMR with EORTC criteria.

The median SUVmax, SUVpeak, MATV, and TLG of the total group at baseline and early and late scan are displayed in the Supplemental Data S1. The results show a decrease of all quantitative PET parameters during treatment compared with baseline, already measurable at the early scan. Furthermore, the largest decrease for all parameters occurred between baseline and 2 weeks.

18F-FDG PET/CT parameters in relation to histopathological response

Histopathologic evaluation of the resected specimens revealed pCR/nearCR in 9/18 (50%) specimens and pPR/pNR in 9/18 (50%). EORTC or PERCIST response measurements did not correspond with pathologic outcome. Only 1/6 patients (17%) with pCR was classified as complete metabolic responder on PET/CT (EORTC and PERCIST). All 8 patients with pathologic nearCR or pPR were classified as PMR. Four patients with pNR were also classified as PMR on PET, 3 of which with only minimal residual uptake in the remaining lymph node metastases.

At baseline, median SUVmax in the pCR/nearCR group was 17.8 (IQR, 12.3–21.6) and in the pPR/pNR group 16.1 (IQR, 11.3–23.6; P = 0.931) (Supplemental Data S1). The median SUVpeak was 13.7 (IQR, 10.0–18.8) in the pCR/nearCR group and 12.6 (IQR, 8.4–18.9; P = 0.863) in the pPR/pNR group. Similarly to SUVmax and SUVpeak, the median MATV and TLG at baseline did not differ significantly between the pCR/nearCR group and pPR/pNR group. However, with regard to MATV at baseline, 3 patients had an MATV between 155 and 914 mL, compared with the other 15, who had an MATV ranged between 10 and 81 mL. All 3 patients with high MATV had pPR/pNR.

Absolute and percentage changes of SUVmax, SUVmean, MATV, and TLG are shown in Figures 1 and 2. In the pCR/nearCR group, after the first 2 weeks of treatment, a decrease of SUVmax and SUVpeak was seen of 63% (IQR, 55%–66%) and 64% (IQR, 54%–66%), respectively. This was not significantly different from the pPR/pNR group in which the decrease of SUVmax was 63% (IQR, 24%–65%; P = 0.888) and of SUVpeak 61% (IQR, 50–65; P = 0.423). As with SUV, MATV also showed a large decrease (60%; IQR, 46–77) in both the pCR/nearCR and the pPR/pNR groups (45%; IQR, 40–84), although not significantly different (P = 0.436).

Figure 1.Absolute changes of PET parameters per patient from baseline.SUV Standardized Uptake Value, MATV Metabolic Activity Tumor Volume, TLG Total Lesion Glycolysis

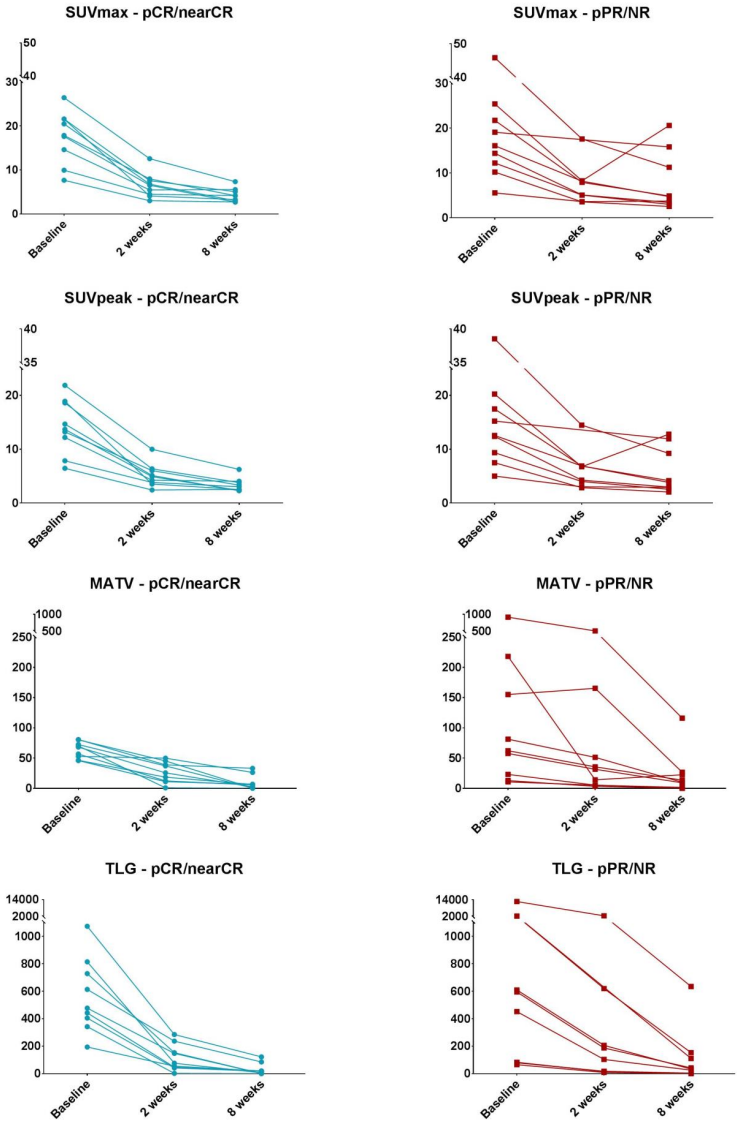
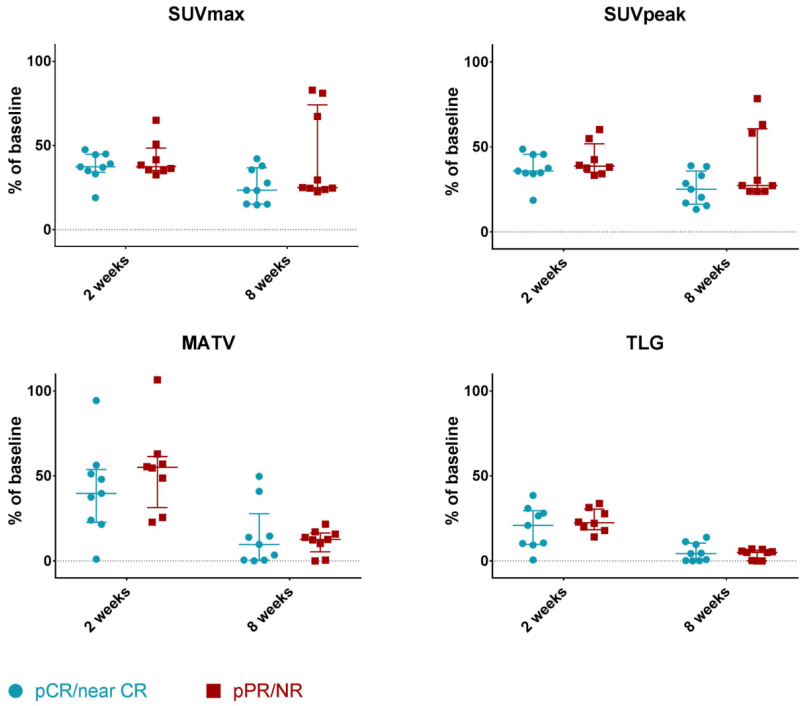


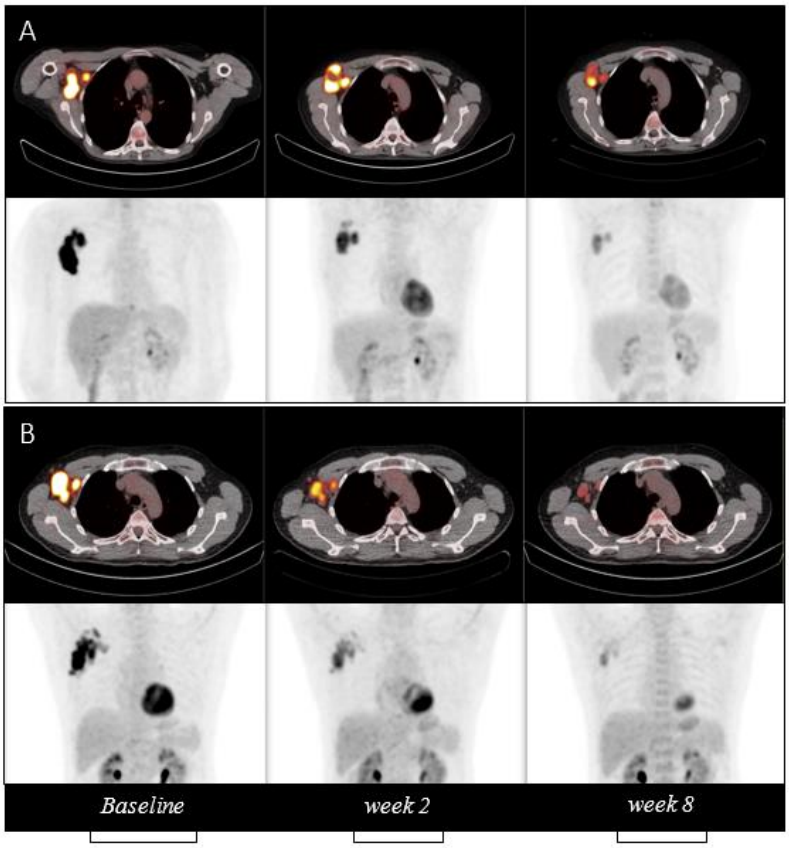
Figure 2. Median and Interquartile Range percentage changes of PET parameters from baseline



SUV Standardized Uptake Value, MATV Metabolic Activity Tumor Volume, TLG Total Lesion Glycolysis.

At 8 weeks after the initiation of treatment, a smaller but further decrease of SUVmax, SUVmean, MATV, and TLG was seen in both pCR/nearCR and pPR/pNR groups. Neither of the parameters was significantly different between the 2 groups. Figure 3 shows the 18F-FDG PET/CT scans of a patient (A) with a pCR in the histopathologic specimen after resection with still significant uptake of 18F-FDG PET/CT at 8 weeks. The histopathologic specimen of this patient showed predominantly lymphoid hyperplasia in addition to widespread necrosis and melanosis. On the contrary, one of the patients (B) showed only faint uptake of 18F-FDG at week 8, and the histologic specimen after surgery revealed partial response (10%–15% viable tumor cells).

Figure 3. ^{18}F-FDG PET/CT transaxial fused and MIP images at baseline and 2 and 8 weeks after starting BRAF/MEK inhibitors.



Lymph node dissection of the axilla after week 8 revealed a complete response in the histologic specimen of patient A, while the histologic specimen of patient B showed a partial response.

In general, patients showed a substantial decrease of FDG uptake. However, in some patients, a deviant course was seen. In 3 patients in the pPR/pNR group, SUVmax and SUVpeak were relatively high at 8 weeks as compared with baseline. Patient 8 already had a low SUVmax and SUVpeak at baseline (5.6 and 5.0, respectively), resulting in a relatively low percentage change at 2 and 8 weeks. Patient 12 had a significant metabolic volume reduction (218 mL at baseline to 22 mL at 8 weeks); however, the 18F-FDG avidity after 8 weeks was still quite high in the remaining tumor volume (SUVmax, 15.8; SUVpeak, 11.9 vs 19.1 and 15.3 at baseline, respectively). Patient 13 had large bulky disease at baseline (MATV, 155 mL), which after 2 weeks of therapy responded with a decrease in FDG avidity but no response in MATV. 18F-FDG uptake at 8 weeks was decreased compared with baseline with additional decrease of tumor burden on CT, which was confirmed with partial response in the histopathologic specimen. However, compared with 18F-FDG PET/CT at 2 weeks, at 8 weeks, an increase of

18F-FDG avidity in several lymph nodes was seen, resulting in a high SUVmax and SUVpeak and indicating progressive disease.

In the pCR/nearCR group, patient 9 had a fast reduction of 18F-FDG avidity after 2 weeks, but no significant changes in tumor shrinkage, resulting in a relatively high MATV after 2 weeks, but a low SUVmax and SUVpeak.

Recurrence

In 8 patients (47%), recurrence occurred with a median time to recurrence of 6 months (range, 3–9 months). One patient experienced locoregional recurrence, being a new subcutaneous metastasis in the scar tissue. Three patients had recurrence in regional lymph nodes, and 4 patients had distant metastases as a first presentation of relapse.

SUVmax, SUVpeak, MATV, and TLG at baseline, 2 weeks, or 8 weeks were not significantly different between the recurrence and nonrecurrence group, nor were absolute or percentage decrease of PET parameters between the different time points (Supplemental Data S1). The histopathologic response after initial surgery before recurrence was as follows: CR n = 1, nearCR n = 2, PR n = 2, and pNR n = 3 (Table 2). In 5 of 9 patients that did not develop recurrence during follow-up, CR after surgery was seen (CR n = 5, nearCR n = 1, PR n = 2, and pNR n = 1).

Table 2. Recurrence in patients with R0 resection and no evidence of disease elsewhere at time of resection.

No.	M/F	Age	Surgery	PA response	Interval surgery-recurrence, mo	Type of recurrence	Diagnostic modality
1	F	53	Axillary and cervical LND	CR	3	Distant, breast	PET/CT
2	M	73	Axillary LND	NR	4	Regional, lnn	PET/CT
3	F	59	Inguinal and iliac LND	NR	4	Distant, brain, lung, liver, bone	Clinical examination, PET/CT
4	F	53	Inguinal and iliac LND	nearCR	6	Distant, intramuscular	PET/CT
5	M	51	Axillary LND	PR	7	Regional, lnn	PET/CT
6	M	76	Axillary and cervical LND	nearCR	8	Regional, lnn	ceCT, PET/CT
7	M	47	Iliac LND	NR	9	Locoregional, subcutaneous	Clinical examination, PET/CT
8	M	25	Axillary LND	PR	9	Distant, lung	ceCT, PET/CT

LND lymph node dissection, CR complete response, PR partial response, NR no response, Lnn lymph nodes, ceCT contrast enhanced CT.

In all 8 patients with recurrent disease, recurrence was detected with 18F-FDG PET/CT, 3 of which as early as 3 to 4 months after surgery. In 2/8 patients (25%), recurrence was first detected with physical examination at 4 and 9 months, respectively. 18F-FDG PET/CT performed shortly thereafter confirmed local recurrence in both patients, and in one of them additional, wide spread metastases in the lungs, liver, and bone. In 2 patients, ceCT was performed for surveillance instead of 18F-FDG PET/CT for unknown reasons. The ceCT revealed recurrence at 9 months in both patients, being a new pulmonary metastasis and locoregional lymph node metastases, respectively. In both patients, the recurrences were FDG avid on 18F-FDG PET/CT, which was performed shortly thereafter to rule out other sites of disease.

18F-FDG PET/CT during follow-up

During follow-up, a total of 95 18F-FDG PET/CT scans were performed in 15 patients. Eight scans were true-positive, one of which recurrence was confirmed by physical examination the same day. Four scans could not distinguish between postoperative inflammation and residual disease even up to 3 months after surgery. Five scans were false-positive and revealed uptake in lymph nodes (2), the thyroid gland (1), nasopharyngeal region (1), and bone (1). All findings were negative for metastases after additional imaging with ultrasound and fine-needle aspiration or MRI. Sensitivity and specificity were 100% and 94.3%, respectively, with a positive predictive value of 61.5% and a negative predictive value of 100%.

Incidental findings were found in 2 patients, being liver steatosis, a benign thyroid nodule, and sarcoidosis in 1 patient and gastritis in another patient.

DISCUSSION

In patients with locally advanced stage III BRAFV600-mutated melanoma, neoadjuvant targeted therapy enables complete resection (R0) of previous borderline resectable and unresectable disease, resulting in better patient prognosis.[5–7] Even so, recurrences still occur, and histopathologic response of the resected specimen might be a predictor for recurrence-free survival in these patients. Because glucose uptake on 18F-FDG PET/CT changes rapidly after starting targeted therapy, noninvasive early metabolic imaging with PET/CT might be able to predict pathologic response or recurrence.

In this prospective study, we measured total tumor burden SUVmax, SUVpeak, MATV, and TLG on 18F-FDG PET/CT to investigate whether absolute uptake at baseline or (early) changes after starting neoadjuvant therapy could distinguish pathologic complete or near-complete responders from pathologic partial or nonresponders. The same parameters were used to predict the appearance of recurrence.

In this study, 9 of 18 patients had pCR or nearCR, but neither baseline PET parameters nor absolute or percentage changes from baseline to 2 or 8 weeks could predict histopathologic response. Similarly, in the 8/17 patients with R0 resection who developed a recurrence, PET parameters could not predict recurrence. However, in the follow-up after surgery, 18F-FDG PET/CT could detect all recurrences, and as early as 3 months after surgery. To our knowledge, no other studies are known in which PET is investigated as a predictive biomarker for pathologic response in melanoma patients treated with BRAF/MEK inhibition.

For pathological response assessment, we used the consensus guidelines presented by the International Neoadjuvant Melanoma Consortium,[8] which classifies histopathologic response as complete, near-complete, partial, or no response. In our study, we investigated PET parameters in 2 groups; pCR/nearCR and pPR/pNR. This has also been used by other neoadjuvant systemic therapy studies.[16,17]

It is questionable whether RECIST can predict pathologic response, because radiologic partial responses on BRAF-targeted therapy may actually have minimal to no viable malignant cells in residual masses seen on imaging. Therefore, it has been suggested that metabolic imaging with serial PET scanning might be a better predictor for response.[18] Despite this hypothesis, in our study, PET parameters could not distinguish pathologic complete or near-complete responders from partial or nonresponders. An explanation could be that metabolic response on 18F-FDG PET/CT is masked by T-cell infiltrates, which is a known phenomenon mimicking tumor metabolism, or the occurrence of cell volume reduction and increased intercellular distance rather than cell death, as presented in a study by Theodosakis et al.[19]

Although we did not find a correlation between 18F-FDG response and pathologic response, some other interesting findings were found. As already shown by McArthur et al,[12] we found the biggest decline of FDG uptake in the first 2 weeks after starting BRAF/MEK inhibition. An advantage of performing a scan early after the initiation of therapy could be the early identification of resistance as was demonstrated in 1 patient. In this patient, 18F-FDG PET/CT at 8 weeks revealed metabolic response compared with baseline, but an increase in FDG avidity compared with the 18F-FDG PET/CT at 2 weeks, suggesting resistance to therapy. Without the 18F-FDG PET/CT at 2 weeks, progressive disease could not have been discovered this early. Another potential interesting finding in our study was that, in patients with a large tumor burden, an MATV higher than 150 mL, no pCR/nearCR was achieved. Although the number of patients was too low to draw any significant conclusions, this might suggest the presence of a certain threshold of MATV above which pathologic CR/nearCR cannot be achieved.

In both the recurrence and nonrecurrence group, pathologic response varied. Even so, in the nonrecurrence group, more patients with pCR were seen than in the recurrence group (5/9 [56%] vs 1/8 [17%]). These results are in line with the INMC pooled analysis of Menzies et al,[17] in which pCR after anti–PD1-based immunotherapy or BRAF/MEK-targeted therapy correlated with RFS.

Preoperative 18F-FDG PET/CT was not predictive for recurrence, which developed within 3 to 9 months after surgery. However, during follow-up, all recurrences were found with 18F-FDG PET/CT, 2 (25%) of which within 3 months. This is in line with the study of Stahlie et al,[20] in which 18F-FDG PET/CT detected early recurrence in 20% of patients who were included in a surveillance protocol after R0 resection of high-risk melanoma. The strength of 18F-FDG PET/CT in relation to recurrent disease therefore might lie in early detection after surgery rather than being predictive before surgery. Although it is known that 18F-FDG PET/CT is less accurate in detecting locoregional recurrences, the value of 18F-FDG PET/CT lies in the high sensitivity for early detection of distant metastases increasing the chance that local therapies with curative intent are still possible. In our study, 18F-FDG PET/CT revealed 3 early recurrences with distant metastases at 3, 4, and 6 months, respectively. One of these recurrences revealed a subcutaneous metastasis by physical examination, but 18F-FDG PET/CT showed widespread disease in lungs, liver, and bone. This emphasizes the value of 18F-FDG PET/CT not only as a tool for early detection of recurrence, but also for determining the extent of disease and therefore therapeutic options.

A limitation of our study is the small number of patients included. Accrual rate for the reductor trail was slower than expected due to a combination of the specific patient population, BRAF/MEK inhibitor availability for unresectable stage III melanoma patients outside of clinical trials, and the changing landscape with adjuvant systemic therapy as the new standard of care.

In conclusion, in this prospective study, SUVmax, SUVpeak, MATV, and TLG on 18F-FDG PET/CT at baseline and during neoadjuvant targeted therapy could not predict pathological response or recurrence in patients with unresectable stage IIIc BRAFV600-mutated melanoma, but a high MATV might suggest pCR/nearCR cannot be achieved. Furthermore, in the follow-up after R0 resection, 18F-FDG PET/CT seems valuable in early detection of recurrence.

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PART II

Adjuvant immunotherapy

Chapter 4

Adjuvant systemic therapy in high-risk melanoma

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ABSTRACT

In resected high-risk melanoma (stage IIB/C-III) the risk of locoregional and/or distant recurrence is substantial and so far adjuvant therapies have been fairly unsuccessful. Interferon showed slight improvements in recurrence-free survival (RFS), but failed to convincingly improve overall survival (OS). In these patients adjuvant therapy with treatments that show promising results in stage IV disease is arising.

Studies using immune checkpoint blockade with anti-CTLA-4 and anti-PD-1 agents reveal convincing RFS benefits. OS rates, however, are not mature yet in most studies. Only ipilimumab has shown an OS benefit but at a high cost of toxicity. Also in studies with adjuvant targeted therapy using BRAF and MEK inhibitors, ensuring results are reported regarding RFS. As possible toxicity cannot be ignored, it is crucial to identify patients who would benefit most from these adjuvant therapies.

In patients with clinically detectable lymph node metastases, studies using neoadjuvant schedules of immunotherapy and targeted therapy have been performed. In phase I and II studies the most optimal schedule of combination immunotherapy was identified and further research on this front will follow in the coming years.

Concluding, after decades of scarce options for patients with high-risk melanoma, recent developments in adjuvant therapy have changed the standard of care for these patients.

INTRODUCTION

The incidence of cutaneous melanoma has been increasing over the past decades. The WHO estimates there will be about 287 000 new cases of melanoma worldwide in 2018, causing over 60 700 deaths this year.[1] Predictive factors associated with survival are Breslow thickness, ulceration, number of positive lymph nodes, tumor burden, distant metastasis and serum lactate dehydrogenase level.[2, 3]

Surgery is the cornerstone in the treatment of melanoma in stage I, II, and III. For localized disease, the standard of care is wide local excision of the primary tumor, with additional safety margins dependent on the Breslow thickness. In addition, sentinel node (SN) biopsy is recommended by most guidelines, to provide information on the lymph node status, for primary melanomas with a Breslow thickness of over 1.0 mm, or less than 1.0 mm with multiple additional risk factors. As both the MSLT-II and DECOG-SLT study showed no survival benefit of CLND compared with the nodal observation by ultrasound, completion lymph node dissection (CLND) in case of a positive SN has become obsolete.[4, 5] Therefore, lymph node dissections are now only reserved for clinically detectable lymph node metastasis by most guidelines.

Despite multiple efforts to improve surgical options and to maintain locoregional control, there is a substantial risk of locoregional and/or distant recurrence in resected high-risk melanoma (stage IIB/C – III) because of the presence of undetectable hematogenous micrometastases already at the time of diagnosis. Together with this risk of recurrence, mortality rates increase with increasing tumor stage. Survival rates for stage III according to the AJCC 8th edition range from 93 to 32%, depending on the number of lymph nodes involved and presence of in-transit or satellite metastases.[2]

For a long time, no additional (systemic) therapy was found to be effective in reducing recurrence and mortality rates in resected high-risk melanoma. However, over the last decade new therapies have become available, first for unresectable stage III-IV melanoma, starting with targeted therapy with BRAF and MEK inhibitors, which have shown encouraging results in these patients.[6-12] Shortly after, immune checkpoint inhibitors against CTLA-4 and PD-1 were introduced and have dramatically changed the landscape of metastatic melanoma treatment.[13-26] Perhaps the spectrum for use of these therapies can be broadened. Therefore, in this review, we will evaluate the effectiveness of immune checkpoint inhibitors and BRAF/MEK inhibitors as adjuvant therapy in resected high-risk melanoma.

ADJUVANT THERAPY

Interferon

Interferon IFN-α-2b was the first and for a long time only therapy approved by the Food and Drug Administration for adjuvant treatment in high-risk melanoma. Its approval in 1995 was mainly based on the results of the ECOG 1684 trial. In this trial both recurrenc- free survival (RFS) and overall survival (OS) were improved in patients treated with IFN-α-2b for 1 year after surgery for T4 or N1 melanomas. Although these results were promising, this treatment did cause severe toxicity and the study was limited by small sample size (n=287).[27]

Two of the largest studies conducted with adjuvant IFN-α-2b were the EORTC 18592 (n=1388) and EORTC 18991 (n=1256). In the EORTC 18952 trial patients with resected stage IIB or III melanoma were randomized to either IFN-α-2b for 13 months, 25 months, or observation. After a median follow-up of 11 years, little effect on the RFS was seen in both treatment arms (13-month arm: hazard ratio (HR) 0.94, p=0,46; 25-month arm: HR: 0.84, p=0.06). No significant effect on OS was observed. Despite these disappointing results, one subgroup did seem to benefit from adjuvant therapy with IFN-α-2b: patients with an ulcerated primary tumor.[28, 29] Subsequently, the EORTC 18991 study randomized patients with resected stage III melanoma to either observation or adjuvant pegylated IFN-α-2b for an intended duration of 5 years. They found a borderline significant better RFS in the pegylated IFN-alpha-2b group of 39.1 versus 34.6% at 7 years (HR 0.87, 95% confidence interval (CI) 0.76-1.00, p=0.055). No advantage in OS was observed and toxicity rates were high, 37% of patients discontinued therapy because of toxicity.[30]

Over the following years several meta-analyses have been performed. These analyses found an advantage in RFS for adjuvant IFN-α-2b in high-risk melanoma, but the advantage in OS was either absent or minor and, therefore, clinically not relevant. Limitations of these meta-analyses were variable inclusion criteria, with different stages of melanoma, and treatment schedules of the included studies. Treatment scheduled differed in dose (low, intermediate or high), induction treatment (with a higher dose and/or more frequent administration of IFN-α-2b), and duration of the maintenance phase.[31-33]

Concluding, slight improvements of RFS and virtually no improvement in OS were seen in patients treated with adjuvant IFN-α-2b. Because of recent changes in the available systemic therapies, IFN-α-2b is outdated and should only be considered as an adjuvant option in countries with limited resources/without access to the new drugs, especially for primary ulcerated melanomas.

Immune checkpoint blockade

The EORTC 18071 study compared high-dose ipilimumab (IPI) 10mg/kg every 3 weeks for four doses and then every 3 months up to 3 years with placebo after complete resection of stage

III (IIIA > 1 mm in SN, IIIB/C, AJCC 7th edition) cutaneous melanoma. This study showed both a convincing 5-year RFS (40.8 vs. 30.3%) and OS (65.4 vs. 54.4%) benefit. Unfortunately, this benefit came with a cost of substantial toxicity: grade 3 or 4 immune-related adverse events occurred (AE) in 41.6% of patients in the IPI arm, compared with 2.7% in the placebo arm and even five (1.1%) of patients died due to immune-related AE.[34, 35]

In an effort to lower these toxicity rates, the three-armed ECOG-1609 trial tested high-dose IPI 10mg/kg versus IPI at the regular dose of 3mg/kg versus IFN-α-2b. Preliminary results suggest that there is no difference in RFS between both IPI arms, with a milder toxicity profile for IPI at 3 mg/kg.[36] Long-term results of this study in terms of RFS of IPI vs. IFN-α-2b and OS results are still pending.

Then, similar to developments in stage IV disease, anti-PD-1 agents were tested in the adjuvant setting. The checkmate 238 study randomized patients who had undergone complete resection of stage IIIB, IIIC, or IV melanoma (AJCC 7th edition) to either anti-PD-1 agent nivolumab (NIVO) 3mg/kg every 2 weeks or high-dose IPI 10mg/kg every 3 weeks for four doses and then every 12 weeks for up to 1 year. They found a 1-year RFS of 70.5% in the NIVO arm and 60.8% in the IPI arm. Toxicity rates were more favorable in the NIVO arm: grade 3 or 4 AEs in 14.4% against 45.9% in the IPI arm. Discontinuation of therapy due to AE occurred in 9.7% of patients in the NIVO group, compared with 42.6% in the IPI group. Therefore, they concluded that adjuvant therapy with NIVO resulted in an improved RFS and a lower rate of severe toxicity, compared with high-dose IPI.[37, 38]

Recently, the EORTC 1325 study, comparing the PD-1 inhibitor pembrolizumab at a fixed dose of 240 mg every 3 weeks with placebo, released its results. This trial was randomized to pembrolizumab or placebo for a total of 18 doses (~1 year) after complete resection of stage III melanoma. The 1-year RFS in patients treated with pembrolizumab was 75.4 versus 61.0% in the placebo group (HR: 0.57, P<0.001) after a median follow-up of 15 months.[39] This study showed a similar rate of grade 3/4 AE of 14.7% when compared with NIVO. These studies show a convincing RFS benefit of adjuvant therapy with immune checkpoint blockade.

BRAF/MEK inhibitors

Another option for treatment in the adjuvant setting is targeted therapy with BRAF and MEK inhibitors. Its use is slightly more limited, as only patients with melanoma harboring a BRAF mutation, which is ~50% of patients, can receive BRAF/MEK inhibitors. Two studies were conducted with BRAF and MEK inhibitors in the adjuvant setting: the BRIM8 and COMBI-AD studies.

The BRIM8 study divided patients into two cohorts: cohort 1 (n=314) included patients with stage IIC, IIIA (SN>1mm) and IIIB melanoma and cohort 2 (n=184) existed of patients with stage IIIC melanoma. In both cohorts patients with $BRAF^{V600}$ mutation-positive melanoma were randomized to receive either vemurafenib or placebo for 1 year, after complete resection of

the tumor. At a median follow-up of 30.8 months in cohort 1 and 33.5 months in cohort 2, an advantage on median disease-free survival (DFS) of vemurafenib was seen in both cohorts. In cohort 2, however, this difference of 23.1 months in the vemurafenib group versus 15.4 months in the placebo group, was not significant (HR: 0.80, 95% CI: 0.54-1.18, p=0.26). In cohort 1 the mean DFS was not reached in the vemurafenib group versus 36.9 months in the placebo group (HR: 0.54, 95% CI: 0.37-0.78, log-rank p=0.001). The authors conclude that the study did not meet its primary DFS endpoints in cohort 2, making the benefit in cohort 1 nonsignificant. The risk reduction in cohort 1 should, therefore, be viewed as exploratory only.[40]

The COMBI-AD study treated patients with a combination of a BRAF and MEK inhibitor. In all, 870 patients with $BRAF^{V600E}$/$BRAF^{V600K}$-mutated, stage III melanoma were randomized to either 12 months of dabrafenib plus trametinib (D+T) or placebo. At a median follow-up of 44 months in the treatment arm and 42 months in the placebo arm, 3- year RFS rates were 59% in the D+T group versus 40% in the placebo group and 4-year RFS was 54 versus 38% (HR 0.49, 95% CI 0.40-0.59). Median RFS was not reached in the D+T arm versus 16.6 months in the placebo arm.[41, 42]

Concluding, these studies show a RFS benefit for the BRAF and MEK combination D+T and suggest a risk reduction for recurrence in patients treated with vemurafenib alone in stage IIC-IIIB melanoma.

Ongoing studies

Currently, three studies involving immune checkpoint inhibitors in the adjuvant setting have not released their results yet: the SWOG S1404, Checkmate 915 and Keynote 716 studies. The SWOG S1404 randomizes patients after complete surgical resection of stage IIIA (N2a), IIIB, IIIC, or IV melanoma among two treatment arms: physician/patient choice of high-dose IFN-α-2b or IPI in arm A and pembrolizumab in arm B.[43] The Checkmate 915 is a randomized phase III study comparing adjuvant combination therapy with NIVO and IPI versus NIVO alone in patients with resected stage IIIB/C/D or IV melanoma (AJCC 8th edition). In both these trials accrual is completed and first results are expected in 2020.[44] Finally, the recently opened Keynote 716 trial randomizes stage II melanoma patients to either a year of fixed-dose pembrolizumab every 3 weeks (maximum of 17 cycles) or placebo. This study is still accruing patients. *Table 1 summarizes all the studies mentioned above.*

Table 1. Overview of adjuvant trials

Study	Design	Stage (AJCC 7th edition)	No. of patients	Median FU	HR RFS	HR OS	2-year RFS	3-year RFS	5-year RFS	IR AE gr 3-4 AE
EORTC 18071	IPI 10mg/kg vs. placebo	IIIA (SN>1mm), IIIB, IIIC	N=951	5.3 years	0,76 (95% CI 0.64-0.89; p<0.001)	0.72 (95% CI 0.58-0.88; p=0.001)	NR? VF: 51% vs. 42%	46.5% vs. 34.8%	40.8% vs. 30.3%	41.6% vs. 2.7%
ECOG-1609	IPI 10mg/kg vs. IPI 3mg/kg vs. HD-IFN	IIIB, IIIC, M1a, M1b	N=1670	3.1 years	NR	NR	NR	54% vs. 56% vs. NR for HD-IFN	NR	57% vs. 36.4% (AE general, most IR)
Checkmate 238	NIVO 3mg/kg vs. IPI 10mg/kg	IIIB, IIIC, IV	N=906	NR, minimum 24 months	HR 0.66, p<0.0001)	NR	62.6% vs. 50.2%	NR	NR	14.4% vs. 45.9%
EORTC 1325	Pembro vs. placebo	III	N=1019	15.1 months	HR 0.57 (98.4% CI 0.43-0.74, p<0.001)	NR	71.4% vs. 53.2% at 18 months	NR	NR	14.7% vs. 3.4%
BRIM8	Vemurafenib vs. placebo	Cohort 1: IIC, IIIA (SN>1mm), IIIB Cohort 2: IIIC	N=498	Cohort 1: 30.8 months Cohort 2: 33.5 months	Cohort 1: 0.54 (95% CI 0.37–0.78, log-rank p=0.0010) Cohort 2: 0.80 (95% CI 0.54-1.18, p=0.26)	NR	62.2% vs. 53.1% Cohort 1: 72.3% vs. 56.5% Cohort 2: 46.3% vs. 47.5%	NR	NR	57% vs. 15% (not IR)
COMBI-AD	Dabrafenib + trametinib vs. placebo	III	N=870	2.8 years	0.49 (95% CI 0.40-0.59; p=0.0006)	0.57 (95% CI 0.42 to 0.79; p=0.0006)**	67% vs. 44%	59% vs. 40%	NR	41% vs. 14%
SWOG S1404	HD-IFN/IPI vs. pembro	III A (N2a), IIIB, IIIC, IV	No results published yet							
Checkmate 915	IPI plus NIVO vs. NIVO	IIIB, IIIC, IIID, IV (AJCC 8th edition)	No results published yet							
Keynote 716	Pembro vs. placebo	II	Accruing patients							

FU: Follow-up; HR: Hazard Ratio; RFS: Recurrence Free Survival; OS: Overall Survival; IR AE: Immune-related Adverse Events; Gr: grade; IPI: Ipilimumab; HD-IFN: High Dose Interferon-alpha-2b; Pembro: Pembrolizumab; * In-transit metastases excluded ** not updated

Another important remaining question for BRAF+ melanoma in terms of adjuvant therapy is: is it better to treat with BRAF/MEK or immunotherapy? No studies have examined this head-to-head yet and it is the question if such a study will ever be performed. For now, choices are being made based on stage IV melanoma experience or cross-trial comparisons, which are always dangerous due to many differences between trials, which do not allow for such comparisons.

Selecting patients

In the studies described above, one of the main inclusion criteria was completely resected melanoma, in most cases consisting of a lymph node dissection. However, this is no longer the standard of care because both the MSLT-II and DECOG-SLT studies showed no survival benefit of CLND compared with the nodal observation by ultrasound.[4, 5] Therefore, lymph node dissections are reserved for clinically detectable lymph node metastasis.

The sentinel node procedure is an important diagnostic tool in determining prognosis, but does it provide enough information if one omits a CLND for a positive SN? The MSLT-II trial showed that additional lymph node metastases were found in 11.5% of patients undergoing a CLND after positive SN. Nonetheless, this does not mean staging would change for all these patients. Additional studies showed that only ~6% of all SN+ cases are upstaged due to additional information provided by the CLND[45]. Moreover, SN tumor burden of more than 1 mm and ulceration of the primary could be an adequate replacement to stratify the risk.[46]

NEOADJUVANT

Neoadjuvant immunotherapy

Besides adjuvant treatment, patients with clinically detectable lymph nodes could also be treated in the neoadjuvant setting, before undergoing the therapeutic lymph node dissection. This could provide some advantages, for example using treatment response as a predictive marker for possible additional adjuvant therapy and reducing presurgical tumor burden. Theoretically neoadjuvant treatment could also allow a stronger immune response to develop, as more tumor antigens will be encountered than in the adjuvant setting.

The phase 1b study comparing neoadjuvant and adjuvant treatment in patients with palpable stage III melanoma was the OpACIN trial. This study randomized 20 patients to either neoadjuvant or adjuvant NIVO 1mg/kg plus IPI 3mg/kg. A high pathologic response rate of 80% was achieved in the neoadjuvant arm and in patients achieving a pathologic response, none had relapsed after a median follow-up of 25.6 months. These promising results came with substantial side effects: grade 3-4 AE occurred in nine out of 10 patients in both treatment arms.[47] Following these findings, the OpACIN-NEO trial was designed examining different neoadjuvant schedules, to reduce toxicity, but preserve efficacy. In arm A

patients received 2x IPI 3mg/kg plus NIVO 1mg/kg every 3 weeks (Q3W); in arm B 2x IPI 1mg/kg plus NIVO 3mg/kg Q3W; and in arm C 2x IPI 3mg/kg Q3W followed immediately by 2x NIVO 3mg/kg Q2W. Accrual to arm C was terminated earlier due to high toxicity rates. In total 86 patients were included in this trial and toxicity profiles were indeed more favorable: grade ≥3 AE occurred in 40, 20 and 50% in arm A, B, and C, respectively. At the same time efficacy was preserved with pathologic response rates of 80 and 77% in arm A and B. To sum up, the dose of IPI 1mg/kg plus NIVO 3mg/kg Q3W would be the most attractive for further research.[48] This is currently happening in an extension cohort: the PRADO study. Patients will be treated with the IPI 1mg/kg plus NIVO 3mg/kg schedule and afterwards only the marked index node will be surgically resected. Depending on the pathologic response subsequent personalized adjuvant treatment with surgery, radiotherapy and/or adjuvant therapy will be performed.[49]

Another study comparing different neoadjuvant treatment schemes was performed by Amaria and colleagues., in which patients received either neoadjuvant NIVO 3mg/kg monotherapy or NIVO 1mg/kg plus IPI 3mg/kg. Accrual was ended early by the Data Safety Monitoring Board due to high toxicity in the combination arm (73% grade 3 trAE) and the observation of disease progression in the NIVO monotherapy arm, preventing surgical resection (2/12 patients, 17%).[50] However, the pathologic complete response rate (pCR) in the combination arm was very similar to the OpACIN and OpACIN-neo studies at 45%.

Neoadjuvant BRAF/MEK inhibitors

Similar to the adjuvant treatment, neoadjuvant studies have been conducted using BRAF and MEK inhibitors in patients with BRAF-mutated melanoma. In a study by Amaria and colleagues, neoadjuvant and adjuvant D+T were combined. Patients were randomized to either surgery or 8 weeks of neoadjuvant D+T, surgery and adjuvant D+T for 44 weeks. The trial was closed to new patient entry after an interim analysis after the inclusion of 21 patients with a median follow-up of 7.1 months showed it was not ethical to assign patients to the standard of care surgery alone. At a median follow-up of 18.6 months an event-free survival of 71% versus 0% was observed with an HR of 0.016 (95% CI: 0.00012-0.14, p<0.001). Pathologic response rates in the neoadjuvant arm were 58% pCR and 17% pathological partial response. Toxicity was similar to previous observations in stage IV disease and did not cause unexpected postoperative complications.[51]

Another study carried out in a slightly different population. Menzies and colleagues performed a phase 2 study in 35 patients with bulky, but resectable stage III melanoma. These patients received D+T for 12 weeks before surgery and continued D+T in the adjuvant setting for 40 more weeks. pCRs were seen in 48% of patients. At a median follow-up of 12.1 months after surgery, 36% patients had relapsed (with a median of 12.9months).[52]

In contrast, the REDUCTOR trial included patients with bulky and unresectable (or high chance of R+) stage III or oligometastatic stage IV melanoma. These patients are treated with neoadjuvant D+T for 8 weeks, after which it is decided whether surgery can be performed on the basis of the evaluation on PET/CT. Interim analyses show that after inclusion of 17 patients with a median follow-up of 22 months, 76% of patients with previous unresectable melanoma could undergo radical surgery. During neoadjuvant treatment, two patients had progressed and were switched to immunotherapy.[53, 54]

Pathologic assessments

An advantage of neoadjuvant therapy is, that it offers the possibility to use the pathologic response as an outcome marker and as a predictor of therapy efficacy for adjuvant therapy. In this light it is important to standardize pathologic assessment and reporting of tumor response. Consensus guidelines have been composed by the International Neoadjuvant Melanoma Consortium and subsequently reported by Tetzlaff and colleagues. In this work, the pathologic response is defined by the % of tumor bed occupied by viable tumor cells (pCR if absent; near pCR if >0% and ≤10%; pathological partial response as ≤50%; and pNR if >50%.[55]

CONCLUSION

After changing the landscape of melanoma stage IV treatment, immune checkpoint blockade and targeted therapy are now also now drastically changing the adjuvant treatment of melanoma. Immunotherapy with anti-CTLA-4 agent IPI showed both a RFS and OS benefit, but at a cost of high severe toxicity rates. Monoclonal antibodies against PD-1 on the other hand showed even better RFS rates, with fewer immune-related AEs. Given these great advantages in RFS, an advantage in OS is expected and these agents will play a big role in adjuvant therapy.

For patients with a melanoma harboring a BRAF-mutation, another option is available for adjuvant therapy: targeted therapy with BRAF and MEK inhibitors. In this area studies have shown an RFS benefit and preliminary data suggests a potential OS benefit.

Besides adjuvant treatment, patients with palpable lymph node metastases have also been included in neoadjuvant studies, with immunotherapy and BRAF/MEK inhibition. These studies show promising results so far and further research is expected in the near future.

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Chapter 5

Interferon-gamma signature as prognostic and predictive marker in macroscopic stage III melanoma

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ABSTRACT

Background: A substantial proportion of patients with macroscopic stage III melanoma do not benefit sufficiently from adjuvant anti-PD-1 therapy, as they either recur despite therapy or would never have recurred. To better inform adjuvant treatment selection, we have performed translational analyses to identify prognostic and predictive biomarkers.

Patients and methods: Two cohorts of patients with macroscopic stage III melanoma from an ongoing biobank study were included. Clinical data were compared between an observation cohort (cohort 1) and an adjuvant intention cohort (cohort 2). RNA sequencing for translational analyses was performed and treatment subgroups (cohort 1A and cohort 2A) were compared for possible biomarkers, using a cut-off based on the treatment-naïve patients. In addition, two validation cohorts (Melanoma Institute Australia (MIA) and University Medical Centre Utrecht (UMCU)) were obtained.

Results: After a median follow-up of 26 months of the 98 patients in our discovery set, median recurrence-free survival (RFS) was significantly longer for the adjuvant intention cohort (cohort 2, n=49) versus the observation cohort (cohort 1, n=49). Median overall survival (OS) was not reached for either cohort, nor significantly different. In observation cohort 1A (n=24), RFS was significantly longer for patients with high interferon-gamma (IFNγ) score (*P*=0.002); for adjuvant patients of cohort 2A (n=24) a similar trend was observed (*P*=0.086). Patients with high B cell score had a longer RFS in cohort 1A, but no difference was seen in cohort 2A. The B cell score based on RNA correlated with $CD20^+$ cells in tumor area, but was not independent from the IFNγ score. In the MIA validation cohort (n=44), longer RFS was observed for patients with high IFNγ score compared with low IFNγ score (*P*=0.046), no difference in RFS was observed according to the B cell score. In both the observation (n=11) and the adjuvant (n=11) UMCU validation cohorts, no difference in RFS was seen for IFNγ and B cell.

Conclusions: IFNγ has shown to be a prognostic marker in both patients who were and were not treated with adjuvant therapy. B cell score was prognostic but did not improve accuracy over IFNγ. Our study confirmed RFS benefit of adjuvant anti-PD-1 for patients with macroscopic stage III melanoma.

INTRODUCTION

Until recently, the standard of care for patients with macroscopic stage III melanoma has been surgery only. Due to the high risk of recurrence, adjuvant therapies (eg, interferon alpha-2b) have been investigated over the past decade, many without demonstrating a significant benefit to patients. Recently, both immune checkpoint inhibition (ICI) and BRAF-targeted therapies have been shown to improve recurrence-free survival (RFS) and are now a standard treatment for patients with resected stage III melanoma.[1-5] However, approximately 80% of patients do not benefit sufficiently from these adjuvant therapies. This issue is presented by two subsets of patients: first, patients who recur despite adjuvant systemic therapy and second, patients who would never have recurred after surgery (and therefore do not require adjuvant treatment). In terms of the number needed to treat, on average five patients need adjuvant therapy in order to prevent one recurrence in stage III melanoma, illustrating the issue of overtreatment, unnecessary toxicity and high healthcare costs.

Selection of adjuvant therapy is currently based on the American Joint Committee on Cancer (AJCC) staging system.[6] Patients with stage IIIA (sentinel node tumor burden >1mm), IIIB and IIIC melanoma, according to the AJCC 7th edition, were included in the randomized clinical trials demonstrating improved outcomes for patients treated with adjuvant systemic therapy compared with placebo or ipilimumab.[1,3] Previous studies have examined the prognostic and predictive performance of additional clinicopathologic features, for example, age, Breslow thickness, ulceration, number of positive lymph nodes, and extracapsular extension, to improve the stratification for adjuvant therapy.[7-10] However, sensitive and specific tissue biomarkers have yet to be identified in stage III melanoma. Adding tissue biomarkers to these clinical characteristics, could lead to a more personalized approach by a more adequate selection of patients, whereby patients are selected to receive adjuvant therapy based on their risk of recurrence and the expected reduction in risk of recurrence from such therapy.

Unraveling the immunologic characteristics of the tumor microenvironment associated with recurrence may reveal insight into possible biomarkers.[11,12] Interferon-gamma (IFNγ) plays an important role in the antitumor response in the tumor microenvironment, and signatures related to IFNγ signaling were evaluated as possible biomarkers in previous studies. These studies have shown the IFNγ signature to be predictive of response in both patients with advanced melanoma treated with anti-PD-1 therapy and patients with stage III melanoma treated with combination ICI in a neoadjuvant setting.[13-15] Therefore we hypothesized that the IFNγ signature could also be useful as a prognostic and/or predictive marker in patients treated with adjuvant therapy.

The aim of this study was to identify biomarkers prognostic and predictive of recurrence after surgery for macroscopic stage III melanoma, both in patients who did and who did not receive adjuvant systemic therapy, in order to predict in whom adjuvant therapy should be omitted, either because of a very low risk of recurrence or due to a lack of benefit.

PATIENTS AND METHODS

Patients

Patients were selected from an ongoing institutional database and biobank study (collecting tumor material from patients with macroscopic stage III melanoma) at the Netherlands Cancer Institute (NKI). Patients included between October 2017 and June 2020 were eligible for selection, when naïve to systemic therapy, complete resection of macroscopic stage III melanoma was performed and sufficient tumor material was available for RNA isolation. Exception for complete resection was made for patients with in-transit metastases who underwent an isolated limb perfusion (ILP), alone or combined with lymph node dissection (LND). Patients with missing data on adjuvant therapy or follow-up were excluded.

Approval and reimbursement of adjuvant systemic therapy in the Netherlands started in December 2018, resulting in two cohorts of similar high-risk patients: prior to availability of adjuvant therapy (observation cohort 1) and thereafter (adjuvant treatment cohort 2) [**Fig. S1**]. Clinical data regarding patient and tumor characteristics, adjuvant therapy, and outcome were collected with a median follow-up of 24 months for both cohorts.

As shown by the screening failures in randomized trials evaluating adjuvant therapy[16], a proportion of patients will not receive adjuvant systemic therapy due to an early recurrence that occurs between surgery and start of adjuvant therapy. These patients were present in our study, as they represent patients faced in daily clinical practice and were therefore included in the clinical data analyses. However, in terms of translational research, analyses were performed for treatment groups: patients who did not (observation cohort) versus who did receive adjuvant therapy (cohort 1A and 2A for RNA sequencing data, cohort 1B and 2B for PD-L1 data, respectively). To perform an equal comparison, patients with early recurrence were excluded from these translational analyses in both groups [**Fig. S1**]. The definition of early recurrence is described in the Supplemental methods.

Tumor samples

Tumor samples were derived from surgical resection material in most patients and in some patients from biopsies prior to surgical procedures (eg, ILP). Per biobank protocol both fresh-frozen (FF) and formalin-fixed paraffin-embedded (FFPE) samples were stored, if feasible. From FF samples that contained sufficient tumor material based on the pathologist's scoring (at least 30% tumor cells of H&E stained cryostat frozen section), RNA was isolated using the AllPrep DNA/RNA/miRNA universal isolation kit (QIAgen, 80224) on the QIAcube, according to the manufacturer's protocol. Details on RNA sequencing can be found in the Supplemental methods.

Immunohistochemistry (IHC) of FFPE tumor samples was performed on a BenchMark Ultra (PD-L1 clone 22C3) or a Discovery Ultra (CD20-CD3 double stain) automated stainer (Ventana

Medical Systems). Detailed methods of IHC are described in the Supplemental methods. PD-L1 expression was scored by a pathologist in a blinded fashion: a score of >1% was considered to indicate PD-L1 positivity.[1,2] Image analysis of CD20-CD3 double-stained samples was performed using HALO software (Module Multiplex IHC v3.0.3). A MiniNet AI classifier was used to define tissue into tumor and non-tumor. Color thresholds were set for $CD20^+$ B cells (yellow), $CD3^+$ T cells (purple) and Melanin, and used in analysis for reliable detection. Cells were segmented using hematoxylin as a nuclear detection.

Validation cohorts

To validate the findings from our cohort, we performed analyses on two validation cohorts.

Validation cohort MIA

A cohort of 44 patients treated with adjuvant anti-PD-1 at Melanoma Institute Australia (MIA) between May 2015 and December 2018 was selected from a retrospective discovery cohort of patients with available resected stage III melanoma tissue.[17] FFPE tumor samples were collected for research purposes with Sydney Local Health District Human Ethics Review Committee approval (Protocol no X15-0454 & 2019/ETH06874 + X17-0312 & HREC/11/RPHA/32) and informed patient consent from the MIA Biospecimen Tissue Bank. RNA sequencing was performed at MIA, which is described in the Supplemental methods. Raw data were made available, on which the same analyses were performed as in the initial cohort.

Validation cohort UMCU

The second validation cohort of 25 patients consisted of melanoma patients treated at the University Medical Centre Utrecht (UMCU). This cohort, similar to the Netherlands Cancer Institute (NKI) study population, consisted of patients who did (n=11) and did not (n=11) receive adjuvant treatment after complete resection of stage III melanoma and patients with early recurrence (n=3). FFPE tumor samples were transported to the NKI, RNA was isolated and sent to CeGaT for sequencing. The same protocols for isolation, sequencing, and analyses were performed as on the initial cohort. In this validation cohort not all non-adjuvantly treated patients were from the preadjuvant treatment era (which is the case in the original study cohort). Patients who decided not to undergo adjuvant treatment despite available adjuvant therapy were included in this cohort.

Statistical analyses

Clinical data

Clinical data were analyzed using IBM SPSS Statistics, V. 27. Baseline and treatment characteristics were compared between patients who did not receive adjuvant systemic therapy (observation cohort 1) and patients who intended to receive adjuvant therapy (cohort 2). Follow-up, RFS and overall survival (OS) were analyzed using Kaplan-Meier estimates and a log-rank test was used to compare cohorts. Follow-up, RFS, and OS were defined as the time between surgery at the time of inclusion in the biobank and recurrence or death, respectively.

Patients not experiencing an event were censored at the time of last follow-up. Cox regression analyses were carried out; correlation was assessed by Spearman's rho test.

RNA sequencing analyses

The previously defined IFNγ[15], Danaher immune cell[18] and micro-environment cell population (MCP-counter)[19] gene expression signatures were analyzed. Cut-offs were calculated based on receiver operating characteristic (ROC) curves, using only observation patients (including patients with an early recurrence) to exclude a treatment effect on risk of disease recurrence. For the immune cell populations of both the Danaher and MCP-counter, z-score of the immune subsets was compared between the treatment groups. Bar and dot plots were generated in GraphPad Prism (V. 9.0.2).

RESULTS

The 98 patients in our discovery set were grouped into two cohorts: cohort 1 included 49 patients who did not receive adjuvant systemic therapy (observation); and cohort 2 included 49 patients who received adjuvant therapy, although some patients did not due to recurrence prior to the planned start (adjuvant intention) [**Fig. S1**]. Patients in cohort 2 were younger (*P*=0.027), more often had a tumor harboring a BRAF mutation (*P*=0.031) and a normal lactate dehydrogenase (LDH) level (*P*=0.009). Also, these patients more frequently underwent an LND only, instead of a LND combined with surgical treatment of in-transit metastases (*P*=0.002) [**Table 1**]. Patients were well balanced between the cohorts for sex and Breslow thickness. In both cohorts, the vast majority of patients had AJCC 8th edition stage IIIC melanoma and S100B levels below the upper limit of normal.

Table 1. Baseline characteristics of all patients intended for adjuvant therapy versus observation

Reported as number (%), percentages may not total 100 due to rounding.

	All patients ***N=98***	**Observation cohort 1** ***N=49***	**Adjuvant intention cohort 2** ***N=49***	***P* value**
Age				0.027
Median	63	69	59	
IQR	54-73	54-76	53-70	
Sex				0.306
Male	57 (58)	31 (63)	23 (47)	
Female	41 (42)	18 (37)	26 (53)	
Site primary				0.055
Extremities	50 (51)	31 (63)	19 (39)	
Trunk	41 (42)	15 (31)	26 (53)	
Head&neck	2 (2)	0	2 (4)	
Acral	1 (1)	0	1 (2)	
Mucosal	2 (2)	2 (4)	0	
MUP	2 (2)	1 (2)	1 (2)	

	All patients ***N=98***	**Observation cohort 1** ***N=49***	**Adjuvant intention cohort 2** ***N=49***	***P* value**
Breslow thickness				0.369
≤1.0mm	7 (7)	2 (4)	5 (10)	
1.01-2.0	25 (26)	13 (27)	12 (25)	
2.01-4.0	37 (38)	21 (43)	16 (33)	
>4.0	21 (21)	8 (16)	13 (27)	
Unknown	8 (8)	5 (10)	3 (6)	
Ulceration				0.067
No	53 (54)	21 (43)	32 (65)	
Yes	29 (30)	17 (35)	12 (25)	
Unknown	16 (16)	11 (22)	5 (10)	
Stage (AJCC 8th edition)				0.718
IIIB	14 (14)	5 (10)	9 (18)	
IIIC	78 (80)	41 (84)	37 (76)	
IIID	4 (4)	2 (4)	2 (4)	
Unknown	2 (2)	1 (2)	1 (2)	
Mutation status				0.031
BRAF	59 (60)	24 (49)	35 (71)	
NRAS	24 (25)	12 (25)	12 (25)	
cKIT	1 (1)	1 (2)	0	
No driver mutations	10 (10)	8 (16)	2 (4)	
Unknown	4 (4)	4 (8)	0	
Type of surgery				0.002
LND	54 (55)	18 (37)	36 (74)	
ITM	20 (20)	13 (27)	7 (14)	
LND + ITM	10 (10)	6 (12)	4 (8)	
ILP ± LND	14 (14)	12 (25)	2 (4)	
S100b				0.905
≤ULN	81 (83)	39 (80)	42 (86)	
>ULN	12 (12)	6 (12)	6 (12)	
Unknown	5 (5)	4 (8)	1 (2)	
LDH				0.009
≤ULN	85 (87)	38 (78)	47 (96)	
>ULN	6 (6)	6 (12)	0	
Unknown	7 (7)	5 (10)	2 (4)	

IQR: interquartile range; MUP: melanoma of unknown primary; LND: lymph node dissection; ITM: in-transit metastasis; ILP: isolated limb perfusion; ULN: upper limit of normal.

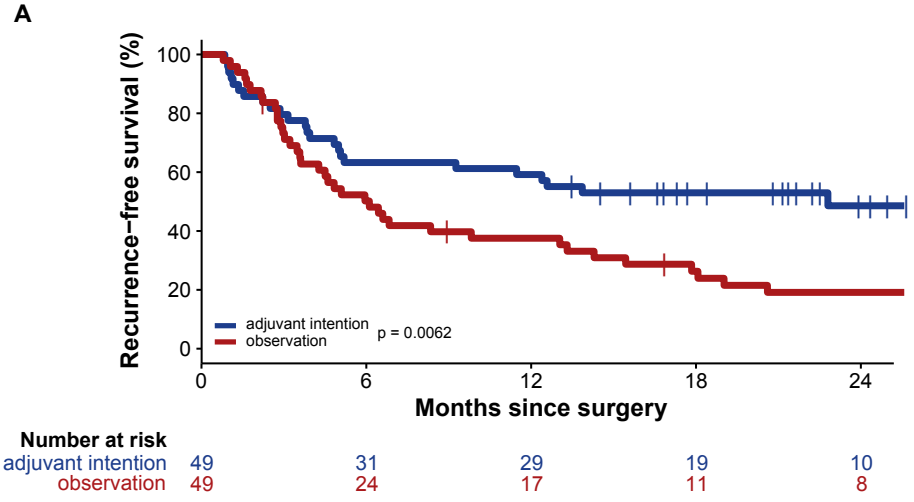
In cohort 2, 10 patients (20%) developed a recurrence before start of adjuvant therapy and therefore 39 patients received at least one dose of adjuvant therapy. One patient initially started adjuvant BRAF-targeted therapy due to temporarily adjusted logistics following the outbreak of the COVID-19 pandemic, but switched to anti-PD-1 because of toxicity. All other patients received adjuvant anti-PD-1. The median time between surgery until the start of adjuvant therapy was 9 weeks (IQR 8-12) [**Table S1**]. At data cut-off, all patients had ceased adjuvant therapy. The main reason for cessation was end of treatment (56%), followed by

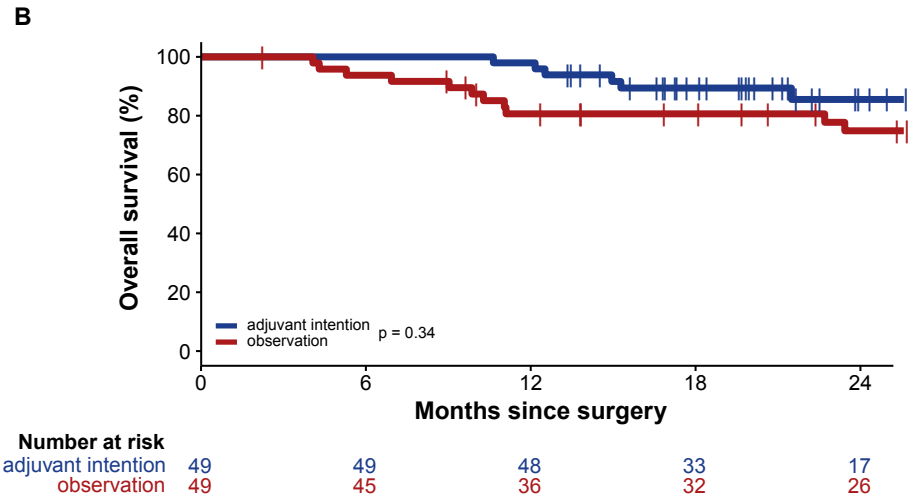
recurrence (26%). Due to the outbreak of the COVID-19 pandemic, 15 patients (39%) had a break in their adjuvant regimen, skipping one or two cycles of treatment.

Median follow-up of the total patient cohort was 26 months (95% CI 23-28). After a median follow-up of 28 months (95%CI 26-30) in cohort 1, median RFS was 6 months (95%CI 4-8). After a median follow-up of 22 months (95%CI 19-25), the median RFS of 23 months (95%CI not reported (NR)) was significantly longer in cohort 2 (*P*=0.006) [**Fig. 1A**]. At 24-months, the RFS rate was 19% for cohort 1 and 49% for cohort 2 (*P*=0.008). Univariable Cox regression analyses for RFS [**Table S2**] demonstrated, besides adjuvant therapy, significance for type of surgery and S100b levels at baseline, but not for LDH levels. In multivariable analyses S100b and adjuvant therapy remained significant contributors.

Median OS was not reached in both cohorts [**Fig 1B**). At 12-months, there was a significant difference in OS rate in favor of adjuvant therapy (81% vs. 98%, *P*=0.006). The 24-months OS rate was 75% vs. 86% in cohort 1 and cohort 2, respectively (*P*=0.158).

Figure 1. Survival curves





A. Recurrence-free survival for patients intended for adjuvant therapy versus observation.
B. Overall survival for patients intended for adjuvant therapy versus observation.

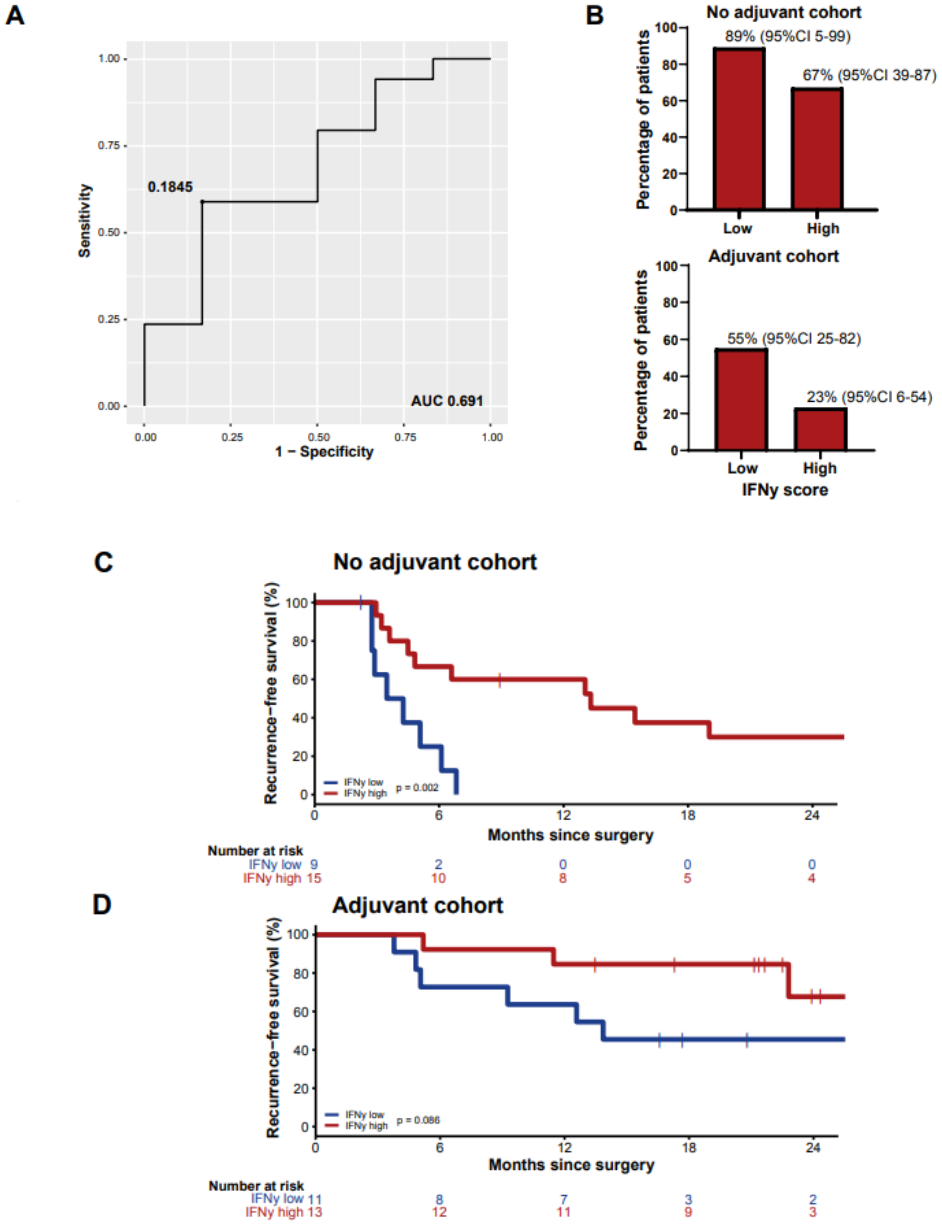
IFNγ score

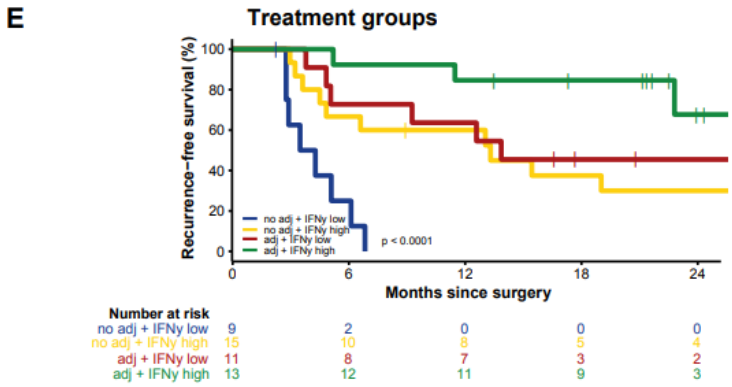
RNA sequencing data were available for the subset of patients in the discovery set of whom RNA was of sufficient quality for sequencing: 24 observation patients (cohort 1A) and 24 adjuvant treatment patients (cohort 2A) [**Fig. S1**]. To perform an equal comparison, patients with early recurrence were excluded from these translational analyses in both groups. Of the patients in cohort 1A, 18 patients had a recurrence, compared with 9 patients in cohort 2A. Baseline characteristics for these cohorts are described in **Table S3**.

The optimal cut-off for the IFNγ score was determined at 0.1845 with a ROC curve, with an area under the curve (AUC) of 0.691 [**Fig. 2A**]. In cohort 1A, 9 patients had low IFNγ score of whom 8 (89%, 95%CI 5-99) had a recurrence, and 15 had a high IFNγ score of whom 10 (67%, 95%CI 39-87) had disease recurrence. In cohort 2A, recurrences occurred in 6/11 patients (55%, 95%CI 25-82) with low IFNγ score and in 3/13 (23%, 95%CI 6-54) patients with high IFNγ score [**Fig. 2B**]. In the patients with an early recurrence, more patients had a low IFNγ score (12 vs. 4 with a high IFNγ score) [**Fig. S2**].

In both cohort 1A (median follow-up 27 months) and cohort 2A (median follow-up of 22 months), a longer RFS for patients with high IFNγ scores was observed, although not statistically significant in the adjuvant cohort [**Fig. 2C-D**]. RFS was significantly improved by adjuvant systemic therapy compared with observation, both in patients with high and especially in patients with low IFNγ score (P<0.001) [**Fig. 2E**]. Patients with low IFNγ score had a median RFS of 4 months (95%CI 2-5) in the observation cohort, which increased to 13 months (95%CI 2-25) in patients receiving adjuvant therapy. In patients with high IFNγ scores, median RFS was 14 months (95%CI NR) versus not reached for patients receiving adjuvant therapy.

Figure 2. Interferon-gamma





A. ROC curve determining the cut-off for the IFNγ score.
B. Bar plots with percentage of patients with recurrence, split for low and high IFNγ score.
C. Recurrence-free survival for patients with low versus high IFNγ score in the no adjuvant cohort.
D. Recurrence-free survival for patients with low versus high IFNγ score in the adjuvant cohort.
E. Recurrence-free survival for patients with low versus high IFNγ score for both the no adjuvant and adjuvant cohort.
IFNγ: interferon-gamma; adj: adjuvant.

IFNγ score was an independent prognostic parameter, as it did not correlate with other known prognostic clinical factors such as T stage, staging according to AJCC 8th edition, Breslow thickness, ulceration and S100B and LDH levels at day of surgery [**Table S4**]. In addition, the observed differences between the cohorts for age, mutation status and surgical treatment were not correlated with IFNγ score either.

Immune cell infiltration

The subsets of immune cells for both the Danaher signature[18] and MCP-counter[19] did not show significant differences between patients with and without a recurrence within the two treatment cohorts 1A and 2A [**Fig. S3 + S4**]. However, full cohorts 1 and 2, including the patients with an early recurrence, demonstrated a difference (*P*=0.014) for the B cell subset within the Danaher signature. Therefore, we decided to expand analyses with the B cell score.

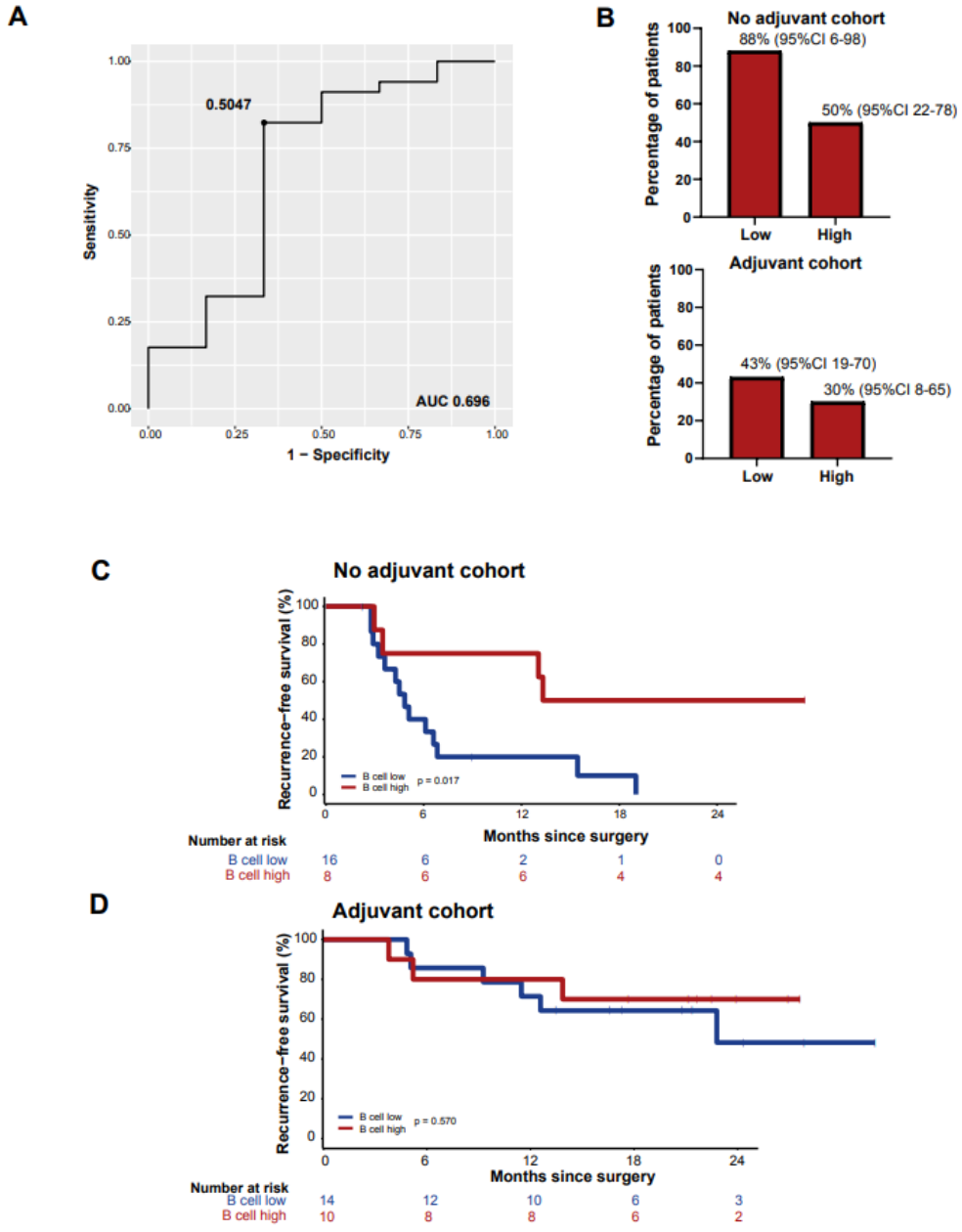
B cell score

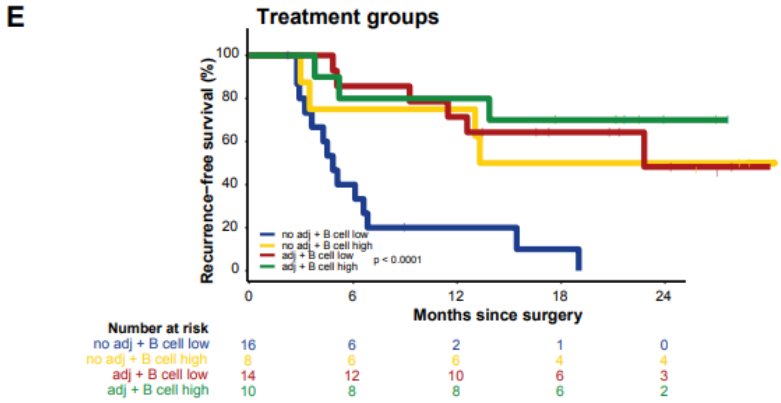
Similar to the IFNγ score, we determined the optimal cut-off at 0.5047 for a low and high B cell score using a ROC curve, with an AUC of 0.696 [**Fig. 3A**]. In cohort 1A, 16 patients with a low B cell score were identified, of whom 14 (88%, 95%CI 6-98) had a recurrence versus 4/8 patients (50%, 95%CI 22-78) with a high B cell score. In cohort 2A, 6/14 patients (43%, 95%CI 19-70) with a low B cell score had a recurrence, versus 3/10 (30% 95%CI 8-65,) of the patients with a high B cell score [**Fig. 3B**]. In the patients with an early recurrence, more patients had a low B cell score (10 vs. 6 with a high B cell score) [**Fig. S5**].

In cohort 1A, RFS was longer in patients with a high B cell score [**Fig. 3C]**. In cohort 2A, there was no difference in RFS [**Fig. 3D]**. For patients of cohort 1B, median RFS was 5 months

(95%CI 4-NR) in patients with low and 13 months (95%CI 13-NR) in patients with high B cell score. Patients with low B cell score had a median RFS of 23 months (95%CI 13-NR) versus not reached for patients with a high score receiving adjuvant therapy [**Fig 3E**].

Figure 3. B cell





A. ROC curve determining the cut-off for the B cell score.
B. Bar plots with percentage of patients with recurrence, split for low and high B cell score.
C. Recurrence-free survival for patients with low versus high B cell score in the no adjuvant cohort.
D. Recurrence-free survival for patients with low versus high B cell score in the adjuvant cohort.
E. Recurrence-free survival for patients with low versus high B cell score for both the no adjuvant and adjuvant cohort.
adj: adjuvant.

To validate if the B cell score based on RNA sequencing data corresponded with B cell presence in the tumor, an IHC CD3/CD20 staining was performed and scored on 46/48 samples used for RNA sequencing (2 samples were not scored due to lack of tumor). In cohort 1A (n=23), the percentage $CD20^+$ cells in tumor region of patients with recurrence was lower, but higher in the stroma region. In cohort 2A (n=23), there was a minimal difference in percentage $CD20^+$ cell in the tumor region, but this was higher for patients without recurrence in the stroma region [**Fig. S6A**]. The B cell score correlated strongly with CD20 staining, the strongest correlation (P<0.001) was seen for the $CD20^+$ cells in the tumor area [**Fig. S6B**].

The Danaher T cell score correlated strongly (p<0.001) with the CD3 staining as well [**Fig S6C**]. As the B cell score and IFNγ score were strongly correlated (P<0.001), these are not independent markers for recurrence [**Fig. S6D**].

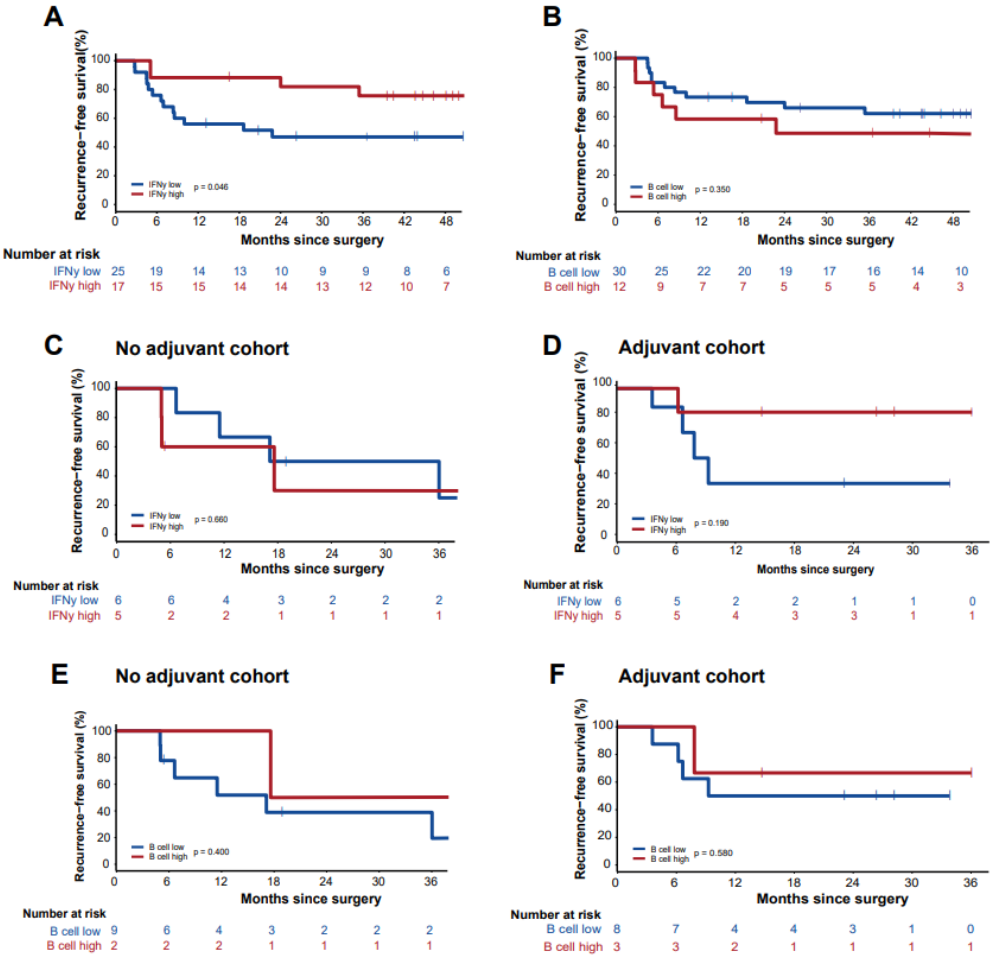
Validation cohorts

We used two validation cohorts to further test our determined cut-offs. Validation cohort MIA consisted of 44 patients treated with adjuvant therapy: 17 had a high and 27 had a low IFNγ score, 36 had a high and 8 had a low B cell score. After a median follow-up of 46 months (95%CI 44-53), RFS was significantly longer for patients with high IFNγ score compared with those with a low score (P=0.046) [**Fig. 4A**], but no difference in RFS was observed according to the B cell score [**Fig. 4B**].

For validation cohort UMCU, in line with our own cohort, three patient groups were determined: patients not receiving adjuvant therapy (n=11), patients receiving adjuvant therapy (n=11) and patients with early recurrence (n=3). Eleven patients had high and 14 had

a low IFNγ score, 7 had high and 18 had a low B cell score. In both the no adjuvant and the adjuvant cohort, no difference in RFS was seen for the IFNγ [**Fig. 4C+D**] or B cell score [**Fig 4E+F**].

Figure 4. Validation cohorts



A. Recurrence-free survival of patients of validation cohort MIA with a low versus high IFNγ score.

B. Recurrence-free survival of patients of validation cohort MIA with a low versus high B cell score.

C. Recurrence-free survival of patients of validation cohort UMCU with a low versus high IFNγ score in the no adjuvant cohort.

D. Recurrence-free survival of patients of validation cohort UMCU with a low versus high IFNγ score in the adjuvant cohort.

E. Recurrence-free survival of patients of validation cohort UMCU with a low versus high B cell score in the no adjuvant cohort.

F. Recurrence-free survival of patients of validation cohort UMCU with a low versus high B cell score in the adjuvant cohort.

PD-L1

PD-L1 staining was performed and scored on 73 tumor samples of the 98 patients in the discovery set; 28 from observation cohort 1 (cohort 1B), 28 from adjuvant treatment cohort 2 (cohort 2B) and 16 patients with an early recurrence [**Fig. S1**]. Samples of 24 patients were not scored due to either not enough tumor in the stained slide or too much pigment present for reliable scoring). Baseline characteristics are displayed in **Table S5**.

For patients who received adjuvant therapy (cohort 2B), 23 (82%) had a high and 5 (18%) had a low PD-L1 score. Fewer patients with a positive PD-L1 score developed a recurrence compared to patients with a PD-L1 negative score (30%, 95%CI 14-53 vs. 80%, 95%CI 30-99). In cohort 1B, this was not as distinct: 71% (95%CI 48-88) of 21 patients with PD-L1 positive score developed a recurrence versus 86% (95%CI 42-99) of 7 patients with a PD-L1 negative score [**Fig. S7A**]. In cohort 1B, 21 patients (75%) had high and 7 (25%) had low PD-L1 score. In the patients with an early recurrence, more patients scored negative for PD-L1 (9 vs. 7 patients with PD-L1 positive score) [**Fig. S7B**].

After a median follow-up of 28 months (95%CI 25-30), median RFS did not differ for patients with PD-L1 positive score versus PD-L1 negative score in cohort 1B (P=0.285) [**Fig. S7C**]. In cohort 2B, RFS was significantly longer (P=0.032) for patients with PD-L1 positive tumors (median RFS not reached), versus 13 months (95%CI 0-29) for PD-L1 negative tumors [**Fig. S7D**]. Both PD-L1 low and PD-L1 high cohorts had an improved RFS when receiving adjuvant therapy in the treatment cohorts [**Fig. S7E**].

DISCUSSION

Our study, focusing on identifying biomarkers for adjuvant treatment selection in macroscopic stage III melanoma, demonstrated IFNγ to be of prognostic value. The lack of predictive value, however, limits its sole use in clinical decision making.

Furthermore, our study demonstrated a significant RFS benefit for adjuvant anti-PD-1 therapy. These real-world data confirm the improved RFS seen with pembrolizumab in the EORTC-1325/Keynote-054 and nivolumab in the Checkmate-238 trials.[1,2] Additionally, our study showed a significantly better 1-year and numerically higher (although not statistically significant) 2-year OS rate for adjuvant therapy, in slightly imbalanced cohorts with a possible selection bias due to the retrospective nature of the study. This is in line with data from the EORTC-1325/Keynote-054 and Checkmate-238 trial, which as of last analyses have not shown an OS benefit.[20]

As reflected in the number needed to treat five for adjuvant anti-PD-1, there is a clear need to identify patients with a higher risk of disease recurrence and a higher chance of benefit of anti-PD-1 prior to commencing adjuvant therapy, as well as those with lower risk and lower benefit. Accurate biomarkers would enable patient selection, so only patients truly in need and with high chance of treatment benefit would receive adjuvant therapy. Furthermore, patients destined to recur despite adjuvant anti-PD-1 therapy could be directed to alternative adjuvant therapies such as BRAF/MEK inhibitors or novel clinical trials, while those would never recur after surgery regardless of any adjuvant therapy can be spared treatment and the risk of toxicities altogether.

PD-L1 has previously been shown to correlate with response and outcome in advanced melanoma and non-small-cell lung cancer, therefore PD-L1 was analyzed in our cohorts.[21-26] PD-L1 did show a predictive value in our study, although this should be interpreted with caution as the PD-L1 low group consisted of very few patients. Our results are in line with previous data: in the EORTC-1325/Keynote-054 trial, in both the PD-L1 positive and negative subgroups, adjuvant pembrolizumab demonstrated a significantly longer RFS than placebo.[1] In the Checkmate-238 trial, RFS was longer for patients with PD-L1 ≥1% treated with either adjuvant nivolumab or adjuvant ipilimumab.[2] Expression of PD-L1 is a controversial biomarker, as both trials demonstrated activity of ICI in PD-L1 low tumors. Moreover, the assessment of PD-L1 is heterogeneous because of different assays used, different cut-offs, and the staining is subject to interobserver variability.

We have investigated the IFNγ signature as it is known to be predictive of response of neoadjuvant combination ICI in macroscopic stage III melanoma.[13-15] In the discovery cohort, IFNγ was an independent prognostic marker in both patients who were and were not treated with adjuvant anti-PD-1 therapy, as patients with low IFNγ score have an inferior RFS compared with patients with high IFNγ score in both cohorts. Comparable, in the COMBI-AD trial, an IFNγ signature score above median was prognostic for prolonged RFS in both patients treated with adjuvant dabrafenib plus trametinib and placebo.[27] In the Checkmate-915, patients receiving adjuvant nivolumab ± ipilimumab with an IFNγ score above median had a longer RFS than patients with IFNγ score below median.[28] Additionally, the Checkmate-238 trial demonstrated favorable RFS and OS outcomes in IFNγ high tumors in both patients treated with adjuvant nivolumab or ipilimumab.[29] In the Checkmate-76K trial of adjuvant nivolumab versus placebo in resected stage IIB/C melanoma, IFNγ was shown to be both prognostic (IFNγ high associated with prolonged RFS regardless of therapy) and predictive (IFNγ high associated with prolonged RFS within the nivolumab group).[30] We are the first, however, to use a cut-off based on a cohort of patients with untreated stage III melanoma and to study the risk of disease recurrence, uninfluenced by treatment effects, since above mentioned trials did not use the cut-off of the placebo group in treatment groups. Our data suggest that patients with low IFNγ score benefit from adjuvant therapy especially: without adjuvant therapy, nearly all develop a disease recurrence within 6 months of surgery, and with

adjuvant treatment RFS is improved to the level of IFNγ high patients not receiving adjuvant therapy. This was also shown in the COMBI-AD trial.[27] However, both the IFNγ low and high groups in our cohort have RFS benefit from adjuvant anti-PD-1 therapy, which makes IFNγ a prognostic and less a predictive biomarker. In the neoadjuvant OpACIN-neo trial, the IFNγ signature did show strong predictive value as it was associated with pathologic response to neoadjuvant ipilimumab plus nivolumab, and responding patients had a significantly longer RFS than patients without pathologic response.[14]

As IFNγ was a strong prognostic factor, we explored possible additional biomarkers. B cells were the strongest marker we could find of the subsets of immune cells defined by Danaher[18] and MCP counter[19], but the IFNγ and B cell scores were strongly correlated with one another in our cohort. Thus, adding B cell analyses to the IFNγ score did not provide an additional predictive effect. It has been shown that B cells can facilitate an antitumor response by releasing pro-inflammatory cytokines, such as IFNγ.[31] This could partly explain the correlation found.

Unfortunately, the validation cohorts lacked similar patient cohorts or patient numbers to validate our data. Validation cohort MIA did show a longer RFS in IFNγ high patients, but consisted only of patients who had received adjuvant therapy. B cell score was not a significant prognostic factor in this cohort. Validation cohort UMCU did have similar patient groups, but the patient numbers were very low and no differences were seen in RFS for both IFNγ and B cell scores. Additionally, this validation cohort included patients deciding not to receive adjuvant therapy, despite availability. This may be based on, for example, comorbidities or risk of recurrence, and may therefore partly explain differences in results.

To our knowledge, our study is the first to use biomarkers with a cut-off identified on a patient population naïve for systemic therapy, in both patients treated with and without adjuvant systemic therapy in the setting faced in daily clinical practice. However, limitations of our study are the retrospective design, the limited patient numbers both in the original dataset, and even more distinctly, in the validation cohorts. Due to the retrospective design, cohorts were defined by the timeframe in which patients were treated, rather than by randomization. This may account for the imbalances between the treatment cohorts as previously described and therefore limits the definitive conclusions drawn from our study.

Another important observation is the rate of early recurrences after surgery, 20% within 12 weeks, usually on the first postoperative scan prior to commencement of adjuvant systemic therapy. This is in line with the previous observations of Bloemendal et al.[16] and is an important consideration when comparing data from neoadjuvant and adjuvant trials, as none of the adjuvant trials have included these early recurrences, because they were considered screen failures. All patients with such aggressive biology are included in neoadjuvant trials and this makes the results of the neoadjuvant SWOG-1801 trial that much more impressive.[32]

In conclusion, we demonstrated that both the IFNγ and B cell scores have prognostic value in stage III melanoma, but we failed to find a single strong predictive biomarker of response. Our study confirmed the high rate of early recurrences in patients with high-risk stage III melanoma, who are intended to start adjuvant therapy. We confirmed the RFS benefit of adjuvant anti-PD-1 in melanoma versus observation and showed at least a numerical OS benefit when looking at all patients.

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PART III

Population-based studies

Chapter 6

Surgery for unresectable stage IIIC and IV melanoma in the era of new systemic therapy

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ABSTRACT

Opportunities for surgical treatment in metastatic melanoma patients have re-emerged due to the development of novel systemic therapeutics over the past decade. The aim of this study is to present data on outcomes of surgery in patients with unresectable stage IIIC and IV melanoma, who have previously been treated with immunotherapy or targeted therapy. Data was extracted from the Dutch Melanoma Treatment Registry (DMTR) on 154 patients obtaining disease control to systemic therapy and undergoing subsequent surgery. Disease control was defined as a complete response (CR), which was seen in 5.2% of patients; a partial response (PR), seen in 46.1% of patients; or stable disease (SD), seen in 44.2% of patients. At a median follow-up of 10.0 months (interquartile range 4-22) after surgery, the median overall survival (OS) had not been reached in our cohort and median progression free survival (PFS) was 9.0 months (95% CI 6.3-11.7). A CR or PR at first follow-up after surgery was associated with both a better OS and PFS compared to stable or progressive disease ($p<0.001$). We conclude that selected patients can benefit from surgery after achieving disease control with systemic therapy.

Keywords: Metastatic melanoma; surgery; systemic therapy; Dutch Melanoma Treatment Registry

INTRODUCTION

Historically the prognosis of patients with unresectable stage III and IV melanoma has been poor, with a median overall survival (OS) of only 6.2 months[1,2]. Some patients with oligometastatic melanoma (up to 3 lesions) can be treated by surgery and achieve long-term survival of around 40% at 5 years, especially those with only 1 lesion and a long interval between their melanoma and the development of stage IV disease[3,4]. However, it is difficult to select the patients that would benefit from such surgery.

Over the past decade the development of new systemic options has drastically changed the treatment of metastatic melanoma and therefore the prognosis of these patients. Immune checkpoint inhibitors (ICI) targeting PD-1 and CTLA-4, either as monotherapy or combined treatment, have shown response rates of approximately 40 to 60% respectively, improving progression free and overall survival[5-18]. BRAF and MEK inhibitors in patients with BRAF mutated melanoma have even higher overall response rates (70%), but fewer durable responses due to the development of resistance[19-25]. This evolution in therapeutic options has also presented new opportunities for surgery in this group of patients.

In some patients experiencing a durable partial response on systemic therapy, resection of remaining lesion(s) can contribute in obtaining a complete response. Additionally, in patients with a partial or complete response developing oligoprogression, resection of the progressive lesion(s) may be performed. However, although these surgeries are already being performed in clinical practice, little evidence has been presented to support this treatment approach.

The aim of this population-based study is to present data on the incidence and outcomes of surgery in patients with unresectable stage III and IV melanoma, who have been treated with immunotherapy or targeted therapy (TT) prior to surgery (no first line surgery included), to provide insight in which patients may benefit from surgery after obtaining disease control with systemic therapy.

PATIENTS AND METHODS

Data were retrieved from the Dutch Melanoma Treatment Registry (DMTR). In this nationwide prospective database, all Dutch patients undergoing treatment for unresectable stage IIIC and IV melanoma are included. This registry was set up to monitor the safety and outcomes of the novel treatments[26]. Registration in the DMTR is a prerequisite for reimbursement, assuring a nationwide coverage. In the current study we included patients from the database who had commenced treatment between the start of the registry (July 2012) and July 2017 to assure sufficient follow-up at data extraction in April 2018.

Patients

Patients who had surgery after obtaining disease control with systemic therapy were selected from the registry. Disease control was defined as stable disease (SD), partial response (PR) or complete response (CR) as the best response to systemic therapy. These responses were non-confirmed investigator-assessed responses, retracted from follow-up data registered in the database, therefore this cannot be considered the same as RECIST measured responses. Progressive disease (PD) was allowed as a most recent status of disease prior to surgery, if these patients initially had a SD, PR or CR as their best response. Patients who had primarily progressive disease and underwent surgery were excluded, as we considered that this would include a substantial number of palliative surgery for symptomatic patients, which was not the focus of this study.

Patients with uveal and mucosal melanoma were excluded, since these subtypes differ in biologic behavior and in their responses to immunotherapy and targeted therapy. Also, patients presenting with brain metastases were excluded from this study, since these patients generally have a different prognosis.

Statistical analysis

Data were analyzed using IBM SPSS Statistics, version 25. Descriptive statistics were used to assess patient, tumor, systemic therapy, surgery and follow-up characteristics. Baseline for patient and tumor characteristics was set at start of systemic therapy. Characteristics of patients treated with ICI were compared to patients treated with targeted therapy using the chi-square test (categorical variables) and t-test (continuous variables). Kaplan-Meier methods and Log-rank tests were applied to calculate and compare progression free survival (PFS) and OS and cox-regression models were used to analyze the influence of different variables. Variables that were (borderline) significant in the univariate analyses (and consisted of sufficient patient numbers) were used in the multivariate cox-regression model. PFS and OS were defined as time between surgery and first disease progression or death, respectively. Patients not experiencing an event were censored at the time of last follow-up.

RESULTS

Patient and tumor characteristics

At the time of data extraction, the DMTR database consisted of 3959 patients, of whom 876 had undergone surgery during their treatment and 463 patients received systemic treatment prior to surgery. After selecting patients obtaining disease control (SD/PR/CR) with systemic therapy, 154 patients remained. Baseline characteristics of these 154 patients and the treatment they received are listed in table 1. The median age of our study population was 58, ranging from 24 to 87. The vast majority of patients had a good performance score, WHO 0 or 1, (91.6%) and a normal lactate dehydrogenase (LDH) level (74.7%), which is a known prognostic factor for survival in metastatic melanoma patients[27]. The percentage of patients with a BRAF mutation was slightly higher than usual in the general patient population (68.8%), most likely due to the selection for response to systemic treatment. Most patients presented with distant metastases: 44.2% with distant metastases only and 34.4% with both distant and locoregional metastases compared to 21.4% of patients presenting with only locoregional metastases. A substantial proportion of patients presenting with distant metastases, had nodal or subcutaneous metastases: 58.2% and 37.2% respectively. The number of metastases before start of systemic treatment was poorly documented, with missing data in 37% of patients, but most patients presented with multiple (>10) lesions.

No differences were seen in baseline characteristics between patients treated with immunotherapy and targeted therapy, except for the location of the metastases (table 1). More patients treated with targeted therapy had locoregional metastases only, compared to patients treated with ICI (31.1% versus 11.4%, p=0.007), of whom a larger percentage were treated for distant metastases (53.2% versus 32.8%).

Table 1. Patient, tumor and treatment characteristics

	Total (n=154)	ICI (n=79)	TT (n=61)	
Characteristic	**n (%)**	**n (%)**	**n (%)**	**P***
Age; years				0.452
Median	58	62	59	
Range	24-87	24-80	34-87	
Sex				0.444
Female	74 (48.1)	35 (44.3)	31 (50.8)	
Male	80 (51.9)	44 (55.7)	30 (49.2)	
WHO performance status				0.543
0	101 (65.6)	52 (65.8)	38 (62.3)	
1	40 (26.0)	21 (26.6)	16 (26.2)	
2	4 (2.6)	3 (3.8)	1 (1.6)	
3	1 (0.6)	0 (0.0)	1 (1.6)	
Unknown	8 (5.2)	3 (3.8)	5 (8.2)	

	Total (n=154)	ICI (n=79)	TT (n=61)	
Characteristic	**n (%)**	**n (%)**	**n (%)**	**P***
Location primary				0.431
Extremity	61 (39.6)	32 (40.5)	23 (37.7)	
Trunk	45 (29.2)	20 (25.3)	20 (32.8)	
Head/Neck	10 (6.5)	4 (5.1)	6 (9.8)	
Acral	9 (5.8)	4 (5.1)	4 (6.6)	
Unknown primary	27 (17.5)	17 (21.5)	8 (13.1)	
Unknown	2 (1.3)	2 (2.5)	0 (0.0)	
Type				0.637
Superficial spreading	63 (40.9)	27 (34.2)	28 (45.9)	
Nodular	27 (17.5)	13 (16.5)	10 (16.4)	
Acral lentiginous	8 (5.2)	5 (6.3)	3 (4.9)	
Lentigo maligna	1 (0.6)	0 (0.0)	1 (1.6)	
Desmoplastic	1 (0.6)	1 (1.3)	0 (0.0)	
Other	5 (3.2)	4 (5.1)	1 (1.6)	
Unknown	20 (13.0)	10 (12.7)	10 (16.4)	
Missing (MUP)	29 (18.8)	19 (24.1)	8 (13.1)	
Breslow thickness				0.788
≤1.0 mm	6 (3.9)	2 (2.5)	3 (4.9)	
1.1-2.0 mm	0 (0.0)	0 (0.0)	0 (0.0)	
2.1-4.0 mm	5 (3.2)	2 (2.5)	3 (4.9)	
>4.0 mm	15 (9.6)	5 (6.3)	6 (9.8)	
Unknown	128 (83.1)	70 (88.6)	49 (80.3)	
Ulceration				0.639
Yes	38 (24.7)	18 (22.8)	13 (21.3)	
No	58 (37.7)	26 (32.9)	28 (45.9)	
Unknown	58 (37.7)	35 (44.3)	20 (32.8)	
Location metastases				0.007
Locoregional	33 (21.4)	9 (11.4)	19 (31.1)	
Distant	68 (44.2)	42 (53.2)	20 (32.8)	
Both	53 (34.4)	28 (35.4)	22 (36.1)	
Number of metastases				0.614
1 lesion	10 (6.5)	6 (7.6)	3 (4.9)	
2-5 lesions	21 (13.6)	13 (16.4)	6 (9.8)	
6-10 lesions	3 (1.9)	3 (3.8)	0 (0.0)	
>10 lesions	63 (40.9)	35 (44.3)	24 (39.3)	
Unknown	57 (37.0)	22 (27.8)	28 (45.9)	
BRAF-mutation				<0.001
Present	106 (68.8)	36 (45.6)	61 (100.0)	
Absent	45 (29.2)	41 (51.9)	0 (0.0)	
Unknown	3 (1.9)	2 (2.5)	0 (0.0)	
LDH				0.095
≤ULN	115 (74.7)	62 (78.5)	43 (70.5)	
>ULN (>250 U/L)	34 (22.1)	14 (17.7)	18 (29.5)	
Unknown	5 (3.2)	3 (3.8)	0 (0.0)	

	Total (n=154)	ICI (n=79)	TT (n=61)	
Characteristic	**n (%)**	**n (%)**	**n (%)**	**P***
S100				0.328
≤ULN	40 (26.0)	21 (26.6)	18 (29.5)	
>ULN (>0,10 ug/L)	59 (38.3)	28 (35.4)	27 (44.3)	
Unknown	55 (35.7)	30 (38.0)	16 (26.2)	
Sequence systemic therapy				0.006
First Line	107 (69.5)	47 (59.5)	49 (80.3)	
Second line	29 (18.8)	23 (29.1)	5 (8.2)	
Third line	11 (7.1)	4 (5.1)	6 (9.8)	
≥ Fourth line	7 (4.5)	5 (6.3)	1 (1.6)	
Type systemic therapy				
ICI	79 (51.3)			
Targeted therapy	61 (39.6)			
Other/unknown	14 (9.1)			
Best response to systemic therapy				0.027
Stable disease	68 (44.2)	33 (41.8)	27 (44.3)	
Partial response	71 (46.1)	42 (53.2)	25 (41.0)	
Complete response	5 (5.2)	3 (3.8)	1 (1.6)	
Unknown	9 (5.8)	1 (1.3)	8 (13.1)	
Status of disease prior to surgery				0.007
Progressive disease	71 (46.1)	45 (57.0)	20 (32.8)	
Stable disease	45 (29.2)	19 (24.1)	19 (31.1)	
Partial response	29 (18.8)	14 (17.7)	15 (24.6)	
Unknown	9 (5.8)	1 (1.3)	7 (11.5)	
Location surgery				0.685
(Sub)cutaneous/LN	127 (82.5)	56 (77.8)	46 (80.7)	
Visceral	27 (17.5)	16 (22.2)	11 (19.3)	
Complication surgery				0.029
None	122 (79.2)	67 (87.0)	42 (68.9)	
Transient	22 (14.3)	6 (7.8)	15 (24.6)	
Requiring intervention	7 (4.5)	4 (5.2)	3 (4.9)	
Permanent damage	1 (0.6)	0 (0.0)	1 (1.6)	
Death	0 (0.0)	0 (0.0)	0 (0.0)	
Status of disease at first follow-up after surgery				0.459
Progressive disease	26 (16.9)	12 (15.4)	11 (18.0)	
Stable disease	49 (31.8)	28 (35.9)	16 (26.2)	
Partial response	18 (11.7)	12 (15.4)	6 (9.8)	
Complete response	49 (31.8)	22 (28.2)	25 (41.0)	
Unknown	12 (7.8)	4 (5.1)	3 (4.9)	

*Difference between group of patients treated with ICI versus patients treated with TT

Treatment

Surgery was performed after the first line of systemic treatment in 69.5% of patients. Little over half of patients (51.3%) were treated with ICI, 39.6% with targeted therapy, and 9.1% of patients with other treatment (in trials) or the given treatment was unknown. Of patients with a BRAF mutation, the majority received targeted therapy (57.5%), the remainder received either immunotherapy (34.0%) or other treatment (8.5%). Patients receiving immunotherapy were roughly evenly divided between anti-PD1 directed therapy (48.7%) and anti-CTLA4 therapy (43.6%) and only a small percentage (7.7%) were treated with combination ICI. Of patients receiving targeted therapy about half (50.8%) were treated with a BRAF inhibitor alone and in the remaining patients (49.2%) it was combined with a MEK inhibitor.

Response

Best response

Only a very small proportion of patients (3.2%) achieved a complete response as best response to systemic treatment prior to surgery and the fractions of patients obtaining a partial response and stable disease as a best response were similar (46.1% and 44.2%).

Most recent disease status prior to surgery

The most recently reported status of disease prior to surgery was PD in 46.1% of patients, versus 29.2% of patients with SD and 18.8% with a PR before surgery. As shown above: the best response to systemic therapy was not necessarily the same as the most recent status of disease prior to surgery. For example, if a patient had a CR upon systemic therapy, but developed a recurrence and was operated for this lesion in due course. Then the best response was CR, but the most recent status of disease prior to surgery was classified as PD. In the vast majority of patients subcutaneous (39.6%) or lymph node (42.9%) metastases were resected and few serious complications occurred.

First evaluation after surgery

In total, 31.8% of patients achieved a complete response at the first new evaluation after surgery, but 16.9% of patients had progressive disease at first follow-up after surgery. A summary of all responses is shown in supplementary figure S1.

Survival outcomes

At a median follow-up of 10.0 months (interquartile range 4-22) after surgery, the median OS had not been reached in our cohort (1-year OS was 70% and 2-year OS 59%) and median PFS was 9.0 months (95% CI 6.3-11.7). Figure 1a and b show Kaplan Meier curves of the PFS and OS of the patients treated with ICI and targeted therapy separately. Supplementary figure S2 shows the PFS and OS of the entire cohort. The time to next treatment has not been shown, since this was similar to the PFS.

Figure 1a. PFS, type of systemic therapy

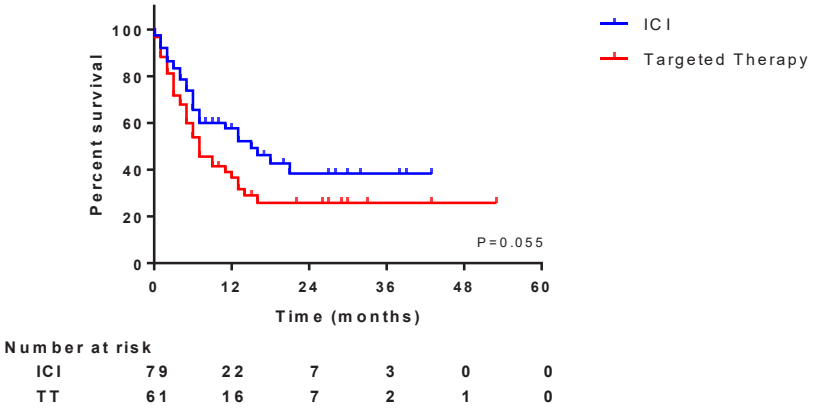
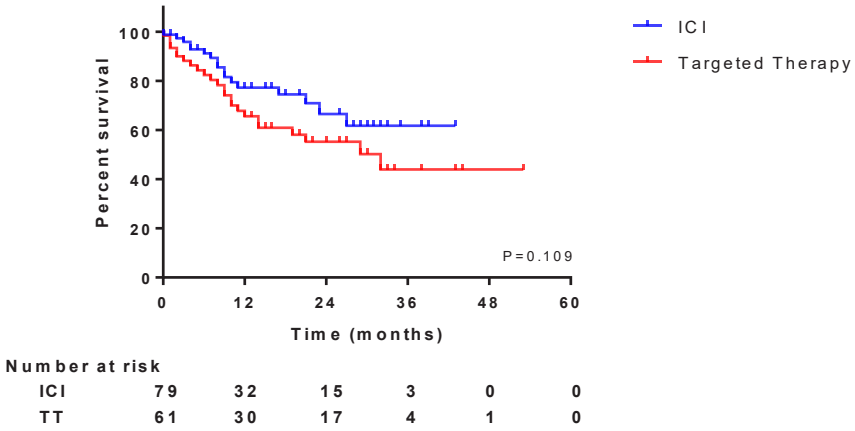


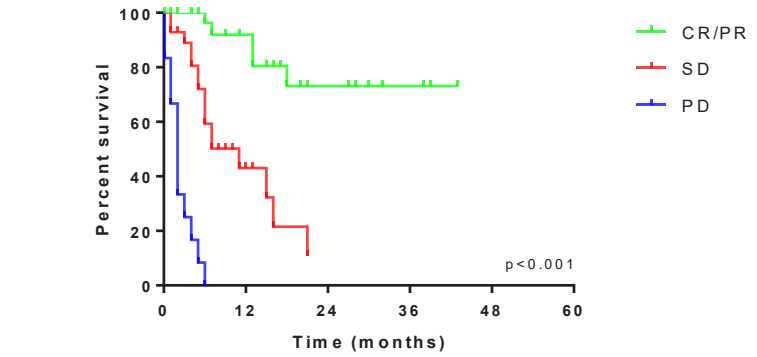
Figure 1b. OS, type of systemic therapy



Since survival could be influenced by the response to systemic treatment, we compared Kaplan Meier curves of these different variables. The influence of these variables was tested in the entire cohort and in patients treated with either ICI or targeted therapy separately. OS and PFS of the entire cohort were not influenced by the best response to systemic treatment. However, in patients treated with ICI, a trend was seen in PFS, favoring patients with a PR compared to patients with SD (supplementary figure S3, CR was not shown due to the very limited number of patients). The most recent status of disease prior to surgery had an impact on PFS and OS. Patients with PD before surgery had a median PFS after surgery of 5.0 months and median OS of 17 months, compared to a not reached median PFS and OS in patients with a PR (p=0.009 and p=0.004). As shown in supplementary figure S4, this impact is seen in patients treated with targeted therapy and is even more pronounced in patients treated with ICI. Also, the status of disease determined at first evaluation after surgery had a significant impact on OS and PFS in the entire cohort and both treatment groups. This is

shown in figure 2 and supplementary figure S5 with a median OS of 7 months in patients with PD compared to 29 months in patients with SD and not reached in patients with a CR or PR after surgery (p<0.001 in both groups). Unfortunately, further follow-up data were missing in a substantial portion of the patients achieving a CR after surgery, and since the outcomes did not significantly differ from patients achieving a PR (p=0.966), these groups were combined.

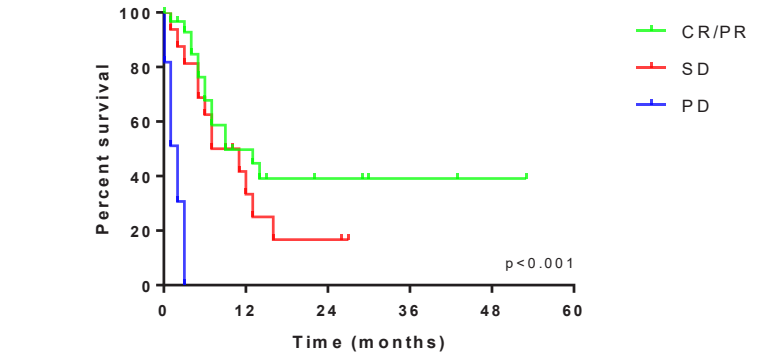
Figure 2a. PFS per status of disease at first follow-up after surgery in patients treated with ICI



Number at risk

CR/PR	35	16	7	3	0	0
SD	28	6	0	0	0	0
PD	12	0	0	0	0	0

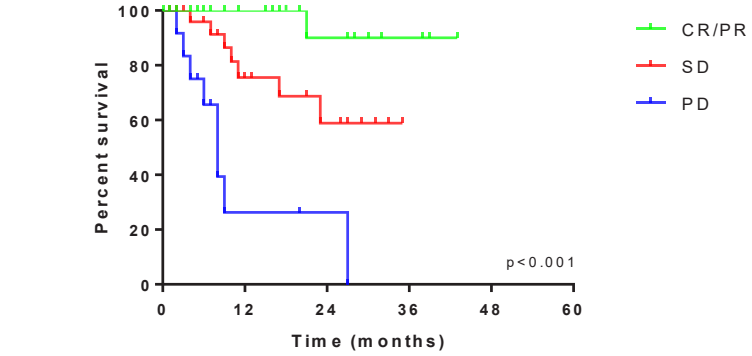
Figure 2b. PFS per status of disease at first follow-up after surgery in patients treated with TT



Number at risk

CR/PR	31	10	4	2	1	0
SD	16	5	2	0	0	0
PD	11	0	0	0	0	0

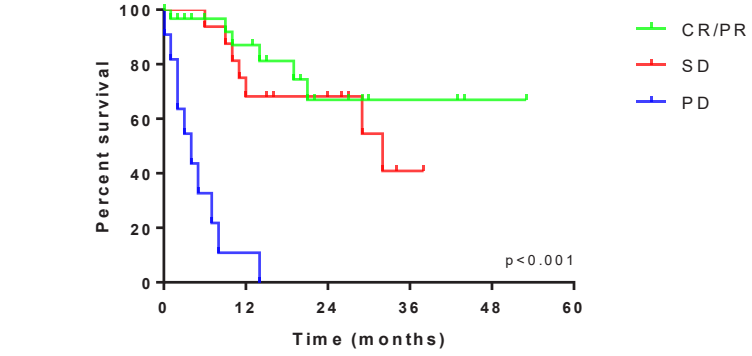
Figure 2c. OS per status of disease at first follow-up after surgery in patients treated with ICI



Number at risk

CR/PR	35	17	8	3	0	0
SD	28	13	6	0	0	0
PD	12	2	1	0	0	0

Figure 2d. OS per status of disease at first follow-up after surgery in patients treated with ICI



Number at risk

CR/PR	31	16	7	3	1	0
SD	16	11	8	1	0	0
PD	11	1	0	0	0	0

Interestingly, the location of the resected lesions had an impact on OS, but not on PFS, as is displayed in figure 3. Unfortunately, it was not possible to differentiate between distant or locoregional subcutaneous and lymph node metastases in this database. However, this shows that after resection of a lymph node or subcutaneous metastasis (either locoregional or distant) patients do experience progression as quickly as after resection of a visceral metastasis, but this does not seem to influence the OS of these patients.

Figure 3a. PFS, location of surgery

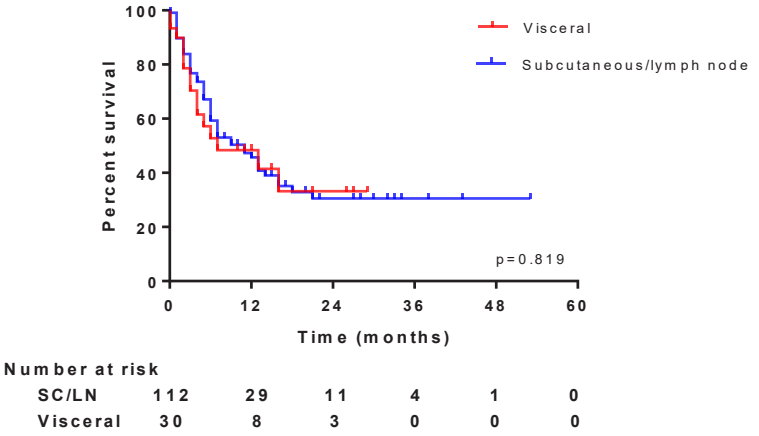
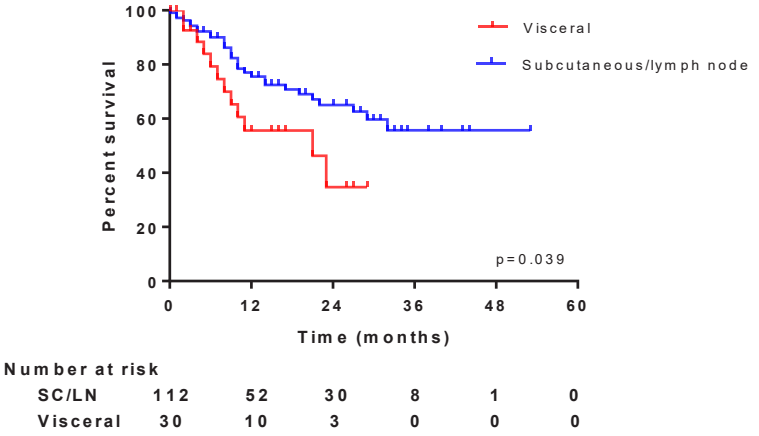


Figure 3b. OS, location of surgery



Other factors that could influence PFS and OS were compared in a univariate Cox regression analysis. Table 2a shows factors that had a significant impact on PFS, OS or both and supplementary table S2 shows all factors. In the univariate analysis type of systemic therapy had no statistically significant impact on PFS or OS, however, a trend favoring ICI might be visible, so this factor was included in further analyses.

All factors that showed (borderline) significance in both PFS and OS univariate analyses, were used to perform a multivariate Cox regression analysis. These results are shown in table 2b. Disease status after surgery is the most convincing factor, which has a significant impact on both PFS and OS. Also, immunotherapy prior to surgery was associated with a PFS and OS benefit compared to targeted therapy, when corrected for other factors in the multivariate analysis.

Table 2a. Univariate analyses (significant results)

		Overall survival			Progression free survival		
Variable		**HR**	**95%CI**	**p**	**HR**	**95%CI**	**p**
Time between primary tumor and current episode				0.030			0.476
	≤1 year	Ref			Ref		
	>1 and ≤5 years	0.32	0.15-0.71	0.005	0.65	0.36-1.17	0.150
	>5 and ≤10 years	0.50	0.23-1.10	0.083	0.91	0.49-1.68	0.753
	>10 years	0.59	0.25-1.39	0.224	0.85	0.43-1.69	0.650
Systemic therapy							
	ICI	Ref					
	Targeted therapy	1.65	0.89-3.07	0.115	1.56	0.97-2.49	0.066
Treatment sequence				0.609			0.015
	First line	Ref			Ref		
	Second line	0.67	0.30-1.51	0.334	0.42	0.21-0.86	0.017
	Third line	1.42	0.55-3.64	0.465	1.79	0.88-3.65	0.109
	Fourth line or more	0.73	0.10-5.40	0.761	0.53	0.13-2.18	0.380
Duration of systemic treatment							
	≤12 months	Ref			Ref		
	>12 months	0.60	0.24-1.53	0.288	0.40	0.18-0.86	0.020
Status of disease prior to surgery				0.003			0.004
	PR	Ref			Ref		
	SD	1.93	0.54-6.96	0.313	1.19	0.55-2.54	0.663
	PD	4.82	1.47-15.83	0.009	.37	1.18-4.75	0.015
Status of disease after surgery				<0.001			<0.001
	CR/PR	Ref			Ref		
	SD	3.08	1.29-7.38	0.012	2.95	1.66-5.23	<0.001
	PD	11.39	4.73-27.47	<0.001	24.20	10.40-56.32	<0.001
Location surgery							
	Subcutaneous/LN	Ref			Ref		
	Visceral	2.02	1.02-3.94	0.045	1.07	0.60-1.88	0.825

Table 2b. Multivariate analyses

	Overall survival			Progression free survival		
Variable	**HR**	**95%CI**	**p**	**HR**	**95%CI**	**p**
Systemic therapy						
ICI	Ref			Ref		
Targeted therapy	3.25	1.48-7.14	0.003	1.89	1.08-3.32	0.026
Status of disease prior to surgery			0.051			0.064
PR	Ref			Ref		
SD	0.69	0.13-3.71	0.669	0.33	0.11-1.00	0.051
PD	2.87	0.55-15.02	0.212	0.69	0.23-2.08	0.514
Status of disease after surgery			<0.001			<0.001
CR/PR	Ref					
SD	6.05	1.64-22.33	0.007	6.61	2.63-16.59	<0.001
PD	18.6	4.54-76.42	<0.001	37.46	12.25-114.51	<0.001
Duration of systemic treatment						
≤12 months	Ref			Ref		
>12 months	0.34	0.09-1.32	0.119	0.40	0.17-0.97	0.042

DISCUSSION

We found that in metastatic melanoma patients obtaining disease control with systemic therapy and undergoing subsequent surgery, the most convincing factor associated with a more favorable outcome was the disease status (CR or PR) at first follow up after surgery. Also, immunotherapy compared to targeted therapy and a duration of systemic therapy of over 3 months seemed to have a positive effect on prognosis.

OS and PFS in our cohort seem to be better than historically, with a median PFS after surgery of 9 months and OS not reached. Howard et al. retrospectively studied patients who had surgery with or without systemic therapy versus systemic therapy alone for stage IV melanoma, all of whom were initially treated in the MSLT-1 trial[4]. They described a survival benefit for surgery with or without systemic therapy versus systemic therapy alone (median OS of 15.8 vs. 6.9 months and 4-year survival of 20.8% vs. 7.0%). However, in this study by Howard most patients had limited disease and in our cohort the majority of patients commenced systemic treatment with >10 lesions. Sosman et al. prospectively analyzed patients undergoing complete resection of stage IV melanoma and found a median recurrence free survival of 5 months and median OS of 21 months[3]. The fact that both studies were conducted in an era without the current effective systemic therapy options explains the difference in outcome with our cohort. Also, it must be noted that median follow-up in the Sosman study was substantially longer than in our cohort (5 years versus 10 months). Follow-up in our cohort is limited, because patients were treated with (novel) systemic therapy first and follow-up was measured from surgery and not from start of systemic therapy.

Selection of patients who could benefit from surgery is crucial and, in this study, we found that expected residual tumor after surgery could be an important selection criterion. Bello et al. described a similar finding, as they had studied 237 patients who had surgery after immunotherapy. They found that a resection to no evidence of disease (NED) was associated with a better survival than residual disease after resection (OS not reached versus 10.8 months, 95% CI 7.3-14.8, p<0.0001).[28] Additionally, they described that OS was associated with the response to systemic therapy: patients with a response or oligoprogression did significantly better than patients with multiple progressive lesions. Unfortunately, in our database, it was not registered how many sites of progression were present in PD cases before surgery. Therefore, we could not distinguish oligoprogression from multiple progressive lesions. Klemen et al. studied patients resected to NED or non-progressive residual disease (NPRD) after progression on immunotherapy and showed a substantial 5-year disease-specific survival of 60% and no significant differences between survival in NED and NPRD patients[29]. They stratified patients for patterns of failure and patients with progression in established tumors had a significant better PFS than patients with new metastases (3-year PFS of 70% versus 6%, p=0.001). Thus, other studies seem to confirm that the expected presence of residual tumor after resection may be an important factor to select the correct patients for surgery.

Imaging may be helpful to select patients prior to surgery. Tan et al described that complete metabolic response on FDG-PET could be useful in predicting long-term benefit and guide discontinuation of therapy in metastatic melanoma patients treated with immunotherapy[30]. Perhaps this could also be used in selecting patients for surgery. However, we are unable to test this hypothesis in our database, since these data were not collected.

This is one of the limitations of our study: the DMTR contains valuable information on metastatic melanoma patients, but there is no possibility to find additional not-registered data. For example, no RECIST response measurements are registered and therefore information on treatment response has to be extracted from status of disease in follow-up. Also, since this is a retrospective study using prospective collected data, selection bias may still occur. Strengths of using data extracted from the DMTR are its nationwide coverage and prospective data-collection by trained datamanagers of real-world data.

The rapid developments in treatment options over the past years have caused some heterogeneity in the registered data. For example, the type of systemic treatment patients in our cohort received does not completely reflect current practice. Anti-CTLA-4 agent ipilimumab was the first immune checkpoint inhibitor available, but anti-PD1 has proven to be superior to ipilimumab and is currently the first choice in most patients[9,13,15]. However, in our cohort 43.6% of patients were treated with ipilimumab. Also, addition of a MEK inhibitor was shown to improve response rates over BRAF inhibitor alone, so this has become standard of care[9,19,20,23,24]. During the first years of the registry, MEK inhibitors were not reimbursed yet and therefore half of the patients in our cohort were treated with a BRAF inhibitor alone.

Targeted therapy is known for its high and quick response rates, where ICI is known to have more durable responses. This may cause a selection bias, because in patients with a worse baseline situation targeted therapy may be preferentially chosen over ICI. However, when comparing baseline characteristics (LDH, performance status, etc.) between patients treated with targeted therapy versus ICI in our cohort, there are no significant differences. Thus, even after correcting for a potential selection bias, surgery after ICI seems to be superior to surgery after TT treatment.

Although several retrospective studies, including ours, do suggest a benefit for surgery after a response to immunotherapy and/or targeted therapy, further studies are warranted. If patients have a deep response with only limited residual lesion(s), stopping therapy, continuing therapy and/or resection can be possible approaches. A randomized trial would be needed to appropriately address this issue.

CONCLUSIONS

Disease status after surgery is the most important prognostic factor for OS and PFS for unresectable stage III / stage IV melanoma patients. Therefore, we recommend that in patients with multiple metastases, surgery is only considered after systemic therapy, when a partial or complete response can be achieved after the resection.

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Chapter 7

Is a history of optimal staging by sentinel lymph node biopsy in the era prior to adjuvant therapy associated with improved outcome once melanoma patients have progressed to advanced disease?

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ABSTRACT

Introduction: Sentinel Lymph Node Biopsy (SLNB) is important for staging in patients with primary cutaneous melanoma. Did having previously undergone SLNB also affect outcomes in patients once they have progressed to metastatic melanoma in the era prior to adjuvant therapy?

Methods: Data were retrieved from the Dutch Melanoma Treatment Registry: a prospectively collected, nationwide database of patients with unresectable stage IIIC or IV (advanced) melanoma between 2012 and 2018. Melanoma-specific survival (MSS) was compared between patients with advanced cutaneous melanoma, previously treated with a wide local excision (WLE) or WLE combined with an SLNB as initial treatment of their primary tumor. Cox regression analyses were used to analyze the influence of different variables on MSS.

Results: In total, 2581 patients were included, of whom 1412 were treated with a WLE of the primary tumor alone and 1169 in whom this was combined with an SLNB. At a median follow-up of 44 months from diagnosis of advanced melanoma, MSS was significantly longer in patients who had previously undergone SLNB (median 23 months [95% confidence interval [CI] 19-29] vs. 18 months [95%CI 15-20] for patients treated with WLE alone, p=0.002). However, multivariate Cox regression did not identify SLNB as an independent favorable prognostic factor for MSS after diagnosis of advanced melanoma.

Conclusion: Prior to the availability of adjuvant systemic therapy, once patients have unresectable stage IIIC or IV (advanced) melanoma, there was no difference in disease outcome for patients who were or were not previously staged with SLNB.

INTRODUCTION

Melanoma treatment has evolved significantly over the past decades. While systemic therapies have become widely available for advanced disease, surgery is still the cornerstone of treatment in localized disease.[1] Nowadays, wide local excision (WLE) of the primary tumor is usually accompanied by a sentinel lymph node biopsy (SLNB) according to the most recent (inter)national guidelines for pT1b melanomas and above, to accurately stage the regional lymph node basin.[2] SLNB in melanoma was first described by Morton et al. and is of paramount prognostic value.[3, 4]

This prognostic value was shown in the MSLT-I trial, which randomized patients with intermediate and thick melanomas to either SLNB or nodal observation. If tumor positive lymph nodes were identified by SLNB, a completion lymph node dissection (CLND) was performed. Although no survival benefit was seen in the SLNB group, the SLNB provided valuable prognostic information: both disease-free survival and melanoma-specific survival (MSS) were significantly better in patients with a negative SLNB (83.2% and 90.2%, respectively) compared with patients with a positive SLNB (53.4% and 72.3%, respectively).[5] These results led to the MSLT-II and DeCOG-SLT studies, which randomized patients to either CLND or observation, after a positive SLNB.[6, 7] Both studies failed to show a survival benefit and CLND is no longer routinely recommended by guidelines in microscopic (SLNB positive) stage III melanoma.[2]

Additionally, with the arrival of new and effective systemic therapies for advanced melanoma, the treatment landscape has shifted from more extensive surgery to extending indications for systemic therapy. Targeted therapy and immune checkpoint inhibition (ICI) have improved prognosis for patients with advanced/metastatic melanoma. A major factor predictive for response is tumor load, irrespective of which treatment was studied.[8-14] This has caused a shift towards adjuvant and neoadjuvant treatments, also demonstrating benefits in terms of relapse-free survival, albeit not yet in overall survival (OS).[15-18] Hence, early identification of recurrence and distant metastases seems valuable.

In this study, we hypothesized that the early identification of lymph node metastasis by SLNB leads to increased awareness of possible metastases during the follow-up period, which could lead to better outcomes for patients once metastasized. Therefore, the aim of this study was to investigate whether outcome in patients once they have progressed to unresectable stage III/IV melanoma is influenced by previous SLNB.

PATIENTS AND METHODS

Data were retrieved from the Dutch Melanoma Treatment Registry (DMTR). In this nationwide prospective database, all Dutch patients undergoing treatment for unresectable stage IIIC and IV metastatic melanoma (hereafter 'advanced melanoma') are included. The goal of the registry is to monitor the safety and outcomes of the novel systemic treatments introduced for melanoma patients over the past decade[19]. Nationwide coverage is assured due to the registration being a prerequisite for reimbursement. In compliance with Dutch regulations, the DMTR was approved by the Medical Ethical Committee and was not considered subject to the Medical Research Involving Human Subjects Act. Patients were offered an opt-out option.

Patients

Patients were included between the start of the registry (July 2012) and December 2018. This time span was chosen to ensure sufficient follow-up at data extraction in December 2020 and to avoid including patients who received adjuvant systemic treatment for SLNB positive melanoma, which was approved in The Netherlands in December 2018. In the DMTR, data on the treatment of advanced melanoma and information on treatment of the primary tumor are registered. Patients treated with a WLE of the primary tumor or WLE accompanied with an SLNB were eligible for inclusion in this study. Patients undergoing adjuvant systemic therapy were excluded. Other exclusion criteria consisted of uveal or mucosal melanoma, melanoma of unknown primary, and macroscopic stage III or IV melanoma at primary diagnosis.

Statistical analysis

Data were analyzed using IBM SPSS Statistics, version 25 (IBM Corporation, Armonk, NY, USA). Two cohorts of patients were analyzed: patients who did undergo SLNB and patients who did not. Patient, tumor and treatment characteristics were analyzed using descriptive statistics. Characteristics of the two cohorts were compared using the Chi-square test for categorical variables and the t-Test or Mann-Whitney U test for continuous variables. Kaplan-Meier estimates were used to calculate follow-up, recurrence-free (RFS), MSS and OS. RFS was defined and calculated as the time from primary tumor to first registered unresectable recurrence (locoregional or distant) or death, as registered in the DMTR. MSS and OS analyses were performed with two different baselines: the date of the primary tumor and the start of registration (diagnosis of unresectable stage III/IV disease), since all patients in our study develop advance disease in due course. Patients not experiencing an event were censored at the time of last follow-up. Log-rank tests were used to compare survival between the two cohorts. Cox regression analysis was used to analyze the influence of different variables on survival. Variables with a p-value of <0.1 in the univariate analyses were used in the multivariate Cox regression models.

RESULTS

Baseline characteristics

In total, 2581 patients included in the DMTR database in December 2018 met the inclusion criteria of this study, of whom 1412 were treated with a WLE of the primary tumor alone and in 1169 patients this was combined with SLNB. Baseline characteristics at the time of primary tumor diagnosis are described in Table 1. Ethnicity data are not collected in the registry. More patients in the SLNB group had primary tumors with unfavorable characteristics, such as high Breslow thickness and ulceration, and fewer patients with a primary melanoma located in the head and neck region underwent SLNB. Of the SLNBs performed, 45% were positive. In the SLNB group, significantly more patients (38%) presented with advanced disease in 2016 or thereafter, compared with those patients (31%) who did not undergo SLNB.

Table 1. Baseline characteristics primary melanoma

Characteristic		All (n=2581)	No SLNB (n=1412)	SLNB (n=1169)	P-value
Sex					0.043
	Female	1113 (43.1)	634 (44.9)	479 (41.0)	
	Male	1467 (56.9)	777 (55.1)	690 (59.0)	
Age (primary), years					0.806
	(median, IQR)	58.0 (46.0-68.0)	57.0 (45.3-68.0)	58.0 (47.0-67.0)	
Breslow					<0.001
	(median, IQR)	2.2 (1.3-3.8)	1.7 (1.0-3.5)	2.8 (1.8-4.0)	
T-stage					<0.001
	T1	392 (15.2)	359 (25.4)	33 (2.8)	
	T2	744 (28.8)	399 (28.3)	345 (29.5)	
	T3	798 (30.9)	300 (21.2)	498 (42.6)	
	T4	513 (19.9)	237 (16.8)	276 (23.6)	
	Unknown	134 (5.2)	117 (8.3)	17 (1.5)	
Location primary					<0.001
	Head & Neck	382 (14.8)	292 (20.7)	90 (7.7)	
	Trunk	1186 (46.0)	614 (43.5)	572 (48.9)	
	Extremity	925 (35.8)	479 (33.9)	446 (38.2)	
	Acral	88 (3.4)	27 (1.9)	61 (5.2)	
Type					<0.001
	Superficial spreading	1359 (52.7)	732 (51.8)	627 (53.6)	
	Nodular	651 (25.2)	291 (20.6)	360 (30.8)	
	Acrolentiginous	64 (2.5)	26 (1.8)	38 (3.3)	
	Lentigo maligna	48 (1.9)	41 (2.9)	7 (0.6)	
	Desmoplastic	25 (1.0)	18 (1.3)	7 (0,6)	
	Other	117 (4.5)	78 (5.5)	39 (3.3)	
	Unknown	317 (12.3)	226 (16.0)	91 (7.8)	
Ulceration					<0.001
	No	1380 (53.5)	782 (55.4)	598 (51.2)	
	Yes	836 (32.4)	354 (25.1)	482 (41.2)	
	Unknown	365 (14.1)	276 (19.5)	89 (7.6)	

Characteristic		All (n=2581)	No SLNB (n=1412)	SLNB (n=1169)	P-value
Satellites					<0.001
	No	2033 (78.8)	1076 (76.2)	957 (81.9)	
	(Micro)satellite	173 (6.7)	79 (5.6)	94 (8.0)	
	Unknown	375 (14.5)	257 (18.2)	118 (10.1)	
SLNB result					N.A.
	Negative			628 (53.7)	
	Positive			537 (45.9)	
	Unknown			4 (0.3)	
Year primary tumor					<0.001
	<2000	175 (6.8)	160 (11.3)	15 (1.3)	
	2000-2009	759 (29.4)	484 (34.3)	275 (23.5)	
	2010-2014	1330 (51.6)	638 (45.2)	692 (59.2)	
	≥2015	316 (12.2)	130 (9.2)	186 (15.9)	

Table 2 shows patient characteristics and the treatment patients received when presenting with advanced disease. Patients in the SLNB group were younger, had a better performance status, less frequently had a tumor harboring a BRAF mutation, and were diagnosed more recently (in 2016 and thereafter) with advanced disease than patients in the no-SLNB group. The majority of patients in the SLNB group were treated with anti-PD-1 as first-line systemic therapy (24.6%), as opposed to patients treated with a WLE of the primary tumor alone who were more likely to be treated with BRAF inhibitors (BRAFi, 32.5%).

Table 2. Baseline and treatment characteristics advanced melanoma

Characteristic		All (n=2581)	No SLNB (n=1412)	SLNB (n=1169)	P-value
Age at DMTR inclusion, years					<0.001
	Median (IQR)	63.0 (53.0-72.0)	64.0 (54.0-73.0)	62.0 (51.0-70.0)	
Year of advanced melanoma					<0.001
	2010-2013	664 (25.7)	384 (27.2)	280 (24.0)	
	2014-2015	1040 (40.3)	597 (42.3)	443 (37.9)	
	≥2016	875 (33.9)	430 (30.5)	445 (38.1)	
BRAF mutation					0.017
	Present	1464 (56.7)	817 (57.9)	647 (55.3)	
	Absent	923 (35.8)	470 (33.3)	453 (38.8)	
	Unknown	194 (7.5)	125 (8.9)	69 (5.9)	
WHO performance status					<0.001
	0	1094 (42.4)	534 (37.8)	560 (47.9)	
	1	767 (29.7)	458 (32.4)	309 (26.4)	
	2	228 (8.8)	137 (9.7)	91 (7.8)	
	3	75 (2.9)	47 (3.3)	28 (2.4)	
	4	15 (0.6	10 (0.7)	5 (0.4)	
	Unknown	401 (15.5)	226 (16.0)	176 (15.1)	

Characteristic			All (n=2581)	No SLNB (n=1412)	SLNB (n=1169)	P-value
LDH-level						0.378
	Normal		1493 (57.8)	798 (56.5)	695 (59.5)	
	Elevated (>250U/L)		911 (35.3)	508 (36.0)	403 (34.5)	
	Unknown		177 (6.9)	106 (7.5)	71 (6.1)	
Brain metastases						0.559
	Present		704 (27.3)	381 (27.0)	323 (27.6)	
	Absent		1660 (64.3)	912 (64.6)	748 (64.0)	
	Unknown		217 (8.4)	119 (8.5)	98 (8.4)	
First line treatment						
	Systemic therapy					<0.001
		Chemotherapy	112 (5.7)	60 (5.8)	52 (5.6)	
		BRAFi	550 (28.0)	336 (32.5)	214 (23.1)	
		Ipilimumab	335 (17.1)	162 (15.7)	173 (18.7)	
		BRAFi + MEKi	312 (15.9)	156 (15.1)	156 (16.8)	
		Anti-PD-1	431 (22.0)	203 (19.6)	228 (24.6)	
		Ipi/nivo	75 (3.8)	36 (3.5)	39 (4.2)	
		Other	139 (7.1)	81 (7.8)	58 (6.3)	
		Unknown	1 (0.1)	0	1 (0.1)	
		T-VEC	6 (0.3)	1 (0.1)	5 (0.5)	
	Surgery					0.303
		No	2053 (82.1)	1107 (81.4)	946 (83.0)	
		Yes	447 (17.9)	253 (18.6)	194 (17.0)	
	Radiotherapy					0.429
		No	1737 (69.5)	954 (70.1)	783 (68.7)	
		Yes	763 (30.5)	406 (29.9)	357 (31.3)	
Second line treatment						
	Systemic therapy					0.245
		Chemotherapy	38 (3.7)	19 (3.5)	19 (3.9)	
		BRAFi	136 (13.2)	73 (13.4)	63 (13.0)	
		Ipilimumab	299 (29.1)	158 (29.1)	141 (29.1)	
		BRAFi + MEKi	138 (13.4)	81 (14.9)	57 (11.8)	
		Anti-PD-1	280 (27.2)	144 (26.5)	136 (28.0)	
		Ipi/nivo	57 (5.5)	22 (4.1)	35 (7.2)	
		Other	78 (7.6)	44 (8.1)	34 (7.0)	
		Unknown	0	0	0	
		T-VEC	2 (0.2)	2 (0.4)	0	
	Surgery					0.305
		No	1012 (90.9)	535 (91.8)	477 (90.0)	
		Yes	101 (9.1)	48 (8.2)	53 (10.0)	
	Radiotherapy					0.030
		No	817 (73.4)	412 (70.7)	405 (76.4)	
		Yes	296 (26.6)	171 (29.3)	125 (23.6)	

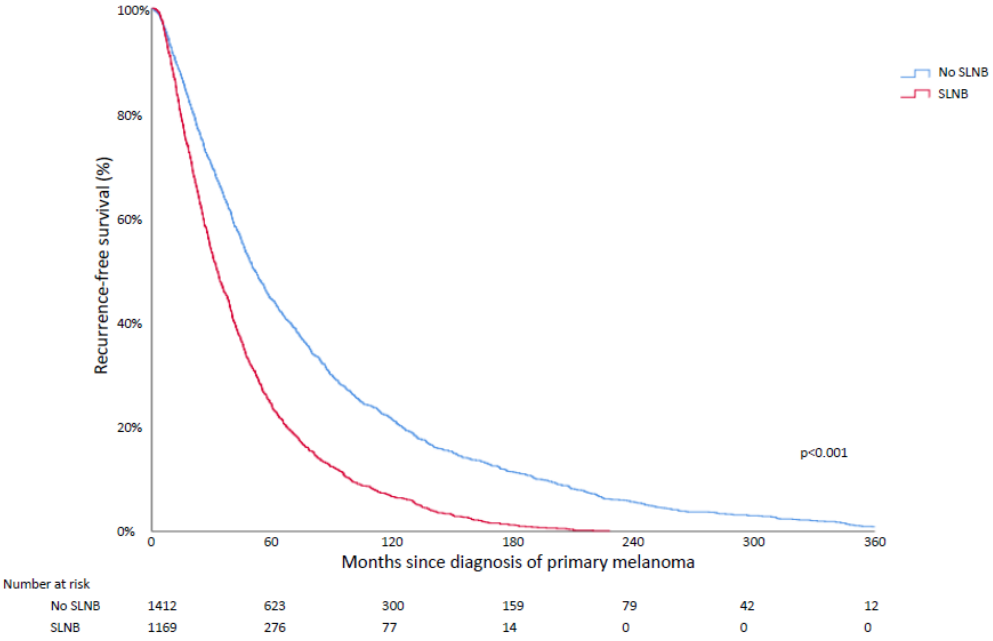
As shown in Table 1, prior to the year 2000 SLNB was rarely performed. With passing time, the proportion of patients undergoing SLNB increased; between 2010 and 2014 a similar number of patients were treated with or without SLNB. Therefore, additional analyses were performed

in the subgroup of patients with a primary tumor in 2010 or thereafter. This patient population will be referred to as 'primary tumor ≥ 2010). Electronic supplementary Table S1 shows the baseline and treatment characteristics of these patients. Similar differences between the SLNB and no SLNB groups were seen in this cohort of patients.

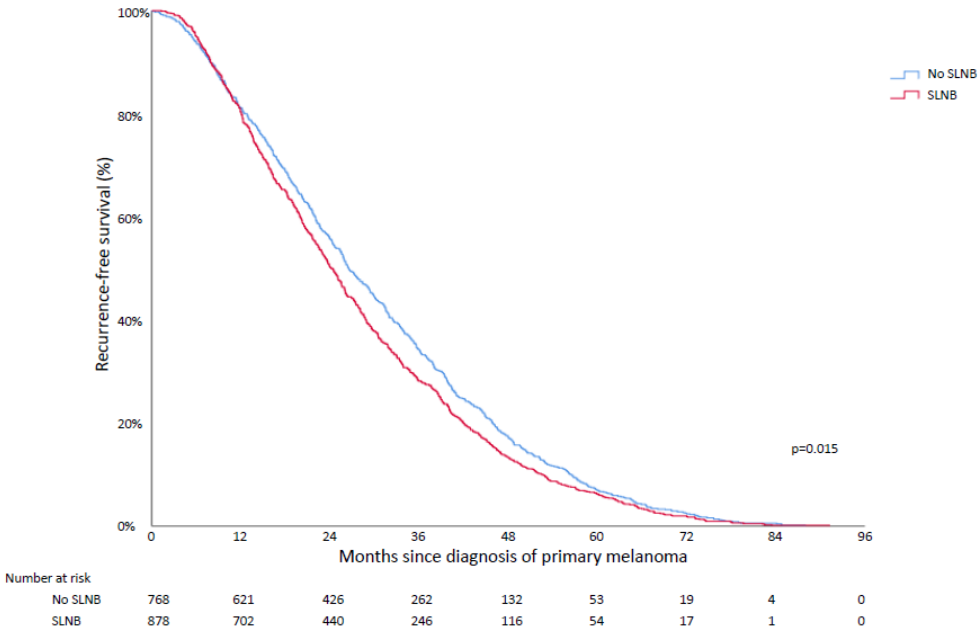
Recurrence-free survival

At data cut-off, median follow-up was 141 months from diagnosis of primary melanoma and 44 months from diagnosis of advanced melanoma. The interval between the primary tumor and first diagnosis of advanced disease (and thus, inclusion in the DMTR registry), was longer in patients treated with WLE alone, showing a median of 51.1 months [95% confidence interval [CI] 47.7-54.5], versus 32.5 months [95%CI 30.0-34.9] in patients undergoing WLE and SLNB (p<0.001) (Fig. 1a). When performing the same analyses in the cohort of patients with a primary tumor in 2010 or thereafter, patients in the SLNB group had a slightly shorter time to first diagnosis of advanced melanoma (Fig. 1b), but this difference was not as distinct as in Fig. 1a, with a median of 26.5 months [95%CI 24.6-28.4] versus 24.2 months [95%CI 22.8-25.5], p=0.015.

Figure 1. Recurrence-free survival



a. All patients: primary tumor to advanced disease

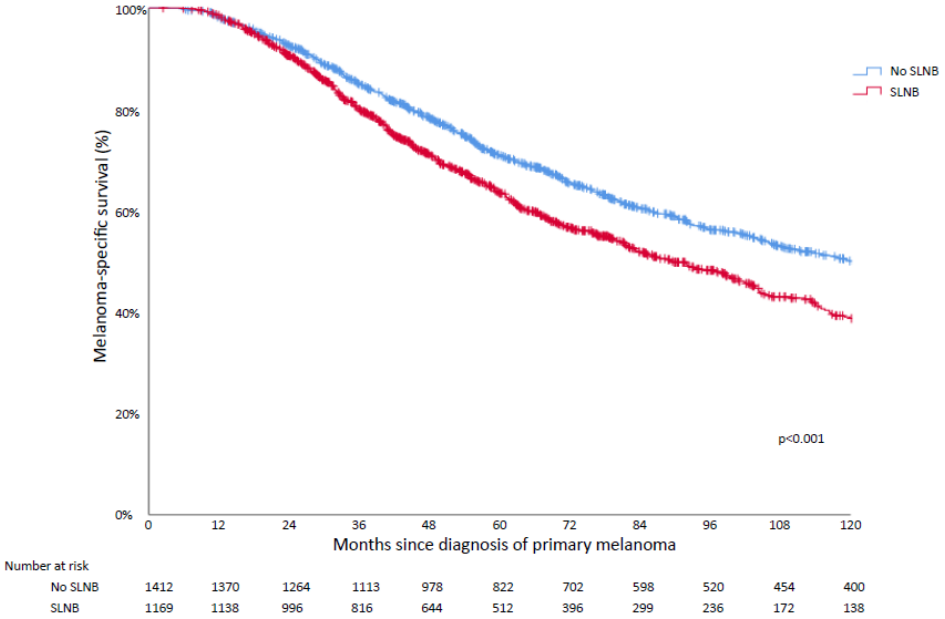


b. Patients with primary tumor ≥2010: primary tumor to advanced disease

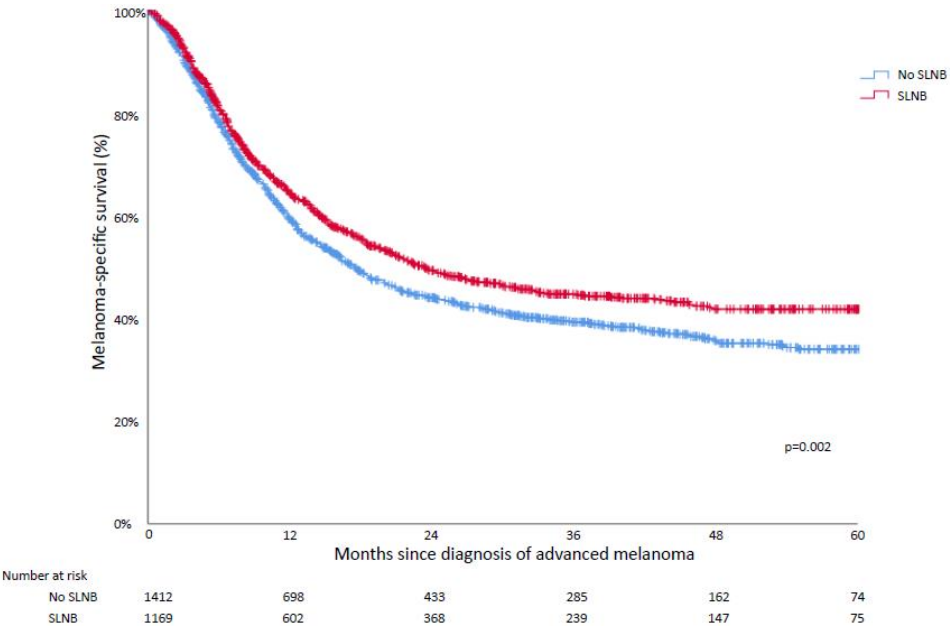
Melanoma-specific survival

MSS from the primary tumor was in favor of patients who did not undergo SLNB: 120.0 months [95%CI 108.9-131.2] versus 89.2 [95%CI 80.3-98.3, p<0.001] (Fig. 2a). However, MSS from diagnosis of advanced melanoma was longer in patients who were previously treated with SLNB (23.3 months [95%CI 18.7-28.1] vs. 17.5 months [95%CI 15.3-19.7] (Fig. 2b). In patients with a primary tumor in 2010 or thereafter, a trend was seen towards a more favorable MSS from primary melanoma in patients who did undergo an SLNB (Fig. 2c). In this cohort, MSS from first diagnosis of advanced melanoma was similar to the entire study population (Fig. 2d). OS results were comparable with MSS, as shown in electronic supplementary Fig. 1.

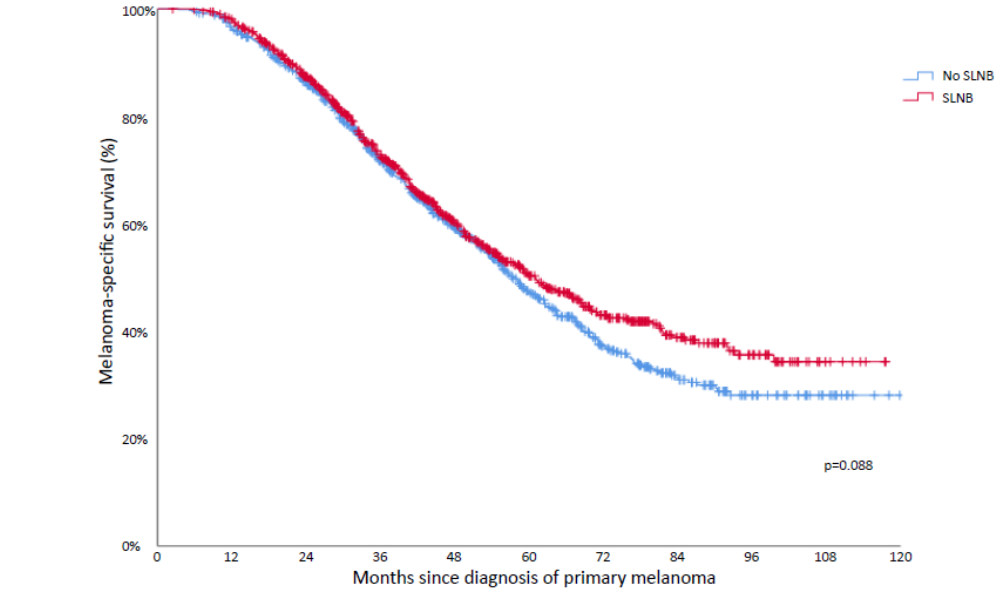
Figure 2. Melanoma-specific survival



a. All patients: from primary tumor



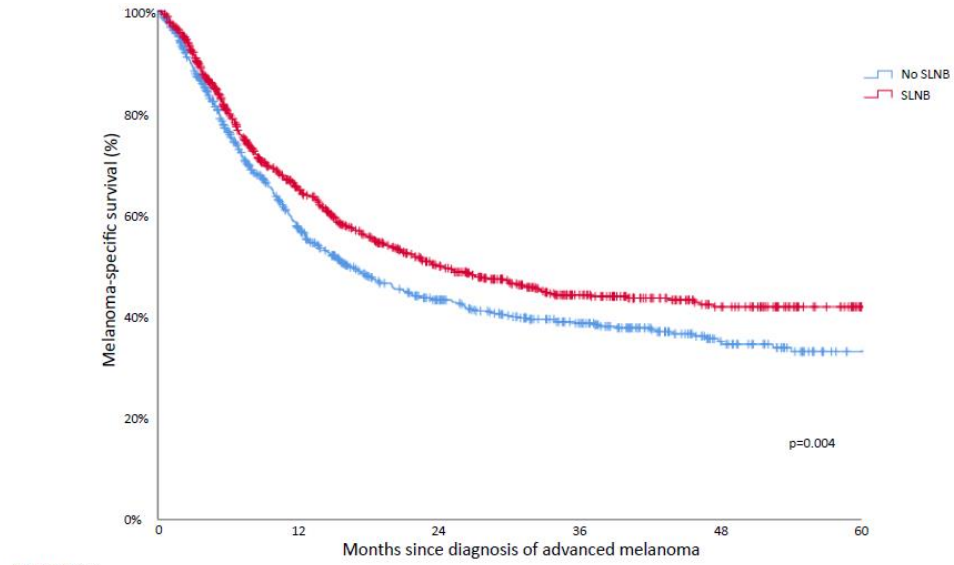
b. All patients: from advanced melanoma



Number at risk

No SLNB	768	728	622	472	344	222	133	71	34	14	2
SLNB	878	848	706	528	359	243	156	83	39	7	0

c. Patients with primary tumor ≥2010: from primary tumor



Number at risk

No SLNB	768	359	209	138	65	26
SLNB	878	444	268	156	90	36

d. Patients with primary tumor ≥2010: from advanced melanoma

Univariate and multivariate analyses

Univariate analyses were performed to identify other factors influencing MSS, with both the diagnosis of the primary tumor and the diagnosis of advanced melanoma as baseline. Electronic supplementary Tables S2a and S2b show the results of the univariate analyses in all patients and in patients with primary tumors in 2010 and thereafter, respectively. Factors associated with the primary tumor, such as Breslow thickness, location, ulceration, and type are influencing MSS starting at diagnosis of the primary tumor, however, these factors are not associated with MSS starting at diagnosis of advanced disease. Apart from SLNB, several other factors are associated with MSS calculated from both baselines, including sex, age, and year of diagnosis of both the primary tumor and advanced disease. Additionally, several patient and treatment characteristics at the time of diagnosis of advanced disease were associated with MSS calculated from both baselines: WHO performance status, lactate dehydrogenase (LDH) level, presence of brain metastases, and type of first-line systemic therapy.

In contrast to the previously shown analyses, SLNB was not associated with MSS from either diagnosis of primary tumor or advanced disease in a multivariate model, in either all patients or patients with a primary tumor in 2010 and thereafter. In the multivariate model including all patients, age, ulceration of primary tumor, year of diagnosis of advanced melanoma, WHO performance status, LDH level, and presence of brain metastases were prognostic factors associated with both MSS starting at diagnosis of both the primary tumor and advanced disease (Table 3). Female sex and a primary melanoma on the extremities were favorable prognostic factors associated with MSS calculated from diagnosis of the primary tumor. In patients with a primary melanoma in 2010 or thereafter, factors impacting MSS from both baselines were age, year of diagnosis of advanced melanoma, WHO performance status, LDH level, and presence of brain metastases (Table 4). Favorable prognostic factors correlating with MSS from the primary tumor were ulceration and year of primary tumor. Presence of a BRAF mutation was associated with better MSS from advanced disease.

Table 3. Multivariate analyses

		MSS primary			MSS advanced disease	
Variable	**HR**	**95%CI**	**P-value**	**HR**	**95%CI**	**P-value**
Sex						
Female	0.91	0.80-1.03	0.119	0.88	0.78-0.99	0.027
Male	Ref			Ref		
Age, primary	1.01	1.01-1.02	<0.001	1.01	1.00-1.01	0.001
Location, primary			0.060			0.589
Trunk	Ref			Ref		
Extremity	0.84	0.73-0.96	0.010	0.94	0.83-1.07	0.381
Acral	1.20	0.71-1.71	0.673	1.07	0.77-1.49	0.682
Head & Neck	0.94	0.78-1.14	0.527	0.91	0.77-1.08	0.278
Breslow	1.00	1.00-1.00	0.232			

		MSS primary			MSS advanced disease	
Variable	**HR**	**95%CI**	**P-value**	**HR**	**95%CI**	**P-value**
Type			0.574			
Superficial spreading	Ref					
Nodular	1.12	0.97-1.30	0.114			
Acrolentiginous	0.87	0.52-1.43	0.574			
Lentigo maligna	0.76	0.44-1.32	0.328			
Desmoplastic	1.05	0.51-2.14	0.898			
Other	0.92	0.69-1.24	0.605			
Unknown	1.05	0.86-1.29	0.639			
Ulceration			0.004			0.029
No	Ref			Ref		
Yes	1.25	1.09-1.44	0.001	1.18	1.03-1.34	0.016
Unknown	0.98	0.80-1.21	0.872	1.16	0.97-1.38	0.097
Satellites			0.253			
No	Ref					
(Micro)satellite	0.93	0.71-1.20	0.575			
Unknown	0.85	0.70-1.04	0.112			
SLNB performed						
No	Ref			Ref		
Yes	0.97	0.85-1.10	0.616	0.90	0.80-1.02	0.100
Year of primary tumor			<0.001			0.168
<2000	Ref			Ref		
2000-2009	11.65	7.73-17.56	<0.001	1.08	0.84-1.38	0.538
2010-2014	70.51	45.07-110.31	<0.001	1.21	0.94-1.57	0.136
≥2015	243.33	145.32-407.43	<0.001	1.04	0.75-1.45	0.799
Year of advanced melanoma			<0.001			<0.001
2010-2013	Ref			Ref		
2014-2015	0.50	0.43-0.57	<0.001	0.75	0.65-0.85	<0.001
≥2016	0.27	0.22-0.31	<0.001	0.55	0.47-0.65	<0.001
BRAF mutation			0.404			
Absent	Ref					
Present	0.97	0.86-1.11	0.698			
Unknown	0.51	0.19-1.38	0.185			
WHO performance status			<0.001			<0.001
0	Ref			Ref		
1	1.61	1.39-1.86	<0.001	1.66	1.45-1.91	<0.001
2	2.06	1.68-2.52	<0.001	2.56	2.11-3.11	<0.001
3	2.45	1.80-3.35	<0.001	4.16	3.10-5.59	<0.001
4	2.51	1.36-4.64	0.003	4.16	2.31-7.49	<0.001
Unknown	1.69	1.40-2.03	<0.001	1.58	1.33-1.89	<0.001
LDH-level			<0.001			<0.001
Normal	Ref			Ref		
Elevated (>250U/L)	1.77	1.57-2.01	<0.001	1.97	1.75-2.22	<0.001
Unknown	0.93	0.67-1.30	0.678	0.99	0.75-1.32	0.970

		MSS primary			MSS advanced disease	
Variable	**HR**	**95%CI**	**P-value**	**HR**	**95%CI**	**P-value**
Brain metastases			<0.001			<0.001
Absent	Ref			Ref		
Present	1.60	1.41-1.82	<0.001	1.77	1.56-2.00	<0.001
Unknown	1.34	0.77-2.33	0.304	1.93	1.14-3.29	0.015

Table 4. Multivariate analyses, patients with a primary tumor in 2010 and thereafter

		MSS primary			MSS advanced disease	
Variable	**HR**	**95%CI**	**P-value**	**HR**	**95%CI**	**P-value**
Age, primary	1.01	1.01-1.02	<0.001	1.01	1.00-1.01	0.024
Breslow	1.00	1.00-1.00	0.411			
Ulceration			0.006			0.364
No	Ref			Ref		
Yes	1.29	1.10-1.51	0.001	1.12	0.95-1.32	0.166
Unknown	1.11	0.84-1.46	0.460	1.10	0.84-1.44	0.506
Sentinel node performed						
No	Ref					
Yes	0.96	0.83-1.11	0.584	0.89	0.76-1.04	0.130
Year of primary tumor						
2010-2014	Ref					
≥2015	3.17	2.50-4.03	<0.001	0.80	0.63-1.02	0.075
Year of advanced melanoma			<0.001			<0.001
2010-2013	Ref			Ref		
2014-2015	0.52	0.43-0.63	<0.001	0.71	0.58-0.86	0.001
≥2016	0.29	0.23-0.36	<0.001	0.53	0.43-0.66	<0.001
BRAF mutation						<0.001
Absent				Ref		
Present				0.75	0.64-0.87	<0.001
Unknown				0.16	0.02-1.17	0.071
WHO performance status			<0.001			<0.001
0	Ref			Ref		
1	1.50	1.25-1.80	<0.001	1.52	1.26-1.83	<0.001
2	1.86	1.44-2.39	<0.001	2.46	1.91-3.18	<0.001
3	2.35	1.62-3.43	<0.001	5.15	3.48-7.62	<0.001
4	2.14	1.00-4.20	0.027	3.12	1.58-6.17	0.001
Unknown	1.55	1.24-1.94	<0.001	1.79	1.42-2.26	<0.001
LDH-level			<0.001			<0.001
Normal	Ref			Ref		
Elevated (>250U/L)	1.93	1.66-2.25	<0.001	2.01	1.72-2.35	<0.001
Unknown	1.01	0.70-1.45	0.964	0.88	0.55-1.39	0.579
Brain metastases			<0.001			<0.001
Absent	Ref			Ref		
Present	1.52	1.29-1.78	<0.001	1.78	1.52-2.10	<0.001
Unknown	1.17	0.52-2.64	0.698	1.14	0.47-2.77	0.768

DISCUSSION

For decades, there has been an ongoing debate on the presence or absence of a therapeutic effect of SLNB for melanoma. In our study, a history of undergoing SLNB as initial staging of the primary melanoma was not associated with an improved outcome once patients were diagnosed with unresectable stage IIIC or IV (advanced) melanoma and were treated with systemic therapy. From 2016 onwards there has been widespread availability of effective systemic therapies for advanced melanoma patients with both BRAF and MEK and immune checkpoint inhibitors.

Our study provides an overview of the implementation of SLNB in common clinical practice in The Netherlands: prior to 2010, the minority of patients diagnosed with primary melanoma were treated with a WLE and SLNB. However, in the last decade, this has shifted towards the majority of patients undergoing SLNB at diagnosis of primary melanoma[20]. Nonetheless, not all patients are undergoing SLNB, despite recommendation in clinical guidelines. Reasons to omit SLNB include patient preference, location of the primary tumor (head and neck), elderly and frail patients, or various other reasons. Additionally, in The Netherlands, primary melanoma excisions are often performed by general practitioners or dermatologists, but surgeons perform SLNB. Therefore, patients need to be referred to a surgeon for re-excision and SLNB after diagnosis, which results in omission in some patients, who undergo re-excision alone by a dermatologist. Unfortunately, the DMTR does not provide data on the reasons to omit SLNB in the treatment of registered patients.

As shown in Fig. 1a, RFS from primary tumor to advanced disease was worse in patients who did undergo SLNB, compared with patients who did not. The most likely explanations are the unfavorable prognostic features of the primary tumor (Table 1) in the SLNB group or a higher awareness of recurrence resulting in more intensive surveillance in those patients who have undergone an SLNB. However, these differences in RFS diminished in the subgroup of patients with a primary melanoma in 2010 or thereafter, even though patients in this subgroup undergoing SLNB also had unfavorable characteristics of their primary tumors (electronic supplementary Table S1a). It is likely the difference between RFS in the subgroup of patients with a primary tumor in 2010 and thereafter and all patients could be explained by the difference in year of diagnosis of the primary tumor. As described previously, the use of SLNB has increased over the years and therefore patients in the SLNB group of the entire cohort were diagnosed with primary melanoma more recently than patients in the no-SLNB group (Table 1). In the subgroup of patients with a primary melanoma in 2010 and thereafter, the year of diagnosis was more evenly spread between patients previously treated with SLNB or not. A less recent diagnosis may indicate a less aggressive biology (more indolent type) of the melanoma. This is supported by the multivariate analyses, which show a strong correlation between the year of diagnosis of the primary tumor and the MSS calculated from this baseline (Table 3).

The MSS calculated from the diagnosis of advanced melanoma is in favor of the SLNB group, both in all patients and in the subgroup of patients with a primary tumor in 2010 or thereafter (Fig. 2b, d). However, SLNB was not associated with MSS in the multivariate analyses of either subgroup (Tables 3, 4). The multivariate model showed other prognostic factors that could explain the differences seen in the initial analysis. For example, a more recent diagnosis of advanced melanoma showed a favorable prognostic characteristic. As shown in previous studies, improving systemic therapies over the past years has improved the prognosis of advanced melanoma patients every year.[21] Combining this information with data from Table 1, showing that more patients in the SLNB group were diagnosed with advanced disease more recently, could partially explain the differences between univariate and multivariate analyses. Additionally, a higher WHO performance status, an elevated LDH level, and the presence of brain metastases were associated with worse MSS in all subgroups. These confounding factors are thought to explain the difference seen in univariate analyses between patients with or without SLNB. We hypothesized that SLNB patients are diagnosed with advanced melanoma earlier and with a lower tumor burden than patients who have never undergone SLNB, despite patients undergoing SLNB having worse prognostic factors to start with.

Finally, this study only included patients who had undergone a WLE with or without SLNB (either positive or negative) as part of their initial staging and at some point developed unresectable stage IIIC or IV (advanced) melanoma. It did not include all patients who underwent SLNB and never developed unresectable stage IIIC or IV (advanced) melanoma, which is an important limitation of this study. Thus, we cannot definitively state that SLNB is not associated with a survival benefit, although it does suggest that SLNB has no therapeutic impact. Additionally, since all patients in this study developed advanced disease, no conclusions can be drawn on whether SNLB would have prevented recurrences in the first place. Therefore this question cannot be answered with the available data.

Previous studies have shown the value of SLNB in melanoma patients, starting with Morton et al.[5] On the contrary, other studies have suggested that the additional value of SLNB, compared with clinicopathological features of the primary tumor alone, was limited.[22-25] The study by El Sharouni et al. has contradicted these suggestions by demonstrating the additional prognostic information provided by SLNB.[4] In contrast with these previous studies, our study focused on the value of the previously performed SLNB in patients once they have already progressed to advanced/metastatic melanoma.

This study is population-based, which is simultaneously a strength and a weakness. The data from the DMTR assure a nationwide coverage and prospective Case Report Form (CRF) data collection by trained data managers, and therefore gives a comprehensive view of the real treatment landscape. However, data are limited to the information collected by the CRF, there is no possibility to retrieve any additional unregistered clinical data (e.g. differences in indication or choice to perform SLNB between different treatment centers, or other

characteristics that may influence MSS). It provides an overview of melanoma treatment over the past decades, but in this study confounding by indication is a threat.

CONCLUSION

Prior to the availability of adjuvant systemic therapy, once patients were diagnosed with unresectable stage IIIC or IV (advanced) melanoma, there was no difference in disease outcome for patients that were or were not originally staged with SLNB. We do recommend SLNB in this day and age for the optimal staging of stage I/II melanoma to allow for appropriate selection for adjuvant systemic therapy (trials).

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PART IV

Concluding remarks

Chapter 8

General discussion

GENERAL DISCUSSION AND FUTURE DIRECTIONS

The melanoma treatment landscape is evolving rapidly. In the past, surgery was the main treatment modality available for melanoma patients and therapeutic options in metastatic disease were scarce. The development of the new systemic therapies, immune checkpoint inhibitors (ICI) and targeted therapy (TT), has brought new opportunities.[1-10] As these systemic therapies demonstrated their value in stage IV melanoma, new indications were explored and this led to the merging of systemic therapy with surgery. This combination has shown promising results, since adjuvant systemic treatment of patients with stage III melanoma reduced the previous substantial risk of recurrence in this patient population.[11-16]

Therefore, in this thesis we have described ways to expand this successful teamwork between surgery and novel systemic therapies. We have studied how systemic therapies can be used to prevent recurrences in high-risk melanoma, how systemic therapy can be used to make patients with unresectable locally advanced melanoma eligible for surgery, and how surgery can complement systemic therapy in patients with metastatic melanoma.

From extensive surgery to extending indications for systemic therapy

Prior to the introduction of the sentinel lymph node biopsy (SLNB), patients with high-risk primary melanoma were sometimes treated with an elective lymph node dissection (ELND). With the discovery of the SLNB, a method for patient selection was introduced and the invasive lymph node dissection and its accompanying complications were preserved for patients with proven lymph node metastases.[17,18] However, the MSLT-II and DeCOG-SLT trials showed no survival benefit of the completion lymph node dissection (CLND) in patients with a positive SLNB and CLND is therefore no longer recommended in patients with a positive SLNB.[19-21] At that time, the SLNB was solely of prognostic value. This was, until the arrival of ICI and TT as adjuvant systemic therapy, making the SLNB crucial in the selection of patients for adjuvant drug therapy.

We have studied the value of the SLNB in patients who progressed to advanced disease in due course. In this study, data from the Dutch Melanoma Treatment Registry (DMTR) gave an overview of the evolving use of the SLNB in the Netherlands. The DMTR is a nationwide prospective database including all Dutch patients undergoing treatment for unresectable stage IIIC (American Joint Committee on Cancer, AJCC 7th edition[22]) and IV metastatic melanoma. We included 2581 patients registered in the DMTR between 2012 and 2018 (prior to the availability of adjuvant systemic therapy). Of these patients with advanced melanoma, 1169 had previously been treated with a SLNB at diagnosis of their primary melanoma, compared to 1412 patients treated with wide local excision (WLE) of the primary tumor alone, reflecting the common uptake of SLNB in the past. The majority of patients in this study were diagnosed with primary melanoma between 2010 and 2014. Our data showed that after 2010, SLNB became common practice in the Netherlands. When comparing melanoma-specific survival (MSS) between patients previously treated with WLE alone or WLE combined with

SLNB, MSS was significantly longer in patients in the latter group (median 23 months versus 18 months, p=0.002). These results, however, were not confirmed by multivariate cox regression and were more likely to have been caused by other prognostic factors, which were not evenly divided between the subgroups. Our study showed that prior to the availability of adjuvant systemic therapy, original staging with SLNB had no survival benefit for patients developing subsequent unresectable stage IIIC/IV melanoma.[23] As some had hypothesized that the earlier detection of stage III disease through SLNB, even in the absence of treatment consequences, might have been beneficial to patients, but unfortunately this was not true for our study.

Opposed to the patient population described in our above-mentioned study, the SLNB has proven its prognostic value in the treatment of primary melanoma patients. Risks of locoregional or distant recurrence and death are substantial in patients with positive SLNB and therefore patients with stage III melanoma, even despite complete surgical resection, are at high risk. Since surgery will never be able to treat undetectable micrometastases, adjuvant systemic therapy has been a topic of interest for a long time, but until the arrival of ICI and TT none of the applied treatments showed sufficient benefit. The first trial to show a recurrence-free survival (RFS) and only trial thus far that has displayed an overall survival (OS) benefit, is het EORTC 18071. In this study, patients with stage III melanoma treated with the high dose anti-CTLA-4 agent ipilimumab (10 mg/kg) after complete surgical resection had a 5-year RFS of 40.8% and OS of 65.4%, versus 30.3% and 54.4% respectively, in patients treated with placebo. However, this treatment schedule was accompanied by severe toxicity: grade 3 or 4 immune-related adverse events in 41.6% and grade 5 (death) in five patients (1.1%).[11] The Checkmate 238 study compared high-dose ipilimumab with the anti-PD1 agent nivolumab, which resulted in an improved 5-year RFS (50% versus 39%, HR 0.72, 95% confidence interval [CI] 0.60-0.86) and distant metastasis-free survival (DMFS, 58% versus 51%, HR 0.79, 95% CI, 0.63-0.99) in patients treated with nivolumab after complete surgical resection of stage III/IV melanoma. Nivolumab had a more favorable toxicity profile, with grade 3 or 4 adverse events in 14.4%, versus 45.9% of patients treated with high-dose ipilimumab.[12,13] Since this study did not incorporate a placebo arm, the investigators have initiated an indirect treatment comparison between the nivolumab arm of the checkmate 238 study and the placebo arm of the EORTC 18071 study. Although the checkmate 238 has yet to show an OS benefit, this indirect comparison showed both RFS and OS benefit of nivolumab over placebo.[24] A different study designed to compare anti-PD1 with placebo was de EORTC 1325 study, resulting in improved 3-year RFS with pembrolizumab versus placebo (63.7% versus 44.1) and improved DMFS (65.3% versus 49.4%).[14,15] The above-mentioned studies included similar, but not identical patient populations: the checkmate 238 included patients with stage IIIB/C and IV melanoma and both the EORTC 18071 and EORTC 1325 included patients with stage IIIA (SLNB tumor burden ≥1.0mm) and IIIB/C melanoma (all AJCC 7th edition). In patients included after a positive SLNB, with a tumor burden of at least 1.0 mm in largest diameter, a complete lymph node dissection (CLND) was required in both EORTC studies. However, this is no longer

standard of care since studies did not show an OS benefit of CLND after a positive SLNB. Therefore, current clinical practice is to offer patients with a positive SLNB adjuvant systemic therapy, and no longer additional surgery. This change in practice of foregoing a CLND is not expected to negatively affect DMFS or OS, because these are determined by distant and not by locoregional recurrences, which are caused by undetectable micrometastases, adequately treated by the adjuvant systemic therapy. There is recent, by proxy evidence, that foregoing a CLND has not been detrimental to the results obtained by adjuvant therapy when comparing the nivolumab arms of Checkmate 238 and Checkmate 915, which both showed a similar 2-year RFS of 63%, but Checkmate 915 no longer required a CLND.[25] The developments in adjuvant systemic therapy have changed the value of the SLNB: starting as a selection tool for lymph node dissection in clinically node negative patients, it now selects patients for adjuvant drug therapy.

These previous data show that over the past decades, melanoma treatment has shifted from aggressive surgical treatment to less invasive surgery combined with systemic therapy to reduce recurrences. By de-escalating from ELND to SLNB, surgeons have made big steps to adequately select patients and tailor treatment. The following steps will be directed at further selecting patients eligible for adjuvant systemic therapy and progressing towards personalized medicine.

Patient selection

Patient selection for adequate surgery and systemic surgery is key: both to give patients sufficient treatment to offer them the best chance of curation, and to omit unnecessary treatment and accompanying side effects in patients who will not benefit. Thus far, clinicopathological features have been the leading factors in selecting patients for adjuvant systemic therapy: AJCC staging and sentinel node tumor burden.[22,26-28] However, even within the patient populations (stage IIIA SN >1.0mm, IIIB, IIIC) now eligible for adjuvant systemic therapy, subgroups can be identified with very limited benefit.[28] To further evolve patient selection, there is a need for biomarkers for response.

At the Netherlands Cancer Institute (NKI), we have conducted a biomarker study including patients with stage III melanoma. Patients were selected from an ongoing biobank study, collecting clinical data and tumor material. Eligible patients had macroscopic stage III melanoma, underwent complete surgical resection and were naïve to systemic therapy prior to inclusion. SLNB positive patients were not included in this biobank, as they did not classify as stage III melanoma (clinical stage I or II) prior to surgery and SLNB samples provided insufficient material for analyses. The cohort consisted of 98 patients in total, of whom 49 did not receive adjuvant systemic therapy, because they were diagnosed before adjuvant drug therapy became available outside of clinical trials and 49 who were intended to receive adjuvant systemic therapy. Analyses of the clinical data showed, similar to the above-mentioned trials, an RFS benefit favoring adjuvant systemic therapy. Previous studies

had identified the interferon-gamma (IFNγ) RNA signature as a promising possible biomarker. In our study, IFNγ showed to be an independent prognostic marker: patients treated with surgery alone harboring an IFNγ high profile had an increased RFS compared to IFNγ low patients, receiving the same treatment. This was also seen in patients treated with adjuvant systemic therapy. This led to the conclusion that both patients with a low and a high IFNγ profile had benefit from adjuvant systemic therapy. Therefore, IFNγ cannot be used to select patients for adjuvant systemic therapy, but is a prognostic marker. Further analyses resulted in B cell score as a possible biomarker. Comparable to IFNγ, this showed to be of prognostic value, but did not predict response to adjuvant systemic therapy.[29]

Broadening indications for surgery

We have described how systemic therapy can complement treatment of melanoma patients by reducing the risk of recurrence after surgical resection. However, in patients not eligible for surgery, systemic therapy can also play a role. Therapeutic lymph node dissection (TLND) is the currently recommended treatment in macroscopic lymph node metastases, but a small proportion of patients presents with bulky and sometimes unresectable disease. We have studied treatment with induction systemic therapy in these patients.

In the REDUCTOR study, patients with unresectable BRAF-mutated locally advanced stage IIIC or oligometastatic stage IV melanoma were treated with BRAF and MEK inhibitors for 8 weeks. After these 8 weeks of dabrafenib plus trametinib, the vast majority of patients (18 of 21 patients, 86%) showed sufficient downsizing to perform a resection, of which 17 were radical resections. A (near) complete response (CR) was seen in 9 out of 18 patients undergoing a resection.[30] A subsequent study showed that ^{18}F-FDG PET/CT could not accurately predict histopathological response or recurrence in this patient population.[31] The REDUCTOR study presented proof that systemic therapy can pave the road to surgery in a selected patient population.

All above-mentioned studies present examples of systemic therapy complementing surgical treatment, but these roles can also be inversed. In patients with unresectable stage III/IV melanoma treated with systemic therapy, responses are often durable, but in some patients progression of a limited number of metastases occurs: oligoprogression. Additionally, some patients experience a durable partial response with a limited number of lesions remaining. In both patient populations, surgery may contribute to a complete response. We have analyzed outcomes of surgery in patients with unresectable stage IIIC and IV melanoma treated with immunotherapy or targeted therapy and included in the DMTR. Patients with disease control (complete or partial response, or stable disease) to systemic therapy and subsequent surgery were selected and at a median follow-up of 10 months, median progression-free survival (PFS) was 9 months and median OS was not reached. A complete or pathologic response at first follow-up after surgery was associated with a better PFS and OS. Therefore, this study showed that selected patients can benefit from surgery after achieving initial disease control

with systemic therapy.[32] Additionally, a study by Versluis et al. analyzed different treatments after solitary progression on ICI: systemic therapy, local therapy, a combination, or active surveillance. Local therapy mainly consisted of surgery (55%), radiotherapy (35%) or surgery plus radiotherapy (5%). They found that for solitary progression after ICI, PFS was higher in patients undergoing surgery and systemic therapy combined, than either treatment alone, and therefore repeatedly shows the synergy between different treatment modalities.[33]

Future directions

This thesis shows that teamwork between surgery and systemic therapy improves melanoma treatment and during the upcoming years, this collaboration will expand. Examples of this expansion are the neoadjuvant trials in macroscopic stage III melanoma. The OpACIN and OpACIN-neo trials showed high pathologic response rates in the lymph node dissection specimen, after neoadjuvant treatment with ipilimumab plus nivolumab. These pathologic responses highly correlated with RFS, as 2-year RFS was 97% in patients with a pathologic response, compared to 36% in nonreponders.[34,35] The following PRADO trial adjusted surgical and adjuvant treatment according to pathologic response in the resected index lymph node after neoadjuvant ipilimumab plus nivolumab. In patients with a complete or partial response adjuvant systemic therapy was omitted. A TLND was only performed in patients with a partial or no pathologic response, which resulted in lower complication rates in patients only undergoing index lymph node resection compared to patients undergoing TLND. Survival outcomes in patients with a complete pathologic response in the index node were not compromised by omitting the TLND, with a 24-month RFS of 93% and this study emphasizes the rationale for response-directed and tailored treatment.[36]

The first phase 3 neoadjuvant trial was the SWOG 1801, which randomized 313 patients between TLND plus 1 year (18 doses) of adjuvant pembrolizumab versus combined neoadjuvant pembrolizumab (3 doses) followed by TLND and adjuvant pembrolizumab (15 doses). Event-free survival was significantly longer in the combined neoadjuvant plus adjuvant group, than in the adjuvant only group: 2-year event-free survival of 72% and 49%, respectively (p=0.004), confirming benefit of neoadjuvant therapy in macroscopic stage III melanoma.[37]

Recently, another neoadjuvant trial has presented its results: the NADINA. This phase 3 trial randomized 423 patients with macroscopic stage III melanoma between neoadjuvant ipilimumab plus nivolumab followed by TLND and TLND followed by adjuvant nivolumab. Adjuvant treatment in the neoadjuvant group was tailored according to pathologic response: only patients with a partial response or nonresponse received adjuvant systemic therapy. The 12-month event-free survival was 83.7% (99% CI 73.8-94.8) in the neoadjuvant group versus 57.2% (99% CI 45.1-72.7) in the adjuvant group. The majority of patients in the neoadjuvant group had a major pathologic response (59.0%). [38] These trials will direct future treatment for patients with macroscopic stage III melanoma.

Conclusions

As described in this thesis, systemic therapies can aid surgery to prevent recurrences in high-risk melanoma, systemic therapy can be used to pave the road to surgery for unresectable and/or locally advanced melanoma, and vice-versa, surgery can assist in selected patients with metastatic melanoma treated with systemic therapy. Concluding, systemic therapy and surgery can be seen as synergistic treatment modalities in treating patients with stage III and IV melanoma.

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Chapter 9

English summary
Nederlandse samenvatting

SUMMARY

Chapter 1 gives the introduction to the thesis, containing general information on melanoma and its treatment. It provides an outline of this thesis and presents the main focus: combining surgery and systemic therapy to enhance melanoma treatment.

The first example of the synergy between surgery and systemic therapy is demonstrated in **chapter 2.** The REDUCTOR trial studied patients with locally advanced melanoma, who were classified as having unresectable disease and therefore not eligible for the standard of care in stage III melanoma: surgery. In this study, 21 patients received short-term induction therapy with BRAF/MEK inhibition, which resulted in sufficient downsizing of the tumor in the majority (18/21) of patients to perform surgery. Of the patients undergoing resection, 9 out of 18 had a pathological (near) complete response. The study described in **chapter 3** aimed to predict pathological response or recurrence by using ^{18}F-FDG PET/CT. Unfortunately ^{18}F-FDG PET/CT could not fulfill this aim, but did seem valuable in detecting recurrence early after radical resection.

As induction targeted therapy showed to be useful in paving the way to surgery in unresectable stage III melanoma, patients remain at high risk for recurrence despite radical resection. **Chapter 4** describes the efforts over the past decades of reducing this risk by using adjuvant systemic therapy, leading to the current standard of care: adjuvant immune checkpoint inhibitors and targeted therapy. These novel systemic therapies have improved recurrence-free survival (RFS) in stage III melanoma patients and overall survival (OS) benefit is thus far preserved to anti-CTLA-4 agent ipilimumab, as OS data of the remaining studies are pending.

However, a subset of patients does not benefit from adjuvant systemic therapy: either patients who would not have recurred without, or patients who recur despite adjuvant therapy. **Chapter 5** discusses the search for prognostic and predictive biomarkers in patients with macroscopic stage III melanoma to adequately select patients for tailored adjuvant therapy. A special target of interest was interferon-gamma (IFNγ), which plays an important role in the antitumor response in the tumor microenvironment and has shown to be predictive of response in patients treated with neoadjuvant immune checkpoint inhibitors. Our study showed that both in patients treated with surgery alone and in patients treated with surgery followed by adjuvant systemic therapy, an IFNγ high profile was correlated with increased RFS. Additionally, both patients with an IFNγ high and low profile showed improved RFS rates when treated with adjuvant systemic therapy, compared to surgery alone. Therefore IFNγ was identified as a prognostic biomarker, but unfortunately it did not predict response to adjuvant systemic therapy.

All previously mentioned chapters describe systemic therapy aiding surgical treatment in stage III melanoma patients, in whom surgery is standard of care. However, in stage IV

melanoma, the standard of care is systemic therapy. Over the past decades, novel systemic therapies have shown durable responses and have drastically changed prognosis in these patients. **Chapter 6** explores the role of surgery in patients with unresectable stage IIIC/IV melanoma showing oligoprogression or a durable partial response with remaining lesions after initial systemic therapy. Patients were included from the Dutch Melanoma Treatment Registry (DMTR), a nationwide database which registers all Dutch patients undergoing treatment for unresectable stage IIIC and IV melanoma. This study showed that selected patients can benefit from surgery after achieving initial disease control with systemic therapy.

Another study carried out with data retrieved from the DMTR, is described in **chapter 7**. In this study the value of sentinel lymph node biopsy (SLNB) in patients who progressed to advanced disease in due course, is evaluated. It showed that undergoing a SLNB, prior to the availability of adjuvant systemic therapy, had no survival benefit for patients developing subsequent advanced melanoma.

In the general discussion, **chapter 8**, arguments are stated to conclude that systemic therapy and surgery can be seen as synergistic treatment modalities in treating patients with stage III and IV melanoma. This is shown in systemic therapies paving the road to surgery in unresectable disease and preventing recurrences in high-risk melanoma, but also surgery aiding in diminishing disease activity after initial control to systemic therapy.

SAMENVATTING

Het inleidende **hoofdstuk 1** geeft een overzicht over de achtergrond en behandeling van melanoom. De hoofdlijnen en de primaire focus van het proefschrift worden in dit hoofdstuk gepresenteerd: het combineren van chirurgie en systeemtherapie om de behandeling van melanoom te verbeteren.

Het eerste voorbeeld van synergie tussen chirurgie en systeemtherapie wordt beschreven in **hoofdstuk 2.** In de REDUCTOR studie werden patiënten behandeld met lokaal gevorderd melanoom, die werden beschouwd als niet-resectabele ziekte en daarom niet in aanmerking kwamen voor de standaardbehandeling van stadium III melanoom: chirurgie. In deze studie ontvingen 21 patiënten kortdurend inductietherapie met BRAF/MEK remmers, waarbij de tumor in de meerderheid (18/21) van de patiënten voldoende afnam om alsnog een operatie te kunnen ondergaan. Van de patiënten die deze resectie ondergingen, hadden 9 van de 18 een pathologische (bijna) complete respons. De studie die wordt omschreven in **hoofdstuk 3** had als doel om de pathologische respons of het optreden van recidieven te voorspellen middels ^{18}F-FDG PET/CT. Helaas bereikte ^{18}F-FDG PET/CT dit doel niet, maar was wel waardevol in het detecteren van een vroeg recidief na een radicale resectie.

Eerder werd gezien dat inductie behandeling met doelgerichte therapie effectief was in het creëren van de mogelijkheid tot een (radicale) resectie bij eerder irresectabel stadium III melanoom. Ondanks een radicale resectie blijft er echter een aanzienlijk recidief risico bestaan bij alle patiënten met stadium III melanoom. **Hoofdstuk 4** geeft een overzicht van de onderzoeken die de afgelopen decennia zijn verricht om dit risico te verminderen middels adjuvante therapie en die hebben geleid tot de huidige standaardbehandeling van adjuvant immuun checkpointremmers en doelgerichte therapie. Deze systemische behandelingen hebben de recidiefvrije overleving van stadium III melanoom patiënten verbeterd. Voordelen voor algehele overleving zijn vooralsnog alleen aangetoond met het anti-CTLA-4 middel ipilimumab. Echter, er is een subgroep van patiënten die geen voordeel heeft van adjuvante systeemtherapie: zowel patiënten die ook zonder aanvullende behandelingen geen recidief hadden ontwikkeld, als patiënten die ondanks adjuvante behandeling alsnog een recidief krijgen. **Hoofdstuk 5** omschrijft een studie naar prognostische en voorspellende biomarkers bij patiënten met macroscopisch stadium III melanoom, om patiënten te kunnen selecteren voor adjuvante therapie op maat. In het bijzonder werd gekeken naar interferon-gamma (IFNγ), wat een belangrijke rol speelt in de antitumor reactie in de tumor microenvironment en een eerder aangetoonde voorspeller van respons is, bij patiënten die behandeld werden met neoadjuvant immuun checkpointremmers. Onze studie toonde aan dat bij zowel patiënten die alleen chirurgie ondergingen als patiënten die daarna ook adjuvante therapie ontvingen, een hoog IFNγ profiel gecorreleerd was met een verbeterde recidiefvrije overleving. Daarnaast hadden zowel patiënten met een hoog als laag IFNγ

profiel een verbeterde recidiefvrije overleving wanneer ze adjuvante systemische therapie ondergingen, vergeleken met chirurgie alleen. Hieruit bleek IFNγ een prognostische biomarker, maar was helaas niet in staat de respons op adjuvante therapie te voorspellen.

Alle bovengenoemde hoofdstukken omschrijven hoe systemische therapie de chirurgische behandeling van patiënten met stadium III melanoom (gouden standaard) kan ondersteunen. Echter, bij patiënten met stadium IV melanoom, bestaat de standaardbehandeling juist uit systemische therapie. De laatste decennia hebben nieuwe systemische behandelingen met kans op langdurige respons de prognose van deze patiënten drastisch verbeterd. **Hoofdstuk 6** bestudeert de rol van chirurgie bij patiënten met irresectabel stadium IIIC/IV melanoom met oligoprogressie of een langdurige partiële respons met resterende laesies, na initiële systemische therapie. Patiënten werden geïncludeerd vanuit de Dutch Melanoma Treatment Registry (DMTR), een nationale database waarin alle Nederlandse patiënten die behandelingen ondergaan voor irresectabel stadium IIIC en IV melanoom worden geregistreerd. Deze studie liet zien dat geselecteerde patiënten baat kunnen hebben bij lokale behandeling met chirurgie na initiële ziektecontrole middels systemische therapie.

Ook **hoofdstuk 7** omschrijft een studie uitgevoerd met data vanuit de DMTR. In deze studie wordt de waarde van een schildwachtklierprocedure bestudeerd, bij patiënten die in het verdere beloop progressie vertonen naar gevorderde ziekte. Deze studie toonde aan dat het ondergaan van een schildwachtklierprocedure, voorafgaand aan het tijdperk van adjuvante systemische therapie, geen overlevingswinst bood aan deze patiënten.

In de discussie, **hoofdstuk 8**, wordt beargumenteerd dat systemische therapie en chirurgie gezien kunnen worden als synergistische behandelmodaliteiten bij het behandelen van patiënten met stadium III en IV melanoom. Systemische behandeling kan chirurgie mogelijk maken bij patiënten met irresectabele ziekte en recidieven voorkomen bij hoog-risico melanoom na chirurgie. Daarnaast kan chirurgie zorgen voor het verdwijnen van ziekteactiviteit na initiële controle met systemische therapie.

Appendices

List of publications
Dankwoord
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LIST OF PUBLICATIONS

Publications in this thesis

Blankenstein SA, Rohaan MW, Klop WMC, van der Hiel B, van de Wiel BA, Lahaye MJ, Adriaansz S; Sikorska K, van Tinteren H, Sari A, Grijpink-Ongering LG, van Houdt WJ, Wouters MWJM, Blank CU, Wilgenhof S, van Thienen JV, van Akkooi ACJ, Haanen, JBAG. **Neoadjuvant cytoreductive treatment with BRAF/MEK inhibition of prior unresectable regionally advanced melanoma to allow complete surgical resection, REDUCTOR: a prospective, single arm, open-label phase II trial.** *Ann Surg 2021 Aug 1;274(2):383-389.*

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CURRICULUM VITAE

Stéphanie Anne-Marie Blankenstein was born on August 24, 1990 in Utrecht, the Netherlands. She grew up in Driebergen, where she attended the Coolsma primary school. At the Revius Lyceum in Doorn she followed a bilingual teaching program, obtaining the Cambridge First Certificate of English in 2005 and completing her high school education in 2008. In 2009 she started Medical School at the Vrije Universiteit Amsterdam, obtaining her medical degree in 2016. Subsequently, she worked as a resident not in training at the surgical oncology department of the Netherlands Cancer Institute/Antoni van Leeuwenhoek. Within the same department, Stéphanie had the opportunity to start as a PhD candidate under the supervision of Dr. Alexander van Akkooi and Prof. dr. John Haanen in 2018, studying the combination of surgery and systemic therapy in melanoma, as described in this thesis.

Following three years of fulltime research, she returned to the clinic as a surgical resident not in training at Gelre Ziekenhuizen and Meander Medisch Centrum, before achieving a surgical resident in training position in 2023 at the Universitair Medisch Centrum Utrecht and Meander Medisch Centrum Amersfoort.