The management of early and late stage melanoma in the modern era

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Daan Jan Willem Rauwerdink

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Management of early and late-stage melanoma in the modern era

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Prof. dr. M.W. Bekkenk[5]

Dr. D.J. Grünhagen[6]

Dr. A.A.M. van der Veldt[7]

Author affiliations

1. Department of Surgical Oncology, Leiden University Medical Center (LUMC), Leiden, The Netherlands.

2. Department of Dermatology, Leiden University Medical Center (LUMC), Leiden, The Netherlands.

3. Department of Medical Oncology, Leiden University Medical Center (LUMC), Leiden, The Netherlands.

4. Department of Surgical Oncology, Netherlands Cancer Institute (NKI), Amsterdam, The Netherlands

5. Department of Dermatology, Amsterdam University Medical Centers (Amsterdam UMC), Amsterdam, The Netherlands.

6. Department of Surgical Oncology, Erasmus Medical Center, Rotterdam, The Netherlands.

7. Department of Medical Oncology, Erasmus Medical Center, Rotterdam, The Netherlands.

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General Introduction

Epidemiology

Melanoma is a malignancy originating from the malignant transformation of melanocytes, skin residing cells that produce melanin pigment. It is the most aggressive form of skin cancer, characterized by its potential for rapid metastasis and high mortality rate if not diagnosed and treated in an early stage.

The incidence of melanoma has increased significantly over the past 50 years, with approximately 325 000 cases globally in 2020.[1,2] In 2020, a total of 150,627 patients in Europe were diagnosed with melanoma, of whom 26,360 patients died from the disease.[3] In the Netherlands, an estimated 7,530 individuals were diagnosed with melanoma in 2021, and approximately 788 patients died from melanoma. Importantly, as the incidence of melanoma is increasing, it is anticipated that more patients will die from melanoma in the coming decades, despite improved therapeutic options.[4]

The main environmental risk factor for melanoma development is exposure to ultraviolet light radiation, a ubiquitous mutagen that induces damage to the DNA in skin cells. Intermittent sun exposure and a history of sunburn in childhood is associated with an increased risk of melanoma development.[5-9] Clinical risk factors associated with an increased risk of melanoma development include pale skin, blue eyes, red hair, more than five dysplastic or 100 common naevi, pre-existing sun-damaged skin (actinic keratosis), a history of skin cancer, and more than five sunburns in childhood.[7]

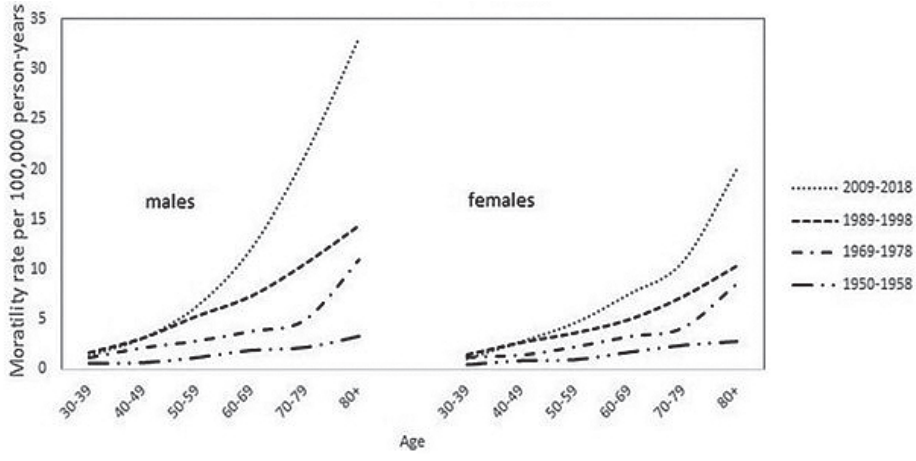

Figure 1. Mortality curves of cutaneous melanoma per 100,000 person–years by age and 10-year calendar periods from 1950–2018 in men and women in the Netherlands – figure derived and adapted from 'van Niekerk et al. Trends and projections in cutaneous melanoma death in the Netherlands from 1950 to 2045. Medicine (Baltimore). 2021 Dec 3;100(48):e27784.'

In addition, a family history of melanoma increases the risk of melanoma development. Approximately 10% of patients diagnosed with melanoma have a positive family history of this malignancy.[9] The most important pathogenic mutation that plays a role in familial melanoma is the pathogenic germline *CDKN2A* mutation. This mutation is identified in 20-40% of individuals with hereditary melanoma. This pathogenic germline mutation is associated with a lifetime risk of merely 70% for developing melanoma.[10] Other identified pathogenic mutations in melanoma predisposition genes, such as *CDK4*, *BAP1*, *TERT*, *POT1*, *ACD*, *TERF21P*, and *MITF*, contribute to another 10% of the causes of hereditary melanoma.[11-17]

Clinical diagnosis

Clinical diagnosis of melanoma involves a comprehensive assessment of skin lesions through visual inspection, dermoscopic evaluation, and histopathological examination. Dermatologists first perform a thorough skin examination to identify suspicious lesions based on asymmetry, border irregularity, color variation, diameter, and evolving characteristics (ABCDE criteria) (figure 2a-b). Atypical lesions commonly harbor at least three of these so-called ABCDE features.[19] However, lesions showing only one ABCDE feature might still be malignant. The outlier or standing-out nevus concept can aid in identifying melanomas: individual patients tend to have their own melanocytic nevus pattern, while melanomas do not fit the individual nevus pattern.[20] In addition to physical examination, dermoscopy enhances visualization of sub-surface skin structures, aiding in the differentiation of benign and malignant lesions (Figure 2c). Confirmatory diagnosis is achieved through excision and histopathological analysis, which identify malignant melanocytes and assess tumor depth and other prognostic features (Figure 2d).[21]

Pathogenesis

Intermittent exposure to ultraviolet radiation causes genetic damage and induces the formation of DNA-damaging reactive oxygen species that affects melanocytes and keratinocytes.[5] Ultraviolet radiation results in a wide range of mutations in DNA, reflecting the high mutational tumor burden that melanoma might have.[22]

The main oncogenic driver mutations in melanoma are *BRAF* (*V600E/K*) and *NRAS* (*Q61R/K*), which result in the hyperactivation of the *MAPK* signaling pathway (figure 3).[22,23] Besides these oncogenic driver mutations, other DNA alterations in tumor suppressor genes can occur, such as *CDKN2A, TP53, PTEN,* and *NF1* mutations.[23,24]

Regarding specific subtypes of melanoma, the subtype lentigo maligna melanoma exhibits a high mutation burden due to chronic ultraviolet radiation exposure, and is often associated with

mutations in the *NF1* gene. In contrast, acral and mucosal melanoma are not associated with chronic ultraviolet radiation exposure, has a low tumor mutation burden, and is more frequently *KIT*-mutated[25].

Understanding the molecular background of cutaneous melanoma is crucial, as it provides insights into oncogenic driver mutations and tumor suppressor genes. Such understanding could help explain the mechanisms responsible for therapy-acquired resistance, paving the way for more effective and personalized treatments.

Clinical overview of cutaneous melanoma.

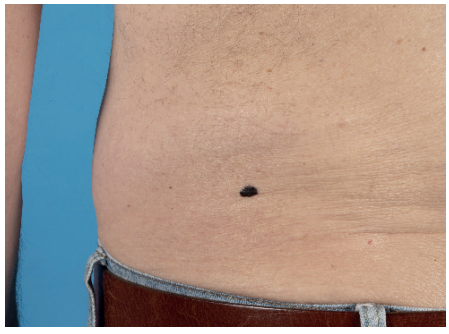

Figure 2a.
Macroscopy of pigmented lesion on the lower trunk.

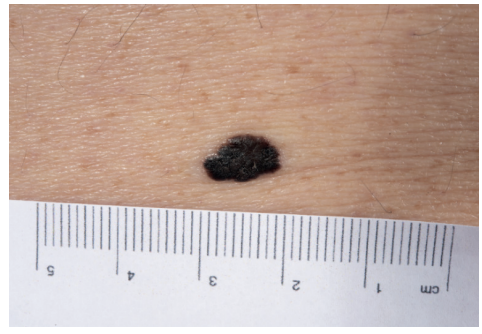

Figure 2b.
Detailed photo shownig a dens pigmentated plaque.

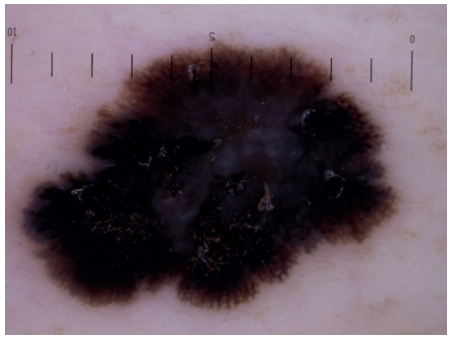

Figure 2c.
Dermoscopy: blue white veil and radiant streaks.

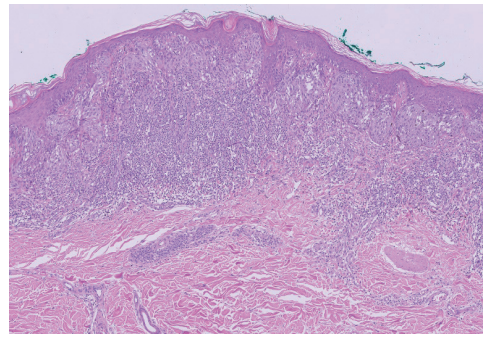

Figure 2d.
Histology: atypical melanocytes, mitotic figures, invasive growth.

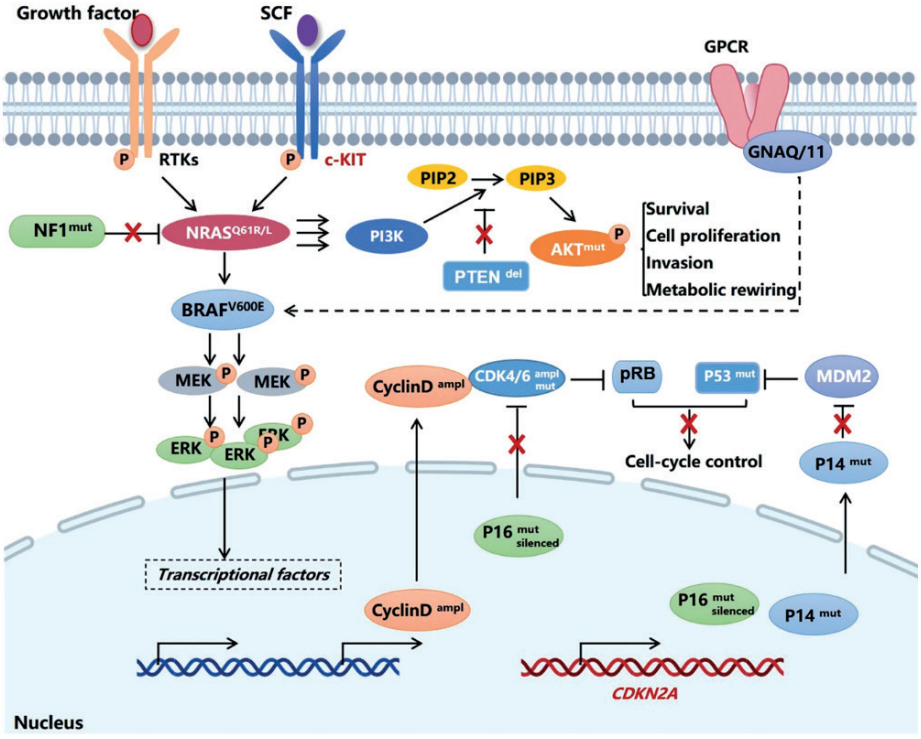

Figure 3. Overview of signal pathway and mutations in driver in melanoma.
Figure adapated from Guo, W., Wang, H. & Li, C. Signal pathways of melanoma and targeted therapy. Sig Transduct Target Ther 6, 424 (2021).

Melanoma Subtypes and Mutations

It is important to assess the exact histologic subtype of melanoma, as the histologic subtype can potentially play a prognostic role in disease recurrence.[26-29] The two major histologic subtypes are superficial spreading melanoma (SSM), covering 70% of the melanoma cases, followed by nodular melanoma (NM) with approximately 20% of the cases. The remaining melanoma cases are of the histologic subtype lentigo maligna melanoma (3-10%), and the subtypes acral melanoma, desmoplastic, and spitz melanoma (1-2%) are less common.[30,31]

NM has worse prognostic tumor characteristics, including a higher Breslow thickness, more often presence of ulceration, higher dermal mitotic rate, and more frequent satellite lesions, compared to SSM.[27,32,33] Primary NM, even corrected for Breslow thickness and the presence of ulceration, is associated with a lower overall survival and a reduced recurrence-free survival rate compared with primary SSM.[34,35]

Besides morphological and histopathological classification, melanoma can be classified based on its genetic mutation profile as well. SSM is most frequently associated with *BRAF* (50%) and *NRAS* (30%) mutations, while the NM subtype is more frequently *NRAS* mutated (50%), and less often associated with *BRAF* mutations (30%).[36] The subtypes acral and mucosal melanoma have a lower mutational rate and are more frequently *KIT* mutated, while desmoplastic melanoma carries the *NF1* mutation more frequently.[11,22,36] It is essential to understand the distinct mutational differences between the melanoma subtypes, as specific mutations and mutational profiles can significantly impact disease prognosis and therapy related outcomes.

Management

In case of suspicious pigmented skin lesions, the therapeutic approach is to perform a diagnostic excision with a 2 mm margin.[37] The diagnosis of melanoma is made based on histopathological assessment.[38] Upon confirmation of melanoma diagnosis, a wide surgical excision is performed with safety margins according to the Breslow thickness of the lesion: for melanomas with a Breslow thickness of less than 2 mm, the margin is 1 cm, and for thicker melanomas, the margin is 2 cm.[37,39]

Further, a sentinel lymph node biopsy, a surgical procedure in which the draining lymph node basin is assessed for metastatic lymphogenic disease involvement, is considered in melanoma cases with a Breslow thickness equal to or greater than 0.8mm, or if ulceration is present.[40,41] The sentinel lymph node biopsy gives an insight into disease prognosis and can have therapeutic consequences.

Historically, the Multicenter Selective Lymphadenectomy Trial-I (MSLT-I) demonstrated that patients with intermediate thickness or thick primary melanoma had an improved locoregional control upon immediate completion lymph node dissection (CLND). Therefore, local guidelines mandated complete lymph node dissection and this was considered the standard of care.[41] In 2017, the results of the MSLT-II trial revolutionized the surgical management of positive sentinel lymph node procedures and ultimately waived CLND. The MSLT-II trial demonstrated that close observation of patients with resected melanoma who had low-burden stage 3 disease and underwent nodal surveillance resulted in a melanoma-specific survival similar to that of patients who underwent immediate CLND.[42] Therefore, in case of a positive sentinel lymph node biopsy, it is advised to only dissect metastatic (e.g., positive) lymph nodes, and complete lymph node dissection procedures are only performed on a case to case basis.

Regarding additional radiological assessment, in case of a positive sentinel lymph node, it is advised to perform additional radiological investigation with a PET-CT scan to rule out distant metastasis. Further, in patients with thicker melanomas (Breslow thickness greater than 4.0mm) regardless of lymph node involvement, additional radiological assessment can be considered.

Staging and prognosis

Melanoma is staged according to the eighth edition of the American Joint Committee on Cancer (AJCC) melanoma staging system, which consists of tumor thickness (T), lymph node involvement (N) and distant metastasis (M) (table 1 and 2). The primary tumor thickness is subdivided into 8 categories (T1a-T4b) and lymph node involvement is further subdivided into 9 categories (N1a – N3c). The anatomic site of metastatic disease determines the grade of the subcategory M (table 1).[43]

Tumor (T)	**Tumor thickness (mm)**	**Ulceration**
T1a	<0.8	
T1b	<0.8	Present
	0.8 - 1.0	Present or absent
T2a	>1.0 - 2.0	Absent
T2b	>1.0 - 2.0	Present
T3a	>2.0 – 4.0	Absent
T3b	>2.0 – 4.0	Present
T4a	>4.0	Absent
T4b	>4.0	Present
Lymph node (N)	**No. Of involved regional lymph nodes**	**Metastasis type**
N0	0	Absent
N1a	1	Clinically occult
N1b	1	Clinically detected*
N1c	0	In-transit, satellite and or microsatellite metastasis
N2a	2-3	Clinically occult
N2b	2-3	Clinically detected
N2c	1	In-transit, satellite and or microsatellite metastasis
N3a	>3	Clinically occult
N3b	>3	Clinically detected
N3c	>1	In-transit, satellite and or microsatellite metastasis
Metastasis (M)	**Anatomic site**	
M0	No distant metastasis	
M1a	Distant metastasis to skin, soft tissue including muscles, and/or nonregional lymph node	
M1b	Lung metastasis with or without M1a sites of disease	
M1c	Non central nerve system visceral sites with or Without M1a or M1b sites of disease	
M1d	Metastasis to central nerve system with or without M1a, M1b, or M1c sites of disease	

Table 1. TNM staging categories.

** Lymph nodes are designated as 'clinically detectable' if they can be palpated on physical exam and are confirmed melanoma by pathology following excision/biopsy.*

Clinical Stage Group	T	N	M
0	Tis	N0	M0
IA	T1a	N0	M0
IB	T1b	N0	M0
	T2a	N0	M0
IIA	T2b	N0	M0
	T3a	N0	M0
IIB	T3b	N0	M0
	T4a	N0	M0
IIC	T4b	N0	M0
IIIA	T1a/b-T2a	N1a, N2a	M0
IIIB	T0	N1b, N1c	M0
	T1a/b-T2a	N1b/c or N2b	M0
	T2b/T3a	N1a-N2b	M0
IIIC	T0	N2b, N2c, N3b, or N3c	M0
	T1a-T3a	N2c or N3a/b/c	M0
	T3b/T4a	Any N ≥N1	M0
	T4b	N1a-N2c	M0
IV	Any	Any	M1
	Skin, soft tissue including muscles, and/or nonregional lymph node		M1a
	Lung metastasis with or without M1a sites of disease		M1b
	Non-CNS visceral metastasis with or without M1a or M1b sites of disease		M1c
	Metastasis to CNS with or without M1a, M1b, or M1c sites of disease		M1d

Table 2. AJCC clinical prognostic stage groups (8th edition).

The Breslow thickness of the primary melanoma is an important prognostic factor, as the 5-year overall survival of thin melanoma (T1a) is considered good with an overall survival of 99%, while the 5-year survival of thicker melanoma (T4b) with no lymph node involvement is associated with a 5-year survival of 82%.[44]

Regarding lymph node involvement, solely microscopic metastatic lymph node disease, stage IIIA, has a relatively good prognosis with a 5-year survival of 84%, while presence of macroscopic lymph node involvement, stage IIIC and stage IIID reflects a relatively poor outcome with a 5-year overall survival of 69% and 32%, respectively (figure 5).[44]

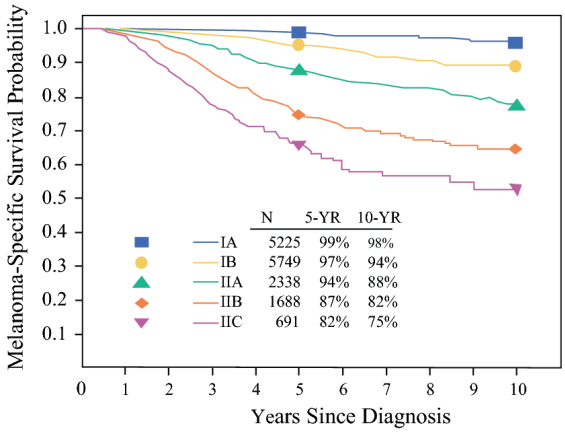

Figure 4. Melanoma stage specific survival per AJCC 8th edition. Figure adapted and derived from 'Gershenwald et al. Melanoma Staging: American Joint Committee on Cancer (AJCC) 8th Edition and Beyond. Ann Surg Oncol 25, 2105–2110 (2018).

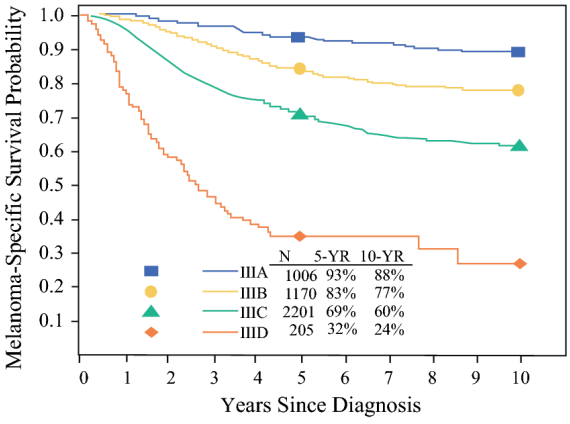

Figure 5. Melanoma specific survival in stage III disease. Figure adapted and derived from 'Gershenwald et al. Melanoma Staging: American Joint Committee on Cancer (AJCC) 8th Edition and Beyond. Ann Surg Oncol 25, 2105–2110 (2018).

Systemic therapy

Immune checkpoint inhibition

The prognosis for metastatic melanoma used to be poor, with a survival rate estimated at just six to nine months in 2000.[45,46] However, the introduction of immune checkpoint therapies has significantly changed the treatment landscape for patients with stage III and IV melanoma. To illustrate this, the FDA approved immune checkpoint inhibition therapy with monoclonal antibody to Cytotoxic T lymphocyte-associated antigen (anti-CTLA-4), as a clinical phase 3 trial demonstrated in 2010 an improved median overall survival of 20 months at a follow-up of 5

year for metastatic melanoma patients.[47] Subsequently, the CheckMate 066 trial published in 2015, demonstrated the effectiveness of the monoclonal antibody against programmed death 1 protein (anti-PD-1, nivolumab) in metastatic melanoma patients without a *BRAF* mutation, and was associated with a median 5-year overall survival of almost 37 months compared to a median 5-year survival of 13 months in patients treated with dacarbazine.[48]

In addition, the KEYNOTE-006 trial showed that the anti-PD-1 antibody pembrolizumab improved overall survival in stage IV melanoma compared to ipilimumab, with a median 5-year overall survival of 39 months and 17 months, respectively.[49]

The combination of ipilimumab and nivolumab has improved outcomes even further, with a higher response rate and a significantly longer median overall survival in patients with metastatic melanoma.[50] To illustrate this, the CheckMate 067 trial investigating the efficacy of ipilimumab/nivolumab combination therapy in metastatic melanoma patients, compared to nivolumab monotherapy and ipilumumab monotherapy, demonstrated a median 5-year overall survival of 52 months for ipilumumab/nivolumab combination therapy, compared to 37 months in the nivolumab monotherapy group and 20 months in the ipilimumab group.[51] These advancements in immune checkpoint therapy have greatly improved the survival rates for patients with advanced melanoma.

Targeted therapy

In addition to checkpoint inhibitors, the introduction of *BRAFV600* inhibitors also revolutionized therapeutic options and survival outcomes. In 2011, a trial with the *BRAF* inhibitor Vemurafenib was associated with an improved response of 84% compared to 64% in the dacarbazine group.[52] Final overall survival results with a median follow-up 50 months demonstrated a median overall survival of 14 months in Vemurafenib treated patients compared to 10 months in dacarbazine treated patients.

Subsequently, the study with vemurafenib and cobimetinib (*MEK* inhibitor) demonstrated that the combination therapy of a *BRAF*- and *MEK* inhibitor was superior to *BRAF* inhibition alone.[53] The median 5-year overall survival for patients receiving cobimetinib plus Vemurafenib was 22.3 months, whereas advanced melanoma patients receiving placebo plus vemurafenib had a median overall survival of 17.4 months.

Consequently, newly initiated trials aimed at developing *BRAF/MEK*i combination therapies. The *BRAF/MEK*i COMBI-AD trial investigating combination of dabrafenib and trametinib (*BRAF/MEK*i) exhibited a median 5-year overall survival of 26 months at a follow-up period of 22 months.[54] In 2018, the COLUMBUS trial demonstrated that the combination of encorafenib/binimetinib in

advanced melanoma is effective and prolonged survival in patients with metastatic melanoma, with a median overall survival of 34 months in the encorafenib plus binimetinib group; 24 months in the encorafenib group, and 17 months in the vemurafenib group, at a follow-up period of 37 months.[55,56]

Novel therapies

Adoptive cell therapy (ACT) using tumor-infiltrating lymphocytes (TILs) is a promising approach to treating metastatic melanoma.[57] This therapy involves isolating TILs from a patient's tumor, expanding them in a laboratory, and then infusing the TILs back into the patient to counter the tumor. A study published in 2022 by Rohaan and colleagues demonstrated that TIL therapy significantly improved overall free survival in patients with advanced melanoma refractory to anti-PD-1 therapy compared to patients who received ipilimumab monotherapy.[58] At a median follow-up period of 33 months, the median overall survival was 25.8 months among patients in the TIL group as compared to 18.9 months among patients who received ipilimumab. This study is significant as it is the first randomized clinical trial to demonstrate the effectiveness of TIL therapy in advanced melanoma.

Another promising novel therapy for melanoma is the use of lymphocyte-activation gene (LAG-3) checkpoint inhibitors, such as relatlimab.[59] LAG-3 is a protein expressed on the surface of T cells, and its blockade can enhance anti-tumor immune responses. The RELATIVITY-047 trial showed improved survival outcomes in previously untreated metastatic melanoma patients treated with relatlimab combined with anti-PD-1 nivolumab. The RELATIVITY-047 trial demonstrated an improved median progression-free survival of 10.1 months in patients treated with relatlimab-nivolumab compared to a progression-free survival of 4.6 months in patients who received nivolumab monotherapy, at a follow-up period of 12 months.[60] Notably, patients with a PD-L1 expression of 1% or less had the most benefit of relatlimab-nivolumab combination therapy, while no difference was observed in the treatment groups in patients with a PD-L1 expression of 1% or more. Hence, this therapy seems to be suitable for patients with a lower PD-L1 expression. Regarding treatment related toxicity, the combination was well-tolerated with manageable side effects.

More studies are currently underway to explore the utilization of personalized vaccines, combination therapies, and innovative strategies in countering melanoma. As the understanding of complex interactions between the immune system and melanoma cancer continues to evolve, more effective and personalized treatments are anticipated.

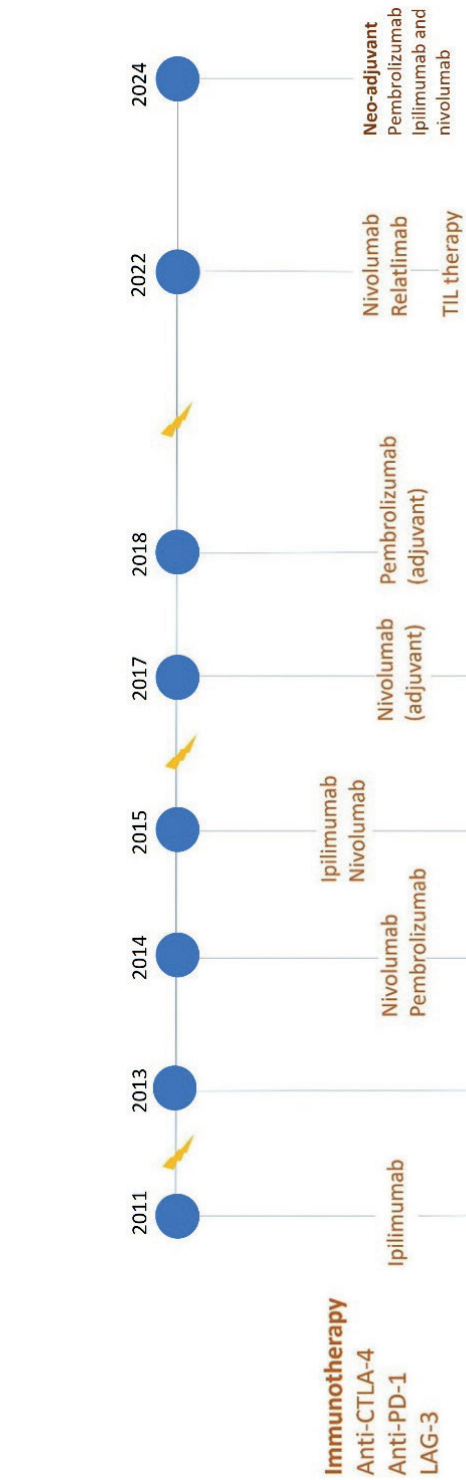

Figure 6. Timeline approved novel therapies for cutaneous melanoma.

Adjuvant therapy

Stage III

The FDA approved adjuvant ipilimumab in 2015 for resected stage III and IV melanoma, as adjuvant ipilimumab was associated with a 5-year rate of recurrence free survival of 40% compared to 30% in the placebo group ($P<0.01$).[61] However, the severe toxicity associated with the high dose of ipilimumab limited its widespread use. Adjuvant nivolumab was FDA approved in 2017 and was associated with an improved recurrence free survival of 71% compared to 61% in the ipilimumab group at a follow-up period of 18 months.[62] Further, the CheckMate 238 trial published in 2017, investigating adjuvant ipilimumab versus adjuvant nivolumab in resected stage III or IV melanoma patients, demonstrated an improved 12-month recurrence free survival of 70% in the adjuvant nivolumab arm and 61% in the adjuvant ipilimumab group.

In 2018 adjuvant pembrolizumab was approved, as this treatment was associated with a recurrence free survival rate of 75.4% at a 12-month survival period versus 61% in the placebo group. Despite these improved local recurrence free survival rates, to date no improved overall survival has yet been reported for adjuvant nivolumab and pembrolizumab, and a longer period of follow-up is required.[63]

Equally, the combination therapy with *BRAF/MEK* inhibitors dabrafenib/trametinib in the adjuvant setting demonstrated a 5-year relapse-free survival rate of 61% in the combination group compared to 43% in the placebo group ($P<0.01$), and the percentage of patient who were alive without distant metastasis was 65% in the dabrafenib/trametinib group compared to 54% in the placebo group.[64] Similar to adjuvant anti-PD-1 treated patients, no prolonged overall survival has been shown up till now, and a longer follow-up is required.

Stage II

The FDA approved the use of adjuvant nivolumab and pembrolizumab for resected stage IIB/C in 2022 and 2023 based on the KEYNOTE-716 trial and CheckMate-76K trial, respectively. The KEYNOTE-716 demonstrated an improved 12-month recurrence free survival of 90% in adjuvant pembrolizumab treated stage IIB/C melanoma patients compared to 83% in the placebo group. The CheckMate-76K trial investigating the risk of disease recurrence in resected stage IIB/C melanoma, showed an improved 12-month recurrence free survival for neo-adjuvant nivolumab (89%) compared to patients who received placebo treatment only (79%).

Additionally, the COLUMBUS-AD study was designed to assess the efficacy of adjuvant encorafenib/binimetinib in stage IIB and IIC melanoma. However, this study was closed prematurely due to slow accrual.[65]

Trial	No. patients	Stage	Adjuvant trial arms	Regimen	RFS, (HR; p)
EORTC-18071	951	IIIA, IIIB, IIIC	Ipilimumab 10 mg/kg *versus* placebo	IV 10 mg/kg every 3 weeks for 4 doses, then every 3 months for up to 3 years	3-year RFS 47% versus 35% (0.76; p<0.01)
CheckMate-238	906	IIIB, IIIC, IV	Nivolumab versus ipi 10 mg/kg	IV 3 mg/kg nivolumab every 2 weeks up to 1 year OR IV ipi 10 mg/kg every 3 weeks for 4 doses and then every 12 weeks up to 1 year	12-month RFS 71% versus 61% (0.65; p<0.01)
KEYNOTE-054	1019	IIIA, IIIB, IIIC	Pembrolizumab versus placebo	IV 200 mg pembrolizumab every 3 weeks up to 18 doses	12-month RFS 75% versus 61% (0.57; p<0.01)
COMI-AD	870	IIIA, IIIB, IIIC	Dabrafenib + trametinib versus placebo	Dabrafenib 150 mg twice per day + trametinib 2 mg once per day up to 1 year	Not reached versus 16.6 (0.51; p<0.01)
KEYNOTE-716	976	IIB, IIC	Pembrolizumab versus placebo	IV 200mg pembrolizumab every 3 weeks for 17 cycles	12-month RFS 90% versus 83% (HR 0.65; p<0.01)
CheckMate-76K	790	IIB, IIC	Nivolumab versus placebo	IV 480 mg or placebo every 4 weeks for 12 months	12-month RFS 89% versus 79% (HR 0.42; p < 0.01)

Table 3. Demonstrating key trial results in adjuvant treated melanoma patients.

These relatively recently approved adjuvant therapies have improved recurrence free survival of adjuvant treated stage II, III and IV melanoma patients. Despite improved local regional disease control in adjuvant treated patients, no overall survival benefit has been shown. Patients with a limited disease load (e.g. stage IIIa) can therefore be excluded from adjuvant treatment, as these patients have considerable good outcomes regardless of adjuvant treatment, and adjuvant therapy might lead to unnecessary exposure to immune-related adverse events, which can be long lasting after treatment with checkpoint inhibitors.[66,69] Additionally, cost-effectiveness analyses and the numbers needed to treat are important to make final decisions about approval of drugs in the early setting of melanoma.[70,71,72] Therefore, adjuvant anti-PD-1 therapy for stage II and stage IIIa melanoma patient, is not widely given nor adopted in clinical practice in the Netherlands. In *BRAF*-positive patients, adjuvant dabrafenib and trametinib might be an alternative therapy, since this treatment does not induce long lasting side-effects.

Neo-adjuvant therapy

In succession to the introduction of novel adjuvant therapeutic options, clinical trials investigating immune checkpoint inhibition, prior to surgery, in the neo-adjuvant setting, have demonstrated promising results.[73] The neo-OpACIN trial assessed the efficacy of the neo-adjuvant nivolumab/ipilimumab versus the same combination in the adjuvant setting in high-risk stage III melanoma, and showed that patients with a pathological response to neo-adjuvant nivolumab/ipilimumab had an improved recurrence-free survival when compared to patients treated in the adjuvant setting.[74] The estimated 5-year RFS rate was 70% for the neoadjuvant arm and 60% for the adjuvant arm. Above all, more T cell clones were present in the neo-adjuvant group than in the adjuvant application group, illustrating the efficacy of inducing immune responses in neo-adjuvant therapy.

In 2020, the PRADO trial reported similar results, with a pathologic response rate of 77% to neo-adjuvant ipilimumab/nivolumab in stage III melanoma, demonstrating the efficacy of immune checkpoint inhibition in the neo-adjuvant setting.[75] Furthermore, at a median follow-up of 28 months, an estimated 2-year recurrence-free survival was 85%.

Additionally, a phase 2 trial investigating (SWOG-trial) neo-adjuvant plus adjuvant versus adjuvant pembrolizumab in clinically detectable, measurable stage IIIB to IVC melanoma revealed a prolonged event-free survival for patients who received neo-adjuvant plus adjuvant pembrolizumab compared to adjuvant-treated patients. Event-free survival at 2 years was 72% in the neoadjuvant-adjuvant group and 49% in the adjuvant-only group.[76] Other trials have demonstrated similar results, implicating the potential neo-adjuvant immune checkpoint inhibition could have.[77]

The NADINA-trial investigated the efficacy of neoadjuvant ipilimumab and nivolumab versus adjuvant nivolumab in macroscopic stage III melanoma (IIIB,IIIC,IIID). In this randomized controlled trial, patients underwent therapeutic lymph node dissection either upon completing neoadjuvant therapy or upfront adjuvant nivolumab therapy. Patients with no major pathological response to neo-adjuvant immunotherapy received additional adjuvant nivolumab. At a follow-up period of 10 months, the 12-month recurrence free survival was 84% and 57% for neo-adjuvant and adjuvant treated patients, respectively. Patients with a major pathological response to neo-adjuvant immunotherapy had a recurrence-free survival of 95%, suggesting that no additional adjuvant therapy is required for these patients.

Although these promising results, a longer follow-up period is needed in order to determine the long term efficacy of neo-adjuvant therapy in treated macroscopic stage III melanoma patients.

Concerning targeted therapy, conducted phase II studies have demonstrated that neo-adjuvant *BRAF/MEK*i with dabrafenib/trametinib improves recurrence free survival in high risk resectable stage III/IV melanoma.[78,79] The phase II NeoCombi study demonstrated that of 35 patients with *BRAF* mutated stage III/IIIC melanoma patients who received neo-adjuvant *BRAF/MEK* inhibition with dabrafenib/trametinib, followed by surgical resection, resulted in a pathological response in 30 (86%) patients.[80] Relapse-free survival at twelve months was 77%, and 24 months relapse-free survival was 43% in the total population. Similarly, the REDUCTOR study in 2021 showed that neo-adjuvant dabrafenib/trametinib can reduce the tumor load significantly, as 17 of 21 treated patients (81%) with unresectable melanoma were eligible for radical resection upon neo-adjuvant therapy completion.[81]

Adverse events

Despite improved outcomes in the (neo-)adjuvant and advanced setting, immune checkpoint inhibition can induce serious immune-related adverse events (irAEs).[82,83] Harmful and life-threatening irAEs can occur, including colitis, hypophysitis, adrenalitis, hepatitis, toxic epidermal necrolysis, and Guillain-Barré.[84,85] These adverse events can occur at any time during therapy and even after completing the treatment course. The majority of the adverse events will resolve eventually with immune suppression; however, some side effects will endure, especially the endocrine side effects.[83,87] Since these irAEs can have a significant impact on the quality of life, the therapy decision-making by the medical oncologist of immune checkpoint inhibition initiation is considered on a case-by-case basis and is done in the context of clinical condition, comorbidity, overall disease load, disease stage, location of metastases, and *BRAF* mutation status, and not all patients will receive treatment beforehand, especially in the adjuvant setting.

Although treatment-related adverse events can be difficult for the patients to handle, the occurrence of specific types of adverse events might resemble whether the patient is responding to therapy or not.[88,89,90] As an example, patients who develop vitiligo or specific endocrine immune-related adverse events have favorable survival outcomes compared to patients who do not develop vitiligo.[91] Regarding the management of adverse events, these are often treated with oral glucocorticoids with or without other second-line immunosuppressants.[86] The dosage of glucocorticoids must be determined carefully, as administering a higher dosage of glucocorticoids for a longer period of time might have its side effects of their own. Importantly, the type of immunosuppressive agent, dosage, and duration of immunosuppression should be assessed thoroughly, as specific immunosuppressors, such as anti-TNF-a, might decrease the efficacy of immunotherapy and have been associated with a decreased survival in melanoma patients treated with immune checkpoint inhibitors.[92]

BRAF/MEKi related adverse events include pyrexia, fatigue, headache, nausea, chills, and diarrhea. Other more serious side effects of *BRAF/MEK* inhibitors include a decreased ejection fraction and chorioretinopathy.[93,94,95] These adverse events will cease with therapy discontinuation. Lastly, the total incidence of treatment related adverse events in *BRAF/MEK* inhibition therapy is higher than in immunotherapy treated patients, yet lower in severity and is more often short-lasting.[96]

Response monitoring

The Response Evaluation Criteria in Solid Tumors (RECIST criteria 1.1) have been introduced to monitor tumor response to systemic therapy in advanced disease. RECIST response is subdivided into complete response (CR, 100% disappearance of (target) lesions), partial response (PR, $\geq$ 30% decrease in target lesions), progressive disease (PD, $\geq$ 20% increase of target lesions, appearance of one or more lesions or unequivocal progression of existing non-target lesions), and stable disease (SD, between $<$ 30% reduction in target lesions and $<$ 20% increase in target lesions) (table 4).[97,98,99] In addition to standard radiological disease monitoring, patients are evaluated clinically while on treatment in order to rule out skin or subcutaneous disease growth, and LDH testing can be performed in order to evaluate disease activity.

Optimal treatment monitoring consists of a multidisciplinary approach, including clinical, radiological and laboratory disease evaluation. Patients should be discussed in case of doubtful results in a multi-disciplinary meeting with specialists from oncologic surgery, medical oncology, radiology, neurology, radiotherapy, ENT and dermatology departments.

Response	Target lesions	Non-target	New lesions
CR	Disappearance of all target lesions	Disappearance of all non-target lesions	No
	Lymph node axis < 10mm	Normalization of tumor marker levels	
PR	30% ≥ decrease in SLD from baseline	No progression	No
PD	≥ 20% increase in SLD from Nadir* with an absolute SoD increase ≥ 5mm	Unequivocally progression in lesion size	Yes, appearance of new unequivocally metastatic lesions
SD	Neither PR nor PD with the Nadir as reference point	Persistence of one or more non-target lesions and/or tumor marks > normal	No

Table 4. Demonstrating response according to RECIST 1.1 criteria. CR: complete response. PR: partial response. PD: progressive disease. SD: stable disease.

* In target lesions, smallest sum of the longest diameter

Aims and outline of this thesis

Melanoma is the most aggressive form of skin cancer, with an increasing incidence and significant mortality despite advances in therapeutic options. Early detection and accurate diagnosis remain crucial for improving patient outcomes. Genetic predisposition, environmental risk factors such as UV exposure, and molecular mutations contribute to melanoma development and progression. Recent advancements in systemic therapy, including immune checkpoint inhibitors and targeted therapies, have revolutionized treatment, yet challenges remain in identifying patients who will benefit most from these therapies.

This thesis aims to explore the molecular, clinical, and prognostic factors that influence melanoma detection, treatment response, and patient survival. By investigating key biomarkers, histologic subtypes, and therapeutic strategies, we seek to enhance diagnostic accuracy and optimize personalized treatment approaches for melanoma patients.

Chapter 2

Large observational studies demonstrate an association between the presence of atypical naevi (AN) and the risk on melanoma development, however, the actual benefit of dermatological surveillance for patients with multiple AN is less clear. Also, it is unknown what specific clinical characteristics are associated with melanoma development in this patient group and, it is unclear whether a melanoma develops from a precursor naevus or from normal appearing skin in these patients.

To shed light on this topic, we evaluated the incidence of melanoma diagnosis during periodic surveillance of patients with multiple atypical naevi or more than 100 common naevi (AN) and analyzed the clinical characteristics associated with melanoma development in this patient group.

Chapter 3

Individuals with a pathogenic germline *CDKN2A* mutation have an estimated life time risk on melanoma development of approximately 70%. In these patients at risk, it is important to assess whether melanoma arises from a pre-existent naevus or develops from normal appearing skin, as this can provide new insights in the clinical management and surveillance of (atypical) naevi.

To answer this question, we analyzed total body photograph in *CDKN2A* germline mutation carriers to detect whether melanoma develops from a preexisting naevus or from normal-appearing skin.

Chapter 4

Published trials investigating the efficacy of immunotherapy in the adjuvant setting mandated completion lymphadenectomy as standard of care for sentinel lymph node biopsy positive disease. As the MSLT-II study demonstrated that an immediate CLND did not improve survival rates, the management strategy to defer CLDN has been widely adopted and is the preferred pathway in the National Comprehensive Cancer Network (NCCN) guidelines. Due to the rapid and concurrent changes in both surgical practice and adjuvant medical therapies, the adjuvant outcomes of patients treated according to the current MSLT-2 guidelines has not been previously assessed.

Chapter 4 reports and discusses the outcomes for adjuvant anti-PD-1 and adjuvant *BRAF/MEK*i treated resected stage III-IV melanoma patients, upon the adoption of the MSLT-2 nodal management strategy.

Chapter 5

The frequency and intensity of adverse events associated with anti-PD-1 therapy (irAEs) differ among melanoma patients receiving adjuvant or advanced treatment, as observed in clinical trial safety analyses. In chapter 5, the incidence, severity and prognostic factors associated with adverse event development is assessed and compared in adjuvant treated melanoma patients compared to systemic treated advanced melanoma patients.

Chapter 6

In depth analysis of additional prognostic factors in metastatic melanoma patients treated with systemic therapy is needed as this can be of aid in determining the optimal treatment. The efficacy of immunotherapy is ought to be lower in melanoma subtypes with a lower mutation rate, such as acral melanoma, and immunotherapy is more effective in melanoma types with a higher mutation rate, which is the case in the histologic subtype desmoplastic melanoma. Unknown is whether the subtype nodular melanoma affects the treatment related overall survival in treated advanced melanoma patients.

Chapter 6 concerns survival outcomes of the histologic subtype superficial spreading melanoma and nodular melanoma treated with first-line immunotherapy or targeted therapy.

Chapter 7

Regarding radiological disease monitoring, nuanced response patterns are poorly detected by current radiographic approaches, such as RECIST 1.1, which has led to other immunotherapy-specific radiographic assessments like immunotherapy response RECIST (iRECIST), which is useful but cumbersome to implement in clinical care. One current clinical challenge is pseudoprogression, a scenario in which tumors will increase in size but eventually regress. Additionally, an individual can have simultaneous regression in some tumors with progression in others, termed mixed response. In other patients, lesions may regress or remain stable for a long period of time (i.e., stable disease), while other patients progress in a single site or organ, termed oligometastatic progression. These heterogeneous responses are challenging and clinical decisions for these situations are made on a case-by-case basis. Therapeutic outcomes and management of these heterogeneous response patterns have not been studied in detail and are warranted for patients, since continuation or change of treatment is dependent on radiological and clinical information.

The management and outcomes of metastatic melanoma patients who develop a mixed response to first line systemic immune checkpoint inhibitors are described in chapter 7.

Chapter 8

In extension to chapter 8, little is known upon the management of heterogeneous responses to immunotherapy in advanced melanoma. Chapter 9 analyzes and discusses the management and outcomes of metastatic melanoma patients with a heterogeneous response to first line systemic immune checkpoint inhibitors.

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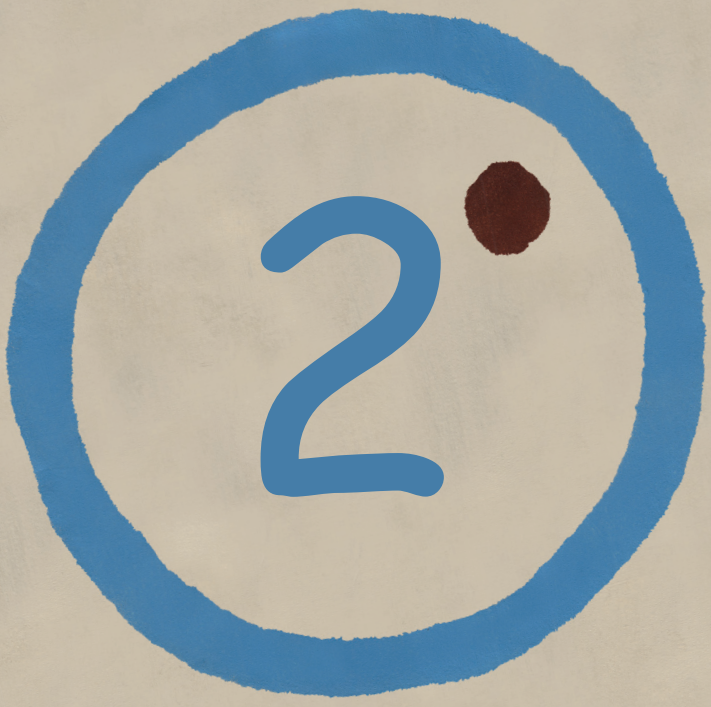
PART

Resectable stage melanoma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Melanoma diagnosis during periodic surveillance of patients with multiple atypical naevi

Authors:
D.J.W. Rauwerdink, R.E.J. Roach, M.A. Etty, N.A. Kukutsch, R. van Doorn

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Letter to the editor

Although large observational studies demonstrate an association between the presence of atypical naevi (AN) and melanoma risk, the actual benefit of dermatological surveillance for patients with multiple AN is less clear[1,2]. Therefore, recommendations for surveillance of such patients vary between countries. The U.K. guideline for the management of cutaneous melanoma recommends that such patients should be taught how to self-examine for changing naevi[3]. A survey among dermatologists from the U.S.A. revealed that 59% recommend annual screening for patients with AN[4]. In the Netherlands, patients with five or more AN commonly undergo long-term yearly surveillance by a dermatologist in addition to receiving skin self-examination instructions[5].

Here we examined the diagnosis of melanoma during periodic surveillance of Dutch patients with multiple AN and analyzed the clinical characteristics of the subset of patients with AN who developed melanoma. All patients with five or more clinically defined AN, based on ABCD criteria, and patients with more than 100 common naevi (collectively referred to as the AN group here) are seen at the dermatology department of Leiden University Medical Center for yearly dermatological consultation and for unscheduled visits when a patient notices suspicious lesions. We performed a cohort analysis of 1131 individuals in the AN group (638 women, 493 men, mean age 41 years) who visited our department between 2011 and 2016 for periodic surveillance consisting of total skin examination with use of dermoscopy, and total body photography in a subset of patients. Patients with familial melanoma, recent melanoma diagnosis or other reasons for surveillance were excluded.

During the follow-up period (total follow-up time 3268 years) melanoma was diagnosed in 39 patients, 17 women and 22 men at a mean age of 48 years (range 22-58). The rate of incident melanoma was 1·1% per follow-up year. Nine patients developed multiple melanomas, resulting in a total of 56 cases of melanoma. There were 46 invasive melanomas and 10 in situ melanomas. Median Breslow thickness of the invasive melanomas was 0·67 mm (range 0·2-2·7 mm). The majority of melanomas was of the superficial spreading type (87%). Melanoma was detected by the dermatologist during routine follow-up examination in 79%, had been noted by the patient first in 18% and had been discovered by the general practitioner in 4%. During the surveillance period 1550 skin lesions were excised, but in a proportion of cases for other reasons such as cosmetic concerns and basal cell carcinoma. Based on total body photographs and histopathology, a reliable conclusion could be drawn if the melanoma had originated from a pre- cursor naevus or de novo from normal appearing skin in 22 cases. In 72% melanoma had developed from an atypical or common naevus, which is higher than reported for melanoma in the general population (29%)[6].

Study of the clinical characteristics of the AN group who develop melanoma might enable more precise delineation of the patient group for whom surveillance would be most beneficial. To identify additional risk factors for melanoma we performed a case–control study on a largely unrelated cohort of 410 patients with AN who had their first visit between 2011 and 2016 and from whom we had collected detailed information on phenotypical risk factors for melanoma. The presence and absence of each clinical risk factor was compared among 85 patients with AN who had developed melanoma previously, at first visit or during periodic surveillance (cases) and 325 patients with AN who never had developed melanoma (controls). The age- and sex-adjusted risk of melanoma was highest among patients with AN with red hair [odds ratio (OR) 4·8] or blonde hair (OR 1·9), more than 100 solar lentigines (OR 3·1) and blistering sunburn during childhood (OR 1·6) (Table 1).

Our results reinforce the notion that dermatological surveil- lance with periodic skin examination is beneficial and a justified strategy for early detection of melanoma in patients with AN. Remarkably, most melanomas were diagnosed by the dermatologist during examination and had not been noticed by the patient in spite of skin self-examination instructions in the majority of patients.

The modifying effects of skin phototype and ultraviolet radiation-induced damage on melanoma risk in patients with AN points to the independence of pigmentation and naevus related factors in melanoma susceptibility. As individuals with AN with red or blonde hair, solar lentigines or a history or sunburn during childhood are at highest risk of developing melanoma, regular surveillance of these patients seems of particular importance. Comprehensive assessment of the health benefits and costs of yearly follow-up of patients with AN compared with skin self-examination on a population-wide scale is required to formulate melanoma-prevention strategies in this patient group at increased risk.

	Overall (n=410)	Melanoma (n=85)	No melanoma (n=325)	OR (95% CI)	OR (95% CI) adjusted for age and sex
Sex					
Women	241	45	196	Reference	
Men	168	40	128	1·4 (0·8–2·2)	
>100 common naevi					
No	131	22	109	Reference	Reference
Yes	258	57	201	1·3 (0·8–2·4)	1·2 (0·7–2·2)
≥ 5 atypical naevi					
No	149	30	119	Reference	Reference
Yes	258	46	179	1·0 (0·6–1·7)	1·0 (0·6–1·8)
Skin type					
I	47	9	38	1·3 (0·5–3·8)	1·9 (0·6–5·8)
II	300	64	236	1·4 (0·6–3·1)	1·7 (0·7–3·9)
III-V	54	8	46	Reference	Reference
Eye colour					
Blue	244	60	184	1·8 (1·1–3·1)	**1·7 (1·0–3·0)**
Other	150	22	128	Reference	Reference
Hair colour					
Red or blonde	20	6	14	2·2 (1·1–4·4)	**2·8 (1·3–6·1)**
Brown or black	375	75	300	Reference	Reference
Sunbathing					
Never	160	33	127	Reference	Reference
Ever	117	31	86	1·1 (0·7–1·9)	1·5 (0·9–2·7)
Sunbed use					
Never	302	72	230	Reference	Reference
Ever	20	3	17	0·5 (0·2–1·3)	0·6 (0·3–1·3)
Blistering sunburn < 20 years					
No	318	59	259	Reference	Reference
Yes	80	19	61	1·7 (1·1–2·7)	**1·6 (1·0–2·5)**
Solar lentigines					
0-40	343	63	280	Reference	Reference
41-100	30	10	20	2·0 (1·4–3·1)	**1·8 (1·2–2·8)**
≥100	35	11	24	4·0 (1·7–9·7)	**3·1 (1·2–7·8)**
Actinic keratoses					
0	353	64	289	Reference	Reference
≥1	22	11	11	4·5 (1·8–10·7)	1·2 (0·4–3·5)

Table 1. Clinical risk factors for melanoma in atypical naevus.

Data missing for some variables. Results in bold are significant. OR, odds ratio; CI, confidence interval.

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3

Melanoma Arising from Nevus Precursors in a Clinical Setting Among CDKN2A Germline Mutation Carriers

Authors:
Daan J W Rauwerdink, Yann Hoogland, Anne M R Schrader,
Thomas P Potjer, Ellen Kapiteijn, Jos A van der Hage, Remco van Doorn

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Letter to the editor

Most melanomas arise from melanocytes in normal-appearing skin with no discernible pigmented lesion. Approximately 30% of melanomas evolve from benign melanocytic naevi.[1] Individuals with a germline pathogenic *CDKN2A* variant, encoding the p16 and p14 tumour suppressor proteins, have an estimated 70% risk of developing melanoma.[2] Carriers of a *CDKN2A* pathogenic variant are recommended to undergo periodic skin examinations to diagnose melanoma at an early stage.[4] Distinguishing melanoma from atypical naevus can be challenging in this patient group. At our department, total body photography (TBP) is routinely used to surveillance these patients, enabling the detection of alterations in existing naevi and the emergence of new lesions.[5]

In *CDKN2A* pathogenic variant carriers, it is unclear what proportion of melanomas develops from pre-existing naevi. To address this question, we studied established carriers of a *CDKN2A* pathogenic gene variant who developed melanoma or melanoma in situ from January 2009 to December 2023 at our department. Germline *CDKN2A* gene variants were detected using sequencing of all coding regions and multiplex ligation-dependent probe amplification. The diagnosis of melanoma was made based on pathological examination. . Lentigo maligna (melanoma) was excluded. TBPs taken at least six months before melanoma development were examined to assess the presence of a pre-existing common naevus or atypical naevus. The pathology slides of the melanomas were reviewed for the presence or absence of a contiguous naevus component, blinded to the clinical data.

In total, 60 new melanomas were diagnosed in 44 patients with a *CDKN2A* pathogenic variant, including 47 cases of invasive melanoma (median Breslow thickness 0.5 mm) and 13 cases of melanoma in situ (Table 1) (Figure 1). The median age at diagnosis was 49 years. TBPs demonstrated the presence of a pre-existing naevus in 47 of 60 cases (78%) at the exact anatomical location of melanoma development. The pre-existing lesion was found to be a common naevus in 31 cases and an atypical naevus in 16 cases, based on its morphology on earlier TBPs. Thirty-seven of 47 invasive melanomas (79%) and 10 of 13 in situ melanomas (77%) originated from a pre-existing naevus. Only 22% of melanomas in this cohort developed de novo from skin with no discernible pigmented skin lesion. When revising the histopathology slides, a contiguous naevus component was only detected in those cases with clinical evidence of a pre-existing naevus as shown by TBP. In the 47 melanomas arising from a pre-existing naevus clinically, pathological examination showed distinct naevus cell nests in 18 cases (38%). The lack of histological evidence of a naevus precursor in the remainder can be explained by sampling error in the histopathology slides or by overgrowing melanoma cells hindering the identification of naevus cell clusters. The disease course of the patients with melanoma evolving from a naevus did not differ from patients with melanoma arising de novo. In this cohort of hereditary melanoma patients, we

found that melanoma developed in the context of a pre-existing naevus in the majority of cases (78%), making use of sequential TBP. This is a higher proportion than reported for sporadic melanoma (30%). We cannot exclude the possibility that the proportion in sporadic melanoma is underestimated since it was concluded solely from pathological examination.[1] In addition, our finding might be associated with the higher number of atypical naevi in carriers of a germline *CDKN2A* pathogenic variant. However, a sizeable proportion of melanomas in the patients arose from common naevi with no atypical features. Alternatively, the high frequency of melanoma emerging from a precursor naevus in these patients might be explained by the sequence of genetic alterations during tumorigenesis. In carriers of a germline inactivating *CDKN2A* gene variant, bi-allelic loss of *CDKN2A* occurs subclonally in the naevus stage, potentially conferring proliferative capacity.[6] In sporadic melanoma, this genetic alteration occurs later in melanoma evolution.[7] The frequent occurrence of nevus-associated melanoma in *CDKN2A* gene variant carriers is notable. A possible limitation of our study is the small number of incident melanomas in our cohort under surveillance. Additionally, our study only included hereditary melanoma patients with fair skin type and sun exposure habits typical for northern Europeans. Although TBP is systematically performed in *CDKN2A* pathogenic variant carriers at our department, it is sometimes omitted in patients with few naevi. It should also be noted that the possibility of slow-growing melanomas mistakenly considered to represent naevi cannot be entirely excluded, despite the minimum interval of six months between photography and melanoma diagnosis. Our finding of frequent melanoma development from naevus precursor lesions is relevant in managing individuals with a *CDKN2A* pathogenic variant, supporting the routine use of TBP in this patient group to facilitate the detection of naevi that have changed in size, shape, or colour. More than previously appreciated, the surgical removal of melanocytic naevi in these high-risk patients may prevent melanoma development.

	Patients (n=44)
Age (median, IQR, year)	49 (42 - 61)
Gender	
Male	25 (57%)
Female	19 (43%)
Skin type	
I	6 (14%)
II	38 (86%)
Naevus phenotype	
> 5 atypical naevi (AN)	9 (21%)
>100 common naevi or >5 AN	19 (43%)
	Melanoma cases (n=60)
Melanoma origin	**47 (78%)**
from naevus	
common naevus	31 (52%)
clinically atypical naevus	16 (26%)
from normal-appearing skin	**13 (22%)**
Histopathology	
No distinct naevus nests	42 (70%)
Residual naevus nests	18 (30%)
Preexisting lesion noticed by the patient	16 (27%)
Melanoma subtype	
Invasive	47 (78%)
Breslow thickness (median, mm)	0.5
In situ	13 (22%)

Table 1. Characteristics of 44 patients with a CDKN2A pathogenic variant and 60 melanomas

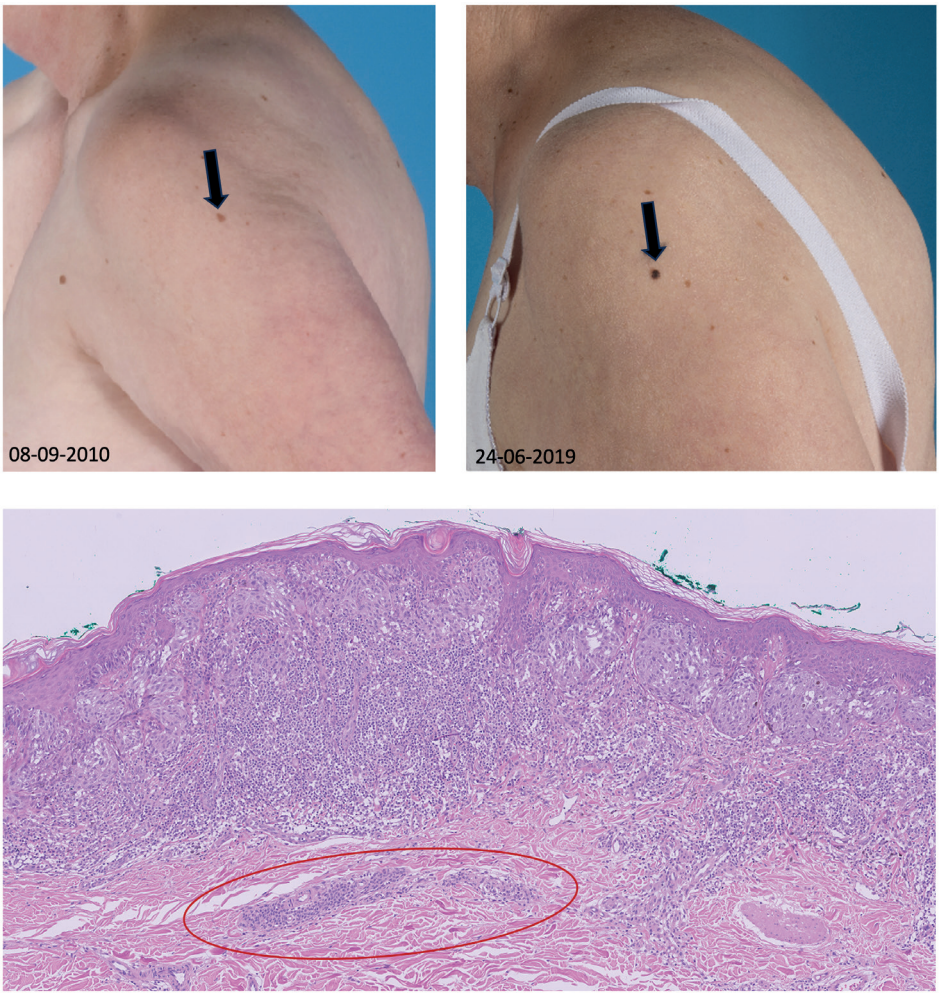

Figure 1. Clinical detailed photo demonstrating the emergence of melanoma in a pre-existent naevus. Histology demonstrated dermal located pre-existing nevus nests and superficially epidermal located melanoma.

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Adjuvant Therapy Failure Patterns in the Modern Era of Melanoma Management

Authors:
Rauwerdink DJW, Molina G, Frederick DT, Sharova T, Carmichael H, Boland GM

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Abstract

Background

The management of patients with resected stage 3 melanoma has changed significantly due to the adoption of the Multicenter Selective Lymphadenectomy Trial (MSLT)-2 guidelines and the recurrence-free survival benefit of adjuvant anti-PD-1 immunotherapy and BRAF/MEK inhibitor (BRAF/MEKi) therapy. Data on recurrence patterns, adjuvant therapy responses, and therapy-associated adverse events (AEs) in the modern era remain scarce.

Methods

This single-institution retrospective study analyzed surgically resected stage 3 and oligometastatic stage 4 melanoma patients who received anti-PD-1 therapy, BRAF/MEK inhibitor (BRAF/MEKi) therapy, or underwent surgery with active surveillance only. The primary endpoint of the study was recurrence-free survival (RFS). Secondary endpoints included the location and clinical characteristics of recurrence, as well as therapy-associated adverse events (AEs).

Results

From a cohort of 137 patients, the study enrolled 102 patients treated with adjuvant anti-PD-1 (n = 46), adjuvant BRAF/MEKi (n = 3), or surgery alone (n = 26). During a mean follow-up period of 17 months, 20% of the ani-PD-1 patients, 13% of the BRAF/MEKi patients, and 42% of the surgery-only patients experienced recurrence. Log-rank testing showed a significantly longer RFS for the patients treated with anti-PD-1 [15.3 months; interquartile range (IQR), 8.2–23.2 months; p = 0.04] or BRAF/MEKi (17.9 months; IQR, 12.5–23 months; p = 0.01) than for those treated with surgery alone (11.9 months; IQR, 7.0–17.6 months). In the anti-PD-1 group, AEs occurred less frequently than in the BRAF/MEKi group (54% vs 80%; p = 0.03).

Conclusions

Adjuvant anti-PD-1 and BRAF/MEKi were associated with significantly improved RFS for the patients with resected stage 3 or 4 melanoma. The BRAF/MEKi group had significantly more AEs than the anti-PD-1 group. This is the first study to characterize real-world recurrence in the post MSLT-2 era of adjuvant therapy for melanoma.

Introduction

In 2014, the Multicenter Selective Lymphadenectomy Trial (MSLT) confirmed the prognostic value of lymphatic nodal evaluation of patients with intermediate-thickness (1.2–3.5 mm) melanoma, and the standard of care for patients with the diagnosis of sentinel lymph node involvement was immediate-completion lymph-node dissection (CLND).[1,2,3,4] In June 2017, the MSLT-2 data demonstrated that close observation of patients with resected melanoma who had low-burden stage 3 disease and underwent nodal surveillance resulted in a melanoma-specific survival similar to that of patients who underwent immediate CLND.[5] In addition, the more recently published long-term follow-up DeCOG-SLT trial showed even more promising results, with no difference in terms of recurrence-free survival (RFS), overall survival, or distant metastases-free survival between nodal surveillance and CLND.[6]

In 2015, systemic ipilimumab treatment was approved by the Food and Drug Administration (FDA) for patients with metastatic melanoma.[7,8] The European Organisation for the Research and Treatment of Cancer (EORTC) 18,071, a randomized, double-blind controlled trial, demonstrated a longer RFS for resected stage 3 melanoma treated with ipilimumab.[9] However, the severe toxicities associated with the high dose of ipilimumab limited its widespread use in the adjuvant setting.[10,11]

In 2017, the FDA approved human immunoglobulin G 4 (IgG4) monoclonal antibody against programmed death 1 (anti-PD-1), which was associated with better RFS and fewer treatment-related adverse events for patients with resected stage 3 melanoma than treatment with ipilimumab.[12] Similarly, trials investigating the efficacy of BRAF plus MEK inhibitors (BRAF/MEKi) in the adjuvant setting also showed improved RFS and overall survival (OS) for resected stage 3 melanoma patients with a BRAF V600 mutation.[13,14] These studies have transformed the management of patients with resected stage 3 or 4 melanoma.

Due to the relatively short period between therapy approval and its adoption, it is unclear how clinical outcomes and drug-related toxicities differ between adjuvant anti-PD-1 and BRAF/MEKi. Additionally, the impact of changes in surgical management during this modern era has not been fully explored. This report describes real-world outcomes for a cohort of stage 3 and resectable 4 melanoma patients from a single high-volume institution after the adoption of the MSLT-2 nodal management strategy and implementation of adjuvant BRAF/MEKi and anti-PD-1 therapies. We compare RFS, location of recurrence, clinical characteristics of recurrence, and therapy-associated adverse events between adjuvant anti-PD-1, BRAF/MEKi, and surgery-only groups.

Methods

Data Source and Study Design

This retrospective review analyzed patients with resected stage 3 and oligometastatic stage 4 melanoma treated at the Massachusetts General Hospital (MGH). Informed consent was obtained from all the patients included in compliance with the Institutional Review Board (IRB).

Patients

Eligible patients 18 years of age or older at the time of diagnosis underwent wide local excision with sentinel lymph node biopsy or oligometastatic resection and were staged according to the eighth edition of the American Joint Committee on Cancer (AJCC), which includes Breslow thickness, tumor ulceration, regional lymph node involvement, and/or distant metastatic disease.[15] The patients had radiographic staging before initiation of adjuvant therapy and a minimum follow-up period of 6 months, defined as the time between definitive resection of all disease sites and last consultation by a treating clinician.

The exclusion criteria ruled out immediate CLND upon positive sentinel node detection, melanoma diagnoses, and treatment before 1 June 2017 (the time that the MSLT-2 guidelines were adopted into MGH surgical practice), incomplete melanoma resection, and missing medical records. All identified patients underwent standard follow-up care at MGH, which consisted of radiographic surveillance every 3 months with computed tomography scan, ultrasound, and physical examination by the treating clinician.

Clinical Variables

Demographic variables including age, gender, and race were collected from the medical charts. Tumor characteristics extracted from the dermatopathologic report were histology, Breslow thickness (mm), ulceration, total number of involved lymph nodes, size of lymph nodes described as micro ($<$ 2 mm) or macro ($>$ 2 mm), and date of surgery. Lymph nodes were assessed using a sentinel lymph node biopsy, with the patients receiving upfront lymphoscintigraphy using technetium-99 m (99mTc)-labeled sulfur colloid. Sections of the biopsy were analyzed by a pathologist using S100 and MelanA immunohistochemistry. The mutational status of the melanoma lesion was identified using Next Gen Sequencing.

The date of disease recurrence was defined as the moment a suspicious lesion was identified, with subsequent histologic confirmation of disease. Recurrence-free survival was calculated as the time from surgery to either the date of first melanoma recurrence or the most recent follow-up evaluation. Reported adverse events were classified using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events, version 5.0.

Treatment

Staged patients with a BRAF V600 mutation received a teaching session by the medical oncologist, in which the patient could consent to undergo adjuvant BRAF/MEKi therapy (dabrafenib 150 mg plus trametinib 2 mg taken orally every day up to 365 days). The patients were evaluated for treatment-related adverse events every 2 weeks by the medical oncology team. Additional follow-up evaluation was performed via telephone surveys conducted by nurse oncologists. In the case of an adverse event with BRAF/MEKi, the dose of therapy could be reduced by half, held temporarily, or stopped permanently.

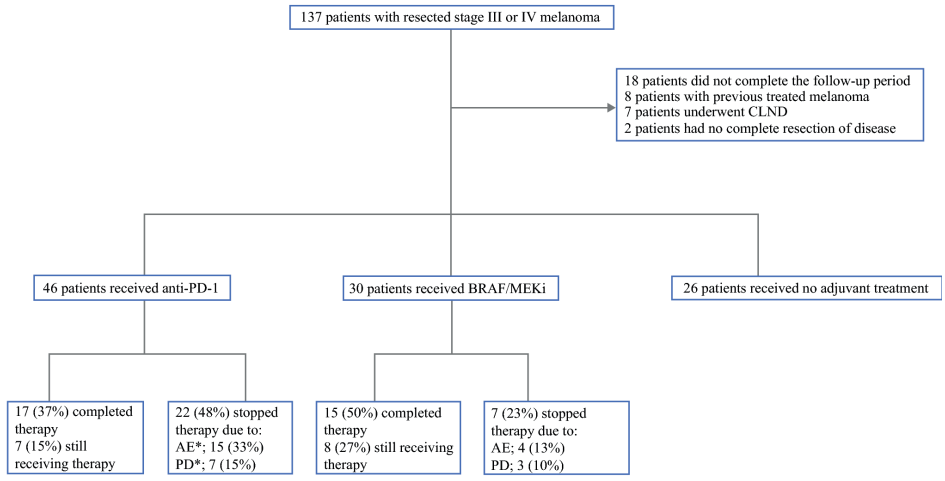
Alternatively, patients receiving immunotherapy attended a teaching session on adjuvant anti-PD-1 therapy, which included nivolumab (240 mg) or pembroluzimab (200 mg), administered via infusion. Pembroluzimab was administered every 3 weeks up to 18 cycles, and nivolumab was administered biweekly for a total of 24 cycles. Anti-PD-1 infusion was either held or stopped definitively in the case of adverse events. Patients who elected not to receive adjuvant therapy had the same follow-up surveillance as patients treated with anti-PD1 or BRAF/MEK. Given the possibility of potential crossover between therapies after recurrence, we assessed RFS instead of overall survival because the patients in the observation arm could become eligible for additional therapy after disease recurrence.

Statistical Analysis

Descriptive analysis was performed to identify frequencies of demographic variables, clinicopathologic variables, and adverse events in each separate patient cohort. Observed frequencies of characteristics were compared between the treatment groups using Chi square and Fisher's exact tests when appropriate. The RFS curves were estimated via the Kaplan–Meier method and compared with the log-rank test for each individual patient cohort. All p values were two-sided, and a p value lower than 0.05 was considered to be statistically significant. All statistical analyses were conducted using IBM SPSS Statistics version 24 (IBM, Armonk, NY, USA, released 2016).

Results

The study identified 137 patients with resected stage 3 ($n = 95$, 93%) or stage 4 M1a ($n = 7$, 7%) melanoma, 35 of whom did not meet the inclusion criteria (figure 1). Among 102 selected patients, 46 received anti-PD-1, 30 received BRAF/MEKi, and 26 were treated with surgery alone during a mean follow-up period of 18 months.



**Abbreviations for AE: Adverse events and PD: Progression of Disease.*

Figure 1. Experimental schema. The study identified 135 patients with stage 3 or resected stage 4 melanoma, 35 of whom did not meet the inclusion criteria. Of the selected patients, 46 received anti-PD-1, 30 had BRAF/MEKi, and 26 underwent surgery alone. AE, adverse events; PD, progression of disease.

For the patients receiving adjuvant therapy, the median number of immunotherapy cycles was 17.5 [interquartile range (IQR), 5.5–24] in the nivolumab group and 9 (IQR, 5.0–18) in the pembrolizumab group. The patients in the BRAF/MEKi group received therapy for an average period of 9 ± 4 months. A full year of anti-PD-1 therapy was completed by 16 patients (35%), whereas the BRAF/MEKi treatment was completed by 15 patients (50%). Anti-PD-1 treatment was stopped due to adverse events for 15 patients (33%) and because of disease recurrence for 7 patients (15%). In the BRAF/MEKi group, adverse events led to therapy discontinuation for four patients (20%), and therapy was stopped due to disease recurrence for three patients (10%). At this writing, eight patients (17%) continue to receive anti-PD-1 therapy, with a median cycle number of 12 (range, 8–18), whereas seven patients (23%) continue to receive BRAF/MEKi therapy, with a mean treatment period of 8 ± 4 months.

Demographic variables such as gender, race, Eastern Cooperative Oncology Group (ECOG) performance status, clinicopathologic characteristics, number of resected lymph nodes, and primary tumor site were similar in the three groups (Table 1). Observed differences between the groups demonstrated that patients who received surgery only were less likely to have macroscopic lymph node involvement and more likely to have thinner primary melanomas than the BRAF/MEKi and anti-PD1 groups ($p = 0.03$ and 0.03, respectively). The patients in the anti-PD-1 cohort had a higher disease stage and thicker melanomas ($p = 0.03$). The patients treated with BRAF/MEKi were younger, as observed in the COMBI-AD-trial, with a median age of 52 years (IQR, 37–61 years), and in our study had a higher proportion of macroscopic lymph node involvement (73%) than the patients who had surgery only (31%) or anti-PD-1 (52%).[13]

	Anti-PD-1 (n=46) n (%)	BRAF/MEK (n=30) n (%)	Surgery only (n=26) n (%)	P-value*
Median age (range) - yr	62 (32 - 78)	52 (21 - 73)	70 (23 - 85)	**0.01**
Sex				
Female	22 (48)	18 (60)	10 (42)	0.38
Male	24 (52)	12 (40)	14 (58)	
Race				
White	43 (94)	29 (97)	24 (92)	0.13
Nonwhite	3 (7)	1 (3)	2 (8)	
ECOG performance status				
0	41 (89)	28 (93)	22 (85)	0.51
1	5 (11)	2 (7)	4 (15)	
BRAF-mutation status				
Positive	4 (9)	30 (100)	5 (19)	**<0.01**
Negative	42 (91)	NA	21 (81)	
Disease stage				
IIIA	4 (9)	9 (30)	6 (23)	**0.05**
IIIB	21 (46)	6 (20)	6 (23)	**0.03**
IIIC	17 (37)	12 (40)	11 (42)	0.59
IIID	NA	2 (7)	1 (4)	0.09
IV	4 (9)	1 (3)	2 (8)	0.64
No. of positive lymph nodes				
1	31 (68)	15 (50)	15 (58)	0.06
2 or 3	6 (13)	10 (33)	8 (31)	0.22
≥4	2 (4)	4 (13)	1 (4)	0.3
Type of lymph node involvement				
Micro	15 (33)	7 (23)	15 (58)	**0.03**
Macro	24 (52)	22 (73)	8 (31)	
Not reported	7 (15)	1 (3)	3 (12)	
Primary tumor site				
Head and neck	9 (20)	5 (17)	4 (17)	0.93
Upper extremities	7 (15)	7 (23)	8 (33)	0.22
Lower extremities	13 (28)	5 (17)	6 (25)	0.51
Trunk	12 (26)	5 (17)	6 (25)	0.93
Not reported	5 (11)	2 (7)	NA	0.24
Median Breslow thickness in mm (range)				
	3.2 (0.6 - 21)	2.2. (0.6 - 9.0)	2.1 (0.6 - 5.5)	**0.03**
Ulceration				
No	19 (41)	20 (67)	14 (54)	0.26
Yes	17 (37)	7 (23)	9 (35)	
Not reported	10 (22)	3 (10)	3 (12)	
Melanoma subtype				
Superficial spreading	19 (41)	17 (57)	9 (35)	0.29
Nodular	5 (11)	2 (7)	5 (19)	0.27
Acral	2 (4)	4 (13)	2 (8)	0.37
Lentigo maligna	4 (9)	NA	2 (8)	0.25
Other	1 (2)	NA	NA	0.55
Not reported	15 (33)	7 (23)	8 (31)	0.63

Table 1. Cohort characteristics

ECOG Eastern Cooperative Oncology Group, N/A not applicable
**Significant p values highlighted in bold type*

Disease recurred for 9 anti-PD-1-treated patients (20%), 4 BRAF/MEKi-treated patients (13%), and 11 surgery-only patients (42%). Descriptive analysis showed a median RFS of 11.9 months (IQR, 7.0–17.6 months) for the surgery-only patients, 15.3 months (IQR, 8.2–23.2 months) for the anti-PD-treated patients, and 17.9 months (IQR, 12.5–23 months) for the BRAF/MEKi-treated patients. Although the estimated median RFS was not reached for the adjuvant treatment groups, the log-rank test showed a significantly longer overall RFS for the patients treated with anti-PD-1 or BRAF/MEKi therapy ($p = 0.04$) than for the surgery-only patients ($p = 0.01$) (figure 2). Given the small number of total events in the RFS analysis, an additional Chi square test was performed to compare overall RFS in the treatment groups, which demonstrated a significantly longer RFS for the patients treated with anti-PD-1 ($p = 0.04$) or BRAF/MEKi ($p = 0.02$).

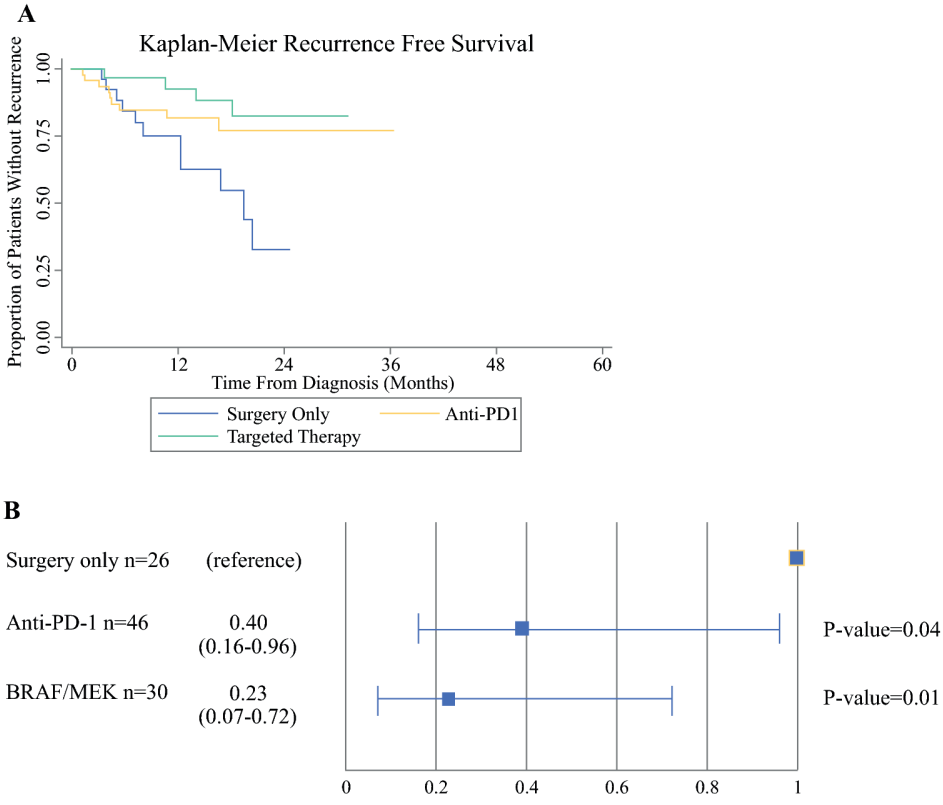


Figure 2. A. Kaplan–Meier recurrence-free survival curve for patients treated with anti-PD-1 ($n = 46$), BRAF/MEKi ($n = 30$), or surgery alone ($n = 26$). The median recurrence-free survival was 11.9 months for the surgery-only patients, 15.3 months for the anti-PD-1 patients, and 17.9 months for the patients treated with BRAF/MEKi. **B.** Hazard ratio showing the hazard of disease recurrence per treatment (surgery only = reference). The hazard ratio is 0.40 ($p = 0.04$) for anti-PD-1 and 0.23 ($p = 0.01$) for BRAF/MEKi.

Local disease recurred for 30% of the patients treated with adjuvant anti-PD-1, 40% of the patients treated with BRAF/MEKi, and 64% of the patients treated with surgery only (Fig. 3), with 59% of the cases detected during physical examination. Distant disease occurred for 70% of the patients treated with anti-PD-1, compared with 40% of the patients treated with BRAF/MEKi and 36% of the patients treated with surgery only. Unsurprisingly, distant recurrence was most commonly detected via computed tomography (CT) (50%). Among the patients with disease recurrence, one patient treated with BRAF/MEKi had simultaneous local and distant recurrences, detected via ultrasound, and one patient treated with anti-PD-1 had local and distant recurrences, detected via CT scan.

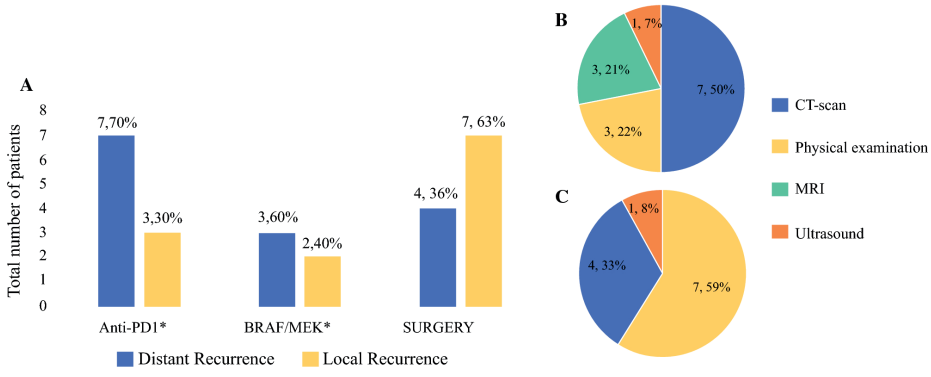


Figure 3. A Location of disease recurrence showing distant and local recurrences per treatment group. **B** Pie chart representing the detection of distant disease recurrences, with computed tomography scan as the most common method (50%). C Pie chart showing local disease recurrences, with physical examination as the most frequent (59%). *One anti-PD-1-treated patient and one BRAF/MEKi-treated patient had simultaneous distant and local disease recurrences.

Regarding the clinical characteristics of the patients with disease recurrence, the BRAF/MEKi patients were more likely be male and to have higher-risk disease (stage 3C or 3D), macroscopic lymph node involvement, and a relatively high median Breslow thickness (table 2). The patients treated with anti-PD-1 who had recurrence were stage 3B (44%) or 3C (56%) and had a high proportion of macroscopic lymph node involvement (78%). The surgery-only group with recurrences had relatively high-risk melanomas [stage 3B (27%), stage 3C (46%), stage 3D (9%), stage 4 (18%)] and included patients with both BRAF-mutated and BRAF wild-type tumors.

	Anti-PD-1 (n=9)	BRAF/MEK (n=4)	Surgery only (n=11)
Median age (range) - yr	67 (44 - 72)	61 (60 - 62)	73 (37 - 85)
Sex - no. (%)			
Female	5 (56)	NA	5 (46)
Male	4 (44)	4 (100)	6 (55)
BRAF-mutation status - no. (%)			
Positive	3 (33)	4 (100)	4 (36)
Negative	6 (67)	NA	7 (64)
Disease stage - no. (%)			
IIIB	4 (44)	NA	3 (27)
IIIC	5 (56)	2 (50)	5 (46)
IIID	NA	2 (50)	1 (9)
IV	NA	NA	2 (18)
Median Breslow thickness - mm (range)			
	4.0 (2.1 - 9.0)	8.0 (2.5 - 8.25)	2.7 (0.8 - 5.5)
Tumor subtype (%)			
Superficial spreading	4 (44)	2 (50)	3 (27)
Nodular	1 (11)	1 (25)	1 (9)
Acral	2 (22)	NA	NA
Lentigo maligna	NA	NA	2 (18)
Unknown	2 (22)	1 (25)	5 (46)
Primary site - no. (%)			
Head and neck	1 (11)	2 (50)	2 (20)
Upper extremities	NA	NA	2 (20)
Lower extremities	7 (78)	1 (25)	2 (20)
Trunk	1 (11)	1 (25)	2 (20)
Ulceration - no. (%)			
No	5 (56)	2 (50)	4 (36)
Yes	3 (33)	1 (25)	4 (36)
Not reported	1 (11)	1 (25)	3 (27)
Size lymph node – no. (%)			
Microscopic	2 (22)	NA	4 (36)
Macroscopic	7 (78)	4 (100)	4 (36)
Not reported	NA	NA	3 (30)

Table 2. Clinical characteristics of patients with disease recurrence.

N/A not applicable

Adverse events of any grade were reported for 25 patients treated with anti-PD-1 [54%; grade 3 or 4 (9%)], leading to treatment interruption for 4 patients (9%) and permanent discontinuation for 15 patients (30%) (Table 3). In the BRAF/MEKi cohort, adverse events of any grade were observed for 24 patients [80%; grade 3 or 4 (20%)], leading to dose interruption for 16 patients (53%), dose reduction for 10 patients (33%), and therapy discontinuation for 6 patients (20%).

The total rate of observed adverse events from any cause was significantly higher in the BRAF/MEKi group (80%) than in the anti-PD-1 cohort (54%) ($p = 0.03$), as was the occurrence of grade

3 or 4 events [BRAF/MEKi (20%) vs anti-PD-1 (9%)]. However, this difference was not statistically significant ($p = 0.18$).

Adverse events related to therapy discontinuation were observed more frequently in the anti-PD-1 group (30%) than in the BRAF/MEKi group (20%), although this difference was not significant ($p = 0.29$). The severe adverse events in the anti-PD-1 group included grade 3 diarrhea, leading to hospitalization in two patients, and one reported case of grade 3 neuropathy, which did not resolve with high-dose corticosteroids treatment. Grades 3 and 4 adverse events in the BRAF/MEKi group consisted of pyrexia, leading to hospitalization in one case, temporary severe hearing loss in two cases, and development of a basal-cell carcinoma in one case.

	Anti-PD-1 (N=46)		BRAF/MEKi (N=30)	
	No. of patients with event (%)			
	Any Grade	**Grade 3 or 4**	**Any grade**	**Grade 3 or 4**
Any treatment-related adverse event	25 (54)*	4 (9)	24 (80)*	6 (20)
Pyrexia	0	0	19 (63)	2 (7)
Diarrhea	7 (15)	3 (7)	0	0
Transaminitis	6 (13)	0	3 (10)	1 (3)
Malaise	0	0	4 (13)	0
Rash	4 (9)	0	2 (7)	0
Fatigue	4 (9)	0	2 (7)	0
Atralgia	4 (9)	0	0	0
Hearing loss	0	0	2 (7)	2 (7)
Infusion related reaction	3 (7)	0	0	0
Neutropenia	0	0	2 (7)	1 (3)
Thyroiditis	2 (4)	0	0	0
Pneumonitis	2 (4)	0	0	0
Leukopenia	0	0	1 (3)	0
Anemia	0	0	1 (3)	0
Oral mucositis	0	0	1 (3)	0
Asthenia	1 (2)	0	0	0
Dyspnea	1 (2)	0	0	0
Vitiligo	1 (2)	0	0	0
Hypophysitis	1 (2)	0	0	0
Neuropathy	1 (2)	1 (2)	0	0
Adverse event leading to dose interruption	4 (9)	NA	16 (53)	NA
Adverse event leading to dose reduction	NA	NA	10 (33)	NA
Adverse event leading to discontinuation	15 (33)	NA	6 (20)	NA

Table 3. Adjuvant Therapy Toxicities.

N/A not applicable
**Statistically significant between the two groups, p-value=0.03.*

Discussion

Despite our relatively small cohort, we report real-world data about outcomes for stage 3 and resectable stage 4 melanoma in this modern era of adjuvant therapy and surgical management. In our practice, 45% of patients received anti-PD-1 immunotherapy, 29% received BRAF/MEKi, and 26% were treated with surgery alone. Although we did not reach the estimated median RFS due to the small total number of events, the patients treated with anti-PD-1 and BRAF/MEKi had a significantly longer overall RFS than the patients who received surgery alone, supporting previous findings from adjuvant trials.[12,14]

The patients in our cohort treated with adjuvant therapy had fewer local recurrences than the patients treated with surgery alone, suggesting that adjuvant therapy may be eradicating occult regional disease. Notably, a higher proportion of distant metastases was seen in the anti-PD-1 group, which must be interpreted carefully because this patient cohort had less favorable clinical characteristics. Overall, the patients with disease recurrences, regardless of therapy, had clinical characteristics associated with a worse outcome, such as stage 3C or 3D disease and macroscopic lymph node involvement defined in previous adjuvant studies.[12,14,16]

Whereas local recurrences were detected was most frequently by physical examination, distant disease was detected primarily with CT scans, supporting the value of both physical examination and cross-sectional imaging during follow-up evaluation. Regarding the timing of disease recurrence, the patients treated with anti-PD-1 had earlier onset of disease recurrence than the patients treated with BRAF/MEKi, although long-term follow-up evaluation is needed.

In terms of adverse events, the patients treated with BRAF/MEKi were more likely to have any adverse event (total, 80%; grade 3 or 4, 20%) than the patients treated with anti-PD-1 (total, 54%; grade 3 or 4, 9%), although the difference in all grade toxicities was the only statistically significant value ($p = 0.03$). This is in line with adjuvant trial data showing a higher frequency of any adverse event with targeted therapy than with anti-PD-1 treatment.[12,14,17]

Interestingly, although fewer patients treated with anti-PD-1 therapy completed a full year of therapy (37%) than those treated with BRAF/MEKi (50%), this was not due to toxicity because no statistically difference was observed in therapy discontinuation due to adverse events between the two groups (anti-PD-1, 33% vs BRAF/MEKi, 20%). Therefore, other factors such as progression of disease also are contributing to adjuvant cessation. Despite the small study cohort, the retrospective nature of the analysis, few disease recurrences in each treatment group, and inherent biases in patient selection, this report describes real-world observational data on adjuvant recurrences and toxicities in this modern adjuvant and surgical era of melanoma management.

Conclusion

Unsurprisingly, the patients with resected stage 3 and low-volume stage 4 melanoma who were treated with adjuvant therapy had a better RFS and a lower risk of local recurrence than the patients treated with surgery alone despite clear selection bias in the allocation of patients to adjuvant versus observation pathways. It also is interesting to note that the surgery-only group had a higher frequency of local recurrences than the adjuvant cohorts, supporting the concept that adjuvant therapies are treating both distant and occult regional disease. The impact of adverse events on outcomes and quality of life have not been fully described, but are increasingly important in the setting of adjuvant therapy. Our findings suggest that both BRAF/MEKi and anti-PD-1 adjuvant therapies are associated with a high frequency of toxicities but overall fairly well tolerated. As expected, the clinical characteristics associated with an increased risk of melanoma recurrence were those of higher-stage disease (IIIB/IIIC, macroscopic nodal involvement, and thicker ulcerated melanoma). Larger cohorts and a longer follow-up period are needed for a full assessment of the impact that adjuvant therapies have on melanoma outcomes.

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5.

Adverse Events in Anti-PD1 Treated Adjuvant and First-Line Advanced Melanoma Patients

Authors:
Rauwerdink DJW, Not OV, de Meza M, Doorn RV, Hage JV, Eertwegh AJMVD, Haanen JB, Aarts MJB, Berkmortel FWPJVD, Blank CU, Boers-Sonderen MJ, Groot JWB, Hospers GAP, Piersma D, van Rijn RS, Stevense-den Boer AM, Veldt AAMV, Vreugdenhil G, Wouters MWJM, Suijkerbuijk KPM, Kapiteijn E

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Abstract

Introduction

The difference in incidence and severity of anti-PD-1 therapy-related adverse events (irAEs) between adjuvant and advanced treated melanoma patients remains unclear, as no head-to-head studies have compared these groups.

Methods

This multi-center cohort study analyzed melanoma patients treated with anti-PD-1 in adjuvant or advanced setting between 2015 and 2021. Comorbidities and ECOG performance status were assessed before treatment, and grade III-IV irAEs were monitored during treatment. Univariate and multivariate regression analyses were conducted to identify factors associated with irAE development.

Results

A total of 1,465 advanced melanoma patients and 908 resected melanoma patients received anti-PD-1 therapy. Adjuvant-treated patients were younger, with a median age of 63 years compared to 69 years in the advanced group ($p<0.01$), and had a better ECOG performance status ($p<0.01$). Comorbidities were seen more frequently in advanced melanoma patients than in those receiving adjuvant treatment, 76% versus 68% ($p<0.01$). Grade III-IV irAEs occurred in 214 (15%) advanced treated patients and in 119 (13%) adjuvant treated patients.

Multivariate analysis showed an increased risk of severe irAE development with the presence of any comorbidity (adjusted OR 1.22, 95% CI 1.02–1.44) and ECOG status greater than 1 (adjusted OR 2.00, 95% CI 1.20–3.32). Adjuvant therapy was not associated with an increased risk of irAE development compared to advanced treatment (adjusted OR 0.95, 95% CI 0.74–1.21) after correcting for comorbidities and ECOG performance score. Anti-PD-1 therapy was halted due to toxicity (any grade irAE) more often in the adjuvant setting than in the advanced setting, 20% versus 15% ($p<0.01$).

Conclusion

Higher ECOG performance status and presence of any comorbidity were independently associated with an increased risk of Grade III-IV irAE in adjuvant and advanced treated melanoma patients. Patients treated in the adjuvant setting did not have an increased risk of developing severe irAEs compared to advanced melanoma patients. These findings are of clinical significance in consulting patients for adjuvant anti-PD-1 treatment.

Introduction

Immune checkpoint inhibition therapy, particularly targeting the programmed death-1 (PD-1) pathway, has revolutionized the treatment landscape for melanoma, yielding significant improvements in patient outcomes across various disease stages. In patients with advanced melanoma (unresectable stage III-IV), the introduction of anti-PD-1 therapies has led to marked improvements in overall survival rates. Furthermore, in the adjuvant setting for melanoma patients who have undergone surgical resection of stage III-IV tumors, anti-PD-1 therapy has demonstrated a substantial benefit in enhancing local recurrence-free survival. By stimulating the immune system to target any residual microscopic disease, these therapies reduce the likelihood of melanoma recurrence after surgery.[1-7] Despite improved outcomes in the adjuvant and advanced setting, immune checkpoint inhibition can induce serious and long-lasting immune-related adverse events (irAEs)[8-11] . Immunotherapy related adverse events can significantly impact the quality of life. Specific irAEs can occur, including fatigue, skin rashes, colitis, nephritis, hepatitis, nausea, and endocrine dysfunctions leading to discomfort, pain, and a reduced ability to perform daily activities, affecting both physical and mental well-being. Severe irAEs often necessitate the interruption or complete discontinuation of anti-PD-1 therapy. Managing irAEs typically requires additional medications, such as corticosteroids (prednisone) or other immunosuppressive drugs. These medications can have their own side effects and complications, further burdening the patient and complicating their treatment regimen.[9-12] While the majority of adverse events tend to resolve over time with the administration of immunosuppressive medication, some side effects can be long lasting.[13] This is particularly true for endocrine-related side effects, which often endure and can require ongoing management.[9,14,15]

Interestingly, clinical trials investigating the efficacy of anti-PD-1 therapy in adjuvant and advanced melanoma patients have demonstrated a slightly higher incidence of treatment-related adverse events in patients receiving adjuvant therapy compared to those with advanced melanoma. This observation suggests that while anti-PD-1 therapy is generally effective across different stages of melanoma, the adverse event development risk profile may vary depending on the treatment setting.[5,6,7,16] Albeit this, it remains a matter of debate whether this holds true in the real-world setting. To date, only a conducted study by Verheijden and colleagues published in 2020, demonstrated a lower risk of severe irAEs in more advanced melanoma patients (stage IV M1c or higher) treated with immune checkpoint inhibitors, compared to less extensive metastatic melanoma patients (stage IV M1a-b).[17] The lower prevalence of immune therapy related adverse events in advanced treated patients could be attributed to the fact that metastatic melanoma produces immune suppressive factors, damping the immune system.[18,19]

This mechanism hypothesizes that patients with completely resected disease, and thus a more active immune system, could potentially develop adverse events more frequently in the

adjuvant setting than patients with metastatic melanoma. The premise is that the robust immune response in these patients, which is essential for the success of adjuvant therapy, might also predispose them to a higher likelihood of immune-related adverse events. In contrast, patients with metastatic melanoma may have a more compromised immune system due to the advanced nature of their disease, which might reduce the incidence of these adverse events. Understanding this differential response is critical for tailoring immunotherapy regimens and managing potential side effects effectively in diverse patient populations.

Despite these assumptions, no study has assessed and compared adverse events in real-world adjuvant and advanced anti-PD-1 treated patients. Additionally, potential confounders such as age, gender, and Eastern Cooperative Oncology Group (ECOG) performance status have not been analyzed and or corrected for in patients receiving treatment in the adjuvant versus advanced setting in daily clinical practice. Assessing these variables is necessary to identify potential factors associated with increased adverse event development, which can help optimize therapy decision-making.[20] For instance, patients with a history of auto-immune disease are more prone to develop disease-specific flare ups, and should be consulted for alternative treatment options (such as BRAF/MEK inhibition) or no anti-PD1 treatment in the adjuvant setting.[21,22]

In this multi-center nationwide register study using prospectively collected data, we assessed and compared demographic variables, ECOG status, comorbidities and type, duration, and severity of irAEs in melanoma patients receiving first-line adjuvant or advanced anti-PD-1 therapy.

Methods

Study design

For this study, we utilized data from the Dutch Melanoma Treatment Registry (DMTR). The DMTR prospectively registers treated patients with advanced melanoma since 2012 and those receiving adjuvant therapy since 2018. Independent data managers, who undergo annual training, register the data, which is subsequently verified and reviewed by treating physicians to ensure high data quality.[23]

This study was designed as a retrospective, observational cohort analysis and included all patients with advanced melanoma (irresectable stage III or IV) or resected stage III-IV melanoma who received anti-PD-1 monotherapy as their first-line treatment. Data was collected in the advanced patient group between January 2015 and September 2020, and from January 2018 to September 2020 in the adjuvant treated patient group. The data set cutoff date was January 2021. The cutoff date of January 2021 provides a sufficient follow-up period to assess the outcomes and adverse events related to anti-PD-1 therapy.

Patients were stratified according to treatment type (adjuvant versus advanced). Comorbidities were assessed prior to treatment initiation, providing a comprehensive baseline for each patient. Severe (grade 3 or higher) immune-related adverse events (irAEs) were meticulously recorded both during treatment and in cases where causality was suspected post-treatment, ensuring thorough monitoring of patient safety and treatment impact.

Moreover, the reason of therapy discontinuation was noted for every individual patients and included progressive disease, therapy completion and therapy discontinuation due to toxicity (any grade irAE).

In compliance with Dutch regulations, the utilization of data from the Dutch Melanoma Treatment Registry (DMTR) for this research received approval from the Medical Ethics Review Committee of Leiden University Medical Center. This research was classified as exempt from the Medical Research Involving Human Subjects Act, meaning that no patient informed consent was required.

Patients Characteristics

Registered demographic variables included age at diagnosis, gender and Eastern Cooperative Oncology Group Performance Status (ECOG). Melanoma was staged according to the eighth edition American Joint Committee on Cancer (AJCC) melanoma staging system.[24]

Comorbidities were documented prior to therapy initiation and consisted of neurological, cardiovascular, pulmonary, gastroenterological, urological, musculoskeletal, infectious, malignancy, rheumatological (cardiomyopathy, scleroderma, sarcoidosis, vasculitis), endocrine (thyroiditis, adrenal insufficiency) and inflammatory bowel disease (Crohn disease, colitis ulcerosa). The variable for preexisting autoimmune diseases encompassed rheumatological, endocrine, and inflammatory bowel disease comorbidities.

Immune therapy related adverse events included grade 3 and 4, according to Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.[18] IrAE's were categorized into the following groups: myelotoxicity, neuropathy, colitis, diarrhea, renal, lung, endocrine, fatigue, cutaneous, and hepatitis. Endocrine adverse events included adrenal insufficiency, thyroid disease, and hypophysitis.

Statistical analysis

Demographic variables were analyzed using descriptive statistics. Categorical variables were compared using the Pearson χ^2 test, while continuous variables were assessed using the Wilcoxon rank-sum test. Univariate regression analysis was conducted to identify significant factors associated with the occurrence of adverse events. Factors identified as significant, along with treatment type, were included in the multivariate regression analysis. Statistical significance was

set at two-sided P-values < 0.05. Data management and statistical analyses were performed using IBM SPSS Statistics version 24 (Armonk, NY, USA).

Results

Baseline characteristics

A total of 1465 advanced melanoma patients received first-line anti-PD-1 therapy with a median age of 69 years (IQR 59-77) and the majority of patients were male (60%) (table 1). The ECOG status was 0 in 818 patients (56%) and ≥1 in 573 patients (39%). Advanced melanoma patients were mostly staged as IV (89%) and irresectable stage III was seen less frequently (11%). An increased LDH level (>250 U/L) was observed in 366 advanced melanoma patients (25%), and 1083 (74%) of the patients had normal LDH levels (<250 U/L). These patients underwent an average of 11 immunotherapy cycles.

	Advanced (N=1465)	Adjuvant (N=908)	P-value
Characteristics			
Age at diagnosis			<0.01
Median (Interquartile range), years	69 (59 - 77)	63 (54 - 72)	
Sex			0.52
Male	882 (60)	523 (58)	
Female	583 (40)	385 (42)	
ECOG performance status			<0.01
0	818 (56)	644 (71)	
1	482 (33)	203 (22)	
>1	91 (6)	14 (2)	
Unknown	74 (5)	47 (5)	
AJCC, 8th edition, stage			<0.01
IIIA	NA	54 (6)	
IIIB	NA	319 (35)	
IIIC	158 (11)	413 (46)	
IIID	NA	12 (1)	
IVa	236 (16)	79 (9)	
IVb	385 (26	30 (3)	
IVc	483 (33)	1 (1)	
IVd	202 (14)	NA	
Unknown	1 (1)	NA	
LDH Levels			
Normal (<250 U/L)	1083 (74)	850 (94)	<0.01
Increased (>250 U/L)	366 (25)	33 (4)	
Missing	16 (1)	23 (3)	
Total mean cycles			
	10.9	9.20	<0.01

Table 1. Patient characteristics.

In the resected melanoma patient group, a total of 908 patients received adjuvant anti-PD-1 therapy. Adjuvant treated patients had a median age of 63 years (IQR 54-72) and the majority of patients had an ECOG performance status of 0 (644 patients (71%)). Adjuvant treated patients were staged IIIB or IIIC (81%) most frequently, while resected stage IV disease was seen less commonly (IVa 9%, IVb 3%, IVc, 1%), and these patients received an average of 9.2 immunotherapy cycles. The LDH values in this patient group was normal (<250 U/L) in the majority of cases (94%), and an elevated LDH value was seen in patients most frequently staged as stage IIIC (52%).

Comparing advanced and adjuvant melanoma patients, the latter were younger ($p<0.01$) and had an ECOG-performance status of 0 ($p<0.01$) more frequently. No difference was observed in gender distribution ($p=0.52$). Advanced melanoma patients received more mean cycles of immunotherapy than adjuvant treated patients, 10.9 cycles versus 9.2 cycles ($p<0.01$). Further, advanced melanoma patients had an increased LDH level more frequently than adjuvant treated patients ($p<0.01$), and these patients were staged IVc the most frequent.

Total median follow-up months, calculated from start to therapy up to last in patient clinic visit, was 11 months in the advanced melanoma group and 12 months in the adjuvant treated group ($p=0.59$).

Comorbidities

Advanced melanoma patients had any comorbidity in 1,128 (77%) cases (Table 2), with cardiovascular (23%) and neurological (17%) comorbidity being the most common. The incidence of other comorbidities (diabetes, pulmonary, gastroenterological, urological, musculoskeletal, infectious) ranged from 2% to 13%.

In the adjuvant-treated group, 627 (69%) patients had any comorbidity, with cardiovascular (14%) and neurological (14%) comorbidity being the most frequent. Other comorbidities (diabetes, pulmonary, gastroenterological, urological, musculoskeletal, infectious) ranged from 2% to 10%.

Comparing comorbidities between the treatment groups, any type of comorbidity ($p<0.01$) and cardiovascular comorbidities ($p=0.02$) were more prevalent in the advanced melanoma group. Other comorbidities, such as neurological, diabetes, pulmonary, gastroenterological, musculoskeletal, and infectious conditions, were similarly distributed between the two groups. Furthermore, a malignant comorbidity, other than melanoma, was observed more frequently in the advanced melanoma group (301 patients, 21%, versus 128 patients, 14%) ($p<0.01$).

Regarding any autoimmune comorbidities, no significant difference was observed between the two treatment groups, with 181 (13%) preexisting auto immune comorbidity in advanced melanoma patients compared to 98 (11%) preexisting auto immune comorbidities in adjuvant-treated

patients (p=0.25). Rheumatologic comorbidities were more common in advanced melanoma patients than in adjuvant-treated patients, 6% versus 4% (p=0.03). Conversely, adjuvant-treated patients had endocrine comorbidities more frequently than advanced melanoma patients, 8% versus 6% (p=0.01).

		Advanced (N=1465)	Adjuvant (N=908)	P-value
Any comorbidity	no	337 (23)	281 (31)	**<0.01**
	yes	1128 (77)	627 (69)	
Neurological	no	1215 (83)	780 (86)	0.34
	yes	250 (17)	128 (14)	
Cardiovascular	no	1135 (77)	779 (86)	**<0.01**
	yes	330 (23)	129 (14)	
Diabetes	no	1286 (88)	811 (90)	0.61
	yes	179 (12)	94 (10)	
Pulmonary	no	1300 (89)	819 (90)	0.38
	yes	165 (11)	89 (10)	
Gastroenterological	no	1313 (90)	839 (92)	0.19
	yes	152 (10)	69 (8)	
Urological	no	1303 (89)	834 (92)	0.13
	yes	162 (11)	74 (8)	
Musculoskeletal	no	197 (13)	806 (89)	0.13
	yes	1268 (87)	102 (11)	
Infectious	no	1426 (98)	906 (98)	0.74
	yes	29 (2)	16 (2)	
Malignancy	no	1164 (79)	780 (86)	**<0.01**
	yes	301 (21)	128 (14)	
Any auto immune disease	no	1284 (87)	810 (89)	0.25
	yes	181 (13)	98 (11)	
IBD	no	1441 (98)	900 (99)	0.12
	yes	24 (2)	8 (1)	
Rheumatologic	no	1371 (94)	869 (96)	**0.03**
	yes	94 (6)	39 (4)	
Endocrine	No	1381 (94)	832 (92)	**0.01**
	yes	84 (6)	76 (8)	

Table 2. Prevalence of comorbidities among study participants.

Characterization of adverse events

Any type of grade III-IV adverse event was seen in 214 (15%) advanced treated patients and in 119 (13%) adjuvant treated patients (table 3) (figure 1), and this did not differ significantly between the two groups (p=0.31). The incidence of specific irAEs (including myelotoxicity, neuropathy, colitis, renal, pulmonary, endocrine, fatigue, cutaneous, hepatitis and other) was equally distributed among the two treatment groups, and the incidence ranged from 1 to 6%.

		Advanced (N=1465)	Adjuvant (N=908)	P-value
Any type of adverse event	No	1251 (85)	789 (87)	0.31
	Yes	214 (15)	119 (13)	
Myelotoxicity	No	1462 (1)	907 (99)	0.66
	Yes	3 (1)	1 (1)	
Neuropathy	No	1462 (99)	905 (99)	0.41
	Yes	3 (1)	3 (1)	
Colitis	No	1414 (96)	875 (96)	0.67
	Yes	53 (4)	33 (4)	
Renal	no	1455 (99)	903 (99)	0.62
	yes	10 (1)	5 (1)	
Pulmonary	no	1451 (99)	904 (99)	0.25
	yes	16 (1)	4 (1)	
Endocrine	no	1405 (96)	870 (96)	0.92
	yes	60 (4)	38 (4)	
Fatigue	No	1456 (99)	904 (99)	0.14
	Yes	9 (1)	2 (1)	
Cutaneous	No	1448 (99)	901 (99)	0.27
	Yes	19 (1)	7 (1)	
Hepatitis	No	1432 (98)	895 (98)	0.10
	Yes	33 (2)	13 (1)	
Other	No	1377 (94)	864 (95)	
	Yes	88 (6)	44 (5)	

Table 3. Type of severe/grade 3+ adverse events and presence or absence in anti-PD-1 treated advanced and adjuvant melanoma patients.

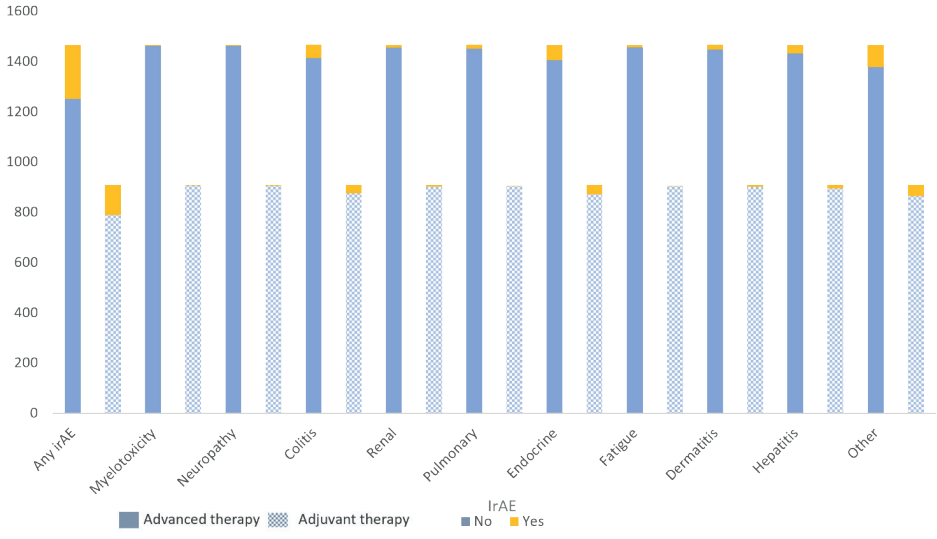

Figure 1. Types of grade III-IV adverse events in advanced and adjuvant melanoma patients treated with anti-PD-1 therapy.

Anti-PD-1 therapy was discontinued due to toxicity (any grade adverse event) more frequently in adjuvant treated patients than in advanced treated patients, 138 (20%) cases versus 196 (15%) cases ($p<0.01$). Additionally, 21 (1%) advanced melanoma patients died due to severe adverse events, whereas no adjuvant-treated patients died from therapy-related causes.

Univariate and multivariate analysis

The primary outcome of the univariate analysis was the occurrence of any type of treatment related severe adverse event (grade III-IV). Univariate analysis demonstrated no significant association between age and gender for grade III-IV irAE development, with an OR of 1.00 (95% CI 0.99 – 1.01) (0.59) and 0.93 (95% CI 0.74 – 1.18) (p=0.98), respectively (table 4). Increased ECOG performance status (>1) and the presence of any type of comorbidity were associated with an increased risk of toxicity development, OR 2.03 (95% CI 1.23 – 3.34) (p=0.01) and 1.22 (95% CI 1.03 – 1.44) (p=0.02), respectively. Univariate analysis for treatment type, demonstrated no increased risk of toxicity development for adjuvant therapy (with advanced therapy taken as reference), OR 0.88 (95% CI 0.69 – 1.12) (p=0.67). The multivariate analysis included the significant variables ECOG, any comorbidity and treatment type.

Increased ECOG performance status (>1) and presence of any comorbidity remained statistically significant with an adjusted odds ratio of 2.00 (95% CI 1.20 – 3.32) (p=0.01) and 1.22 (95% CI 1.02 – 1.44) (p=0.01), respectively. Multiple variable analysis demonstrated no association between grade III-IV irAE development and type of therapy setting (advanced versus adjuvant), with an adjusted odds ratio of 0.95 (95% CI 0.74 – 1.21) (p=0.39).

	Univariate (OR, 95% CI)	**Multivariate (OR, 95% CI)**
Age	1.00 (0.99 - 1.01)	
Gender		
Male	Reference	
Female	0.93 (0.74 -1.18)	
ECOG		
0-1	Reference	
>1	2.11 (1.23 - 3.49)	2.00 (1.20 - 3.32)
Comorbidity		
Absent	Reference	
Any	1.24 (1.04 - 1.46)	1.22 (1.02 - 1.44)
Treatment Type		
Advanced	Reference	
Adjuvant	0.88 (0.69 - 1.12)	0.95 (0.74 - 1.21)

Table 4. Uni- and multivariate regression analysis assessing clinical factors associated with irAE occurrence.

Discussion

To our knowledge, this is the first and largest population-based study to compare adverse events in adjuvant and advanced melanoma patients treated with anti-PD-1 therapy.

In our cohort, 212 (15%) advanced melanoma patients and 130 (13%) adjuvant treated patients developed grade III/IV irAEs during median follow-up periods of 11 and 12 months, respectively. Multivariate analysis adjusted for ECOG status and comorbidities showed no increased risk of adverse event development in the adjuvant setting.

Adjuvant treated patients were younger and generally healthier, with 71% of the patients having an ECOG status of 0, compared to 56% in the advanced group ($p<0.01$). Cardiovascular comorbidities were seen less frequently in adjuvant treated patients ($p<0.01$), while endocrine comorbidities were more common ($p=0.01$). Rheumatologic comorbidities were more frequent in advanced melanoma patients ($p=0.03$). The observed difference in rheumatologic and endocrine comorbidity distribution, might be due to the age difference in the treatment groups, as the incidence of rheumatologic comorbidities increases in older patients, and specific endocrine comorbidities (thyroid dysfunction) can be more prevalent in younger patients.[25] The total prevalence of pre-existing autoimmune diseases was higher in advanced melanoma patients, albeit not significantly different ($p=0.25$).

To explain the observed clinical differences between the treatment groups: the need for anti-PD-1 therapy in the adjuvant setting may be less urgent, while in the advanced setting, advanced anti-PD-1 can be essential and might be initiated regardless of deteriorated ECOG performance status, higher age, or the presence of additional comorbidities. In addition, it is important to note that adjuvant immunotherapy has only been shown to improve recurrence-free survival, and no prolonged overall survival has yet been observed. Patients with poorer health and pre-existing risk factors for adverse events are potentially less likely to be selected for adjuvant therapy, as the potential cons (adverse event development) may outweigh the benefits.

In our cohort, grade 3-4 irAEs were observed in 13% of adjuvant anti-PD-1 patients and 15% of advanced melanoma patients, with no significant difference between the groups ($p=0.31$). A study by de Meza and colleagues, using the same data registry, reported a higher incidence of 18% in adjuvant treated patients.[26] The higher incidence may be due to their longer follow-up period (median follow-up of 18 months vs. median follow-up of 12 months in our study), allowing more time for irAEs to develop. Previous studies have indeed shown that irAEs can occur after therapy cessation and that the incidence of irAEs can increase with a prolonged follow-up.[9]

Albeit similar incidence of irAEs in both treatment groups, adjuvant treated anti-PD-1 patients discontinued therapy due to toxicity (any grade irAE) more frequently than advanced treated

patients. An explanation for the observed difference can be that anti-PD-1 treatment is preventive rather than curative. In contrast, advanced melanoma patients might continue treatment despite adverse events due to the critical nature of their therapy. This could result in a comparable overall toxicity profile, as early discontinuation in the adjuvant setting reduces prolonged exposure to toxicity.

Similar to our findings, clinical trials reported comparable incidences of grade 3-4 irAEs with adjuvant nivolumab. The CheckMate 238 trial for adjuvant treated stage III melanoma (median follow-up of 18 months) reported 14%, and the CheckMate 76K trial for adjuvant treated stage II melanoma (median follow-up of 12 months) reported an incidence of 10%. For advanced melanoma, the CheckMate 066 trial (median follow-up of 17 months) showed a 12% incidence. The KEYNOTE-054 study on adjuvant pembrolizumab for resected stage III melanoma reported 15%, while the KEYNOTE-006 study on pembrolizumab for advanced melanoma reported a 13% incidence.[2,9,11]

The onset of irAEs is multifactorial. Established clinical factors that contribute to irAE development in advanced melanoma patients treated with immune checkpoint inhibitors include a deteriorated ECGO status and preexisting autoimmune diseases.[12,20,27,28] In addition, the extent of metastatic disease might damp the immune system activity and could thereby potentially reduce the irAE development in advanced melanoma patients.[18,19,20] To elucidate this, melanoma tumor can evade immune-mediated destruction through immunosuppressive mechanisms that inhibit T cell activation. However, some tumors still exhibit high levels of CD8+ T cells despite being suppressed by tumoral factors. This elevated T cell level may result from innate immunosuppressive mechanisms such as indoleamine-2,3-dioxygenase (IDO), PD-L1/B7-H1, tryptophan 2,3-dioxygenase (TDO), and FoxP3+ regulatory T cells.[30,32] These pathways are driven by the innate immune system rather than activated by tumor cells.

Interestingly, the expression of IDO and TDO is associated with reduced tumor-infiltrating immune cells and poor responses in malignancies. Furthermore, the expression of IDO and/or TDO could potentially decrease the efficacy of immune checkpoint inhibition, making these mechanisms significant targets for addressing melanoma patients who do not respond to anti-PD-1 therapy.[33] Moreover, a recently published study demonstrated that the molecule heme plays a role in the activation of IDO and TDO, and therefore could potentially be a therapeutic target as well.[33,34]

These tumoral immune response remains a complex, multifactorial challenge. Patients with widespread metastatic cancer may be exposed to higher levels of immunosuppressive factors than patients rendered disease-free. Despite this, the innate immune system plays an important role, which might still induce a similar frequency of adverse event development in metastatic melanoma patients as compared to adjuvant-treated patients.

In our multivariate analysis, we assessed the effect of therapy setting (adjuvant versus advanced), corrected for factors associated with an increased risk on irAE development (ECOG performance status, and comorbidities). We did not show an increased risk of adverse event development in the adjuvant setting. Potential selection bias may influence our results, as patients with a higher tumor load might be more frequently selected for anti-CTLA-4/anti-PD-1 combination therapy rather than anti-PD-1 monotherapy. Albeit this, anti-PD-1 treatment can induce an immune response, irrespective of the disease stage. In addition, adjuvant treated patients may have microscopic residual disease, resulting in a vigorous immune response similar to that seen in patients with detectable metastatic disease. The uniform mechanism of action, may potentially lead to similar incidence of immune-related adverse events (irAEs) across different stages of melanoma.

Our findings indicate that ECOG performance status and the presence of any comorbidity are independently associated with an increased risk of adverse event development in both adjuvant and advanced melanoma patients treated with anti-PD-1 therapy. Patients with these pre-existing risk factors for adverse event development, should receive thorough counseling on whether to pursue adjuvant anti-PD-1 therapy initiation, especially since no improvement in overall survival has been reported for melanoma patients treated with adjuvant anti-PD-1.

Limitations of our study include that the data registry only captures grade 3-4 irAEs and the retrospective nature of the study. Despite these limitations, our data were collected prospectively by independent data managers and reviewed by treating physicians. Also, this is the largest real-world study to date investigating grade III-IV irAEs, with extensive descriptions of patient comorbidities.

Conclusion

In this real-world study, our data demonstrated that an ECOG score $>$ 1 and the presence of any type of comorbidity were associated with an increased risk of immune therapy related adverse event development in resected and irresectable stage III-IV melanoma patients undergoing immunotherapy. Anti-PD-1 treatment in the adjuvant setting, compared to the advanced setting, was not significantly associated with an increased risk of grade III-IV adverse event development. Anti-PD-1 therapy was halted due to toxicity (any grade irAE) more frequently in the adjuvant setting, potentially as treatment might be less essential as compared to advanced anti-PD-1 treated melanoma patients. These findings hold clinical importance in advising patients about the potential benefits of adjuvant anti-PD-1 therapy.

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PART

Irresectable stage melanoma


6.

Systemic Therapy in Advanced Nodular Melanoma versus Superficial Spreading Melanoma: A Nation-Wide Study of the Dutch Melanoma Treatment Registry

Authors:
Daan Jan Willem Rauwerdink, Remco van Doorn, Jos van der Hage, Alfonsus J M Van den Eertwegh , John B A G Haanen, Maureen Aarts, Franchette Berkmortel, Christian U Blank, Marye J Boers-Sonderen, Jan Willem B De Groot, Geke A P Hospers, Melissa de Meza, Djura Piersma, Rozemarijn S Van Rijn, Marion Stevense, Astrid Van der Veldt, Gerard Vreugdenhil, Michel W J M Wouters, Karijn Suijkerbuijk, Monique van der Kooij, Ellen Kapiteijn.

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Abstract

Introduction

Nodular melanoma (NM) is associated with a higher locoregional and distant recurrence rate compared with superficial spreading melanoma (SSM). It is unknown whether the efficacy of systemic therapy is limited.

Methods

Here, we compare the efficacy of immunotherapy and BRAF/MEK inhibitors (BRAF/MEKi) in advanced NM to SSM. Patients with advanced stage IIIc and stage IV NM and SSM treated with anti-CTLA-4 and/or anti-PD-1, or BRAF/MEKi in the first line, were included from the prospective Dutch Melanoma Treatment Registry. The primary objectives were distant metastasis-free survival (DMFS) and overall survival (OS).

Results

In total, 1086 NM and 2246 SSM patients were included. DMFS was significantly shorter for advanced NM patients at 1.9 years (CI 95% 0.7–4.2) compared with SSM patients at 3.1 years (CI 95% 1.3–6.2) ($p < 0.01$). Multivariate survival analysis for immunotherapy and BRAF/MEKi demonstrated a hazard ratio for immunotherapy of 1.0 (CI 95% 0.85–1.17) and BRAF/MEKi of 0.95 (CI 95% 0.81–1.11).

Conclusion

A shorter DMFS for NM patients developing advanced disease compared with SSM patients was observed, while no difference was observed in the efficacy of systemic immunotherapy or BRAF/MEKi between NM and SSM patients. Our results suggests that the worse overall survival of NM is mainly driven by propensity of metastatic outgrowth of NM after primary diagnosis.

Cutaneous melanoma is a highly heterogeneous cancer comprised of distinct histologic subtypes based on cell of origin, role of ultraviolet radiation exposure, pattern of oncogenic mutations, and type of histological growth.[1,2] The two major histologic subtypes are superficial spreading melanoma (SSM), covering 70% of the cases, followed by nodular melanoma (NM) with approximately 20% of the cases, whereas the majority of the remaining melanoma cases are of the histologic subtype lentigo maligna melanoma (3–10%) and the histologic subtype acral melanoma is less common.[2,3] It is important to underline the exact histologic subtype of melanoma, as the histologic subtype can potentially play a prognostic role in disease recurrence. NM, in general, has worse prognostic tumor characteristics. including a higher Breslow thickness, ulcerative status, higher dermal mitotic rate, and more frequent satellite lesions.[3,4] The histologic subtype NM is associated with a vertical growth rate and tends to grow more rapidly compared with SSM. As for the mutation profile, NM is more frequently NRAS mutated, while SSM harbors the BRAF mutation more often. Molecular analysis shows that NM contains a lower mutational load compared with SSM, illustrating the distinct biologic molecular background.[5,6,7] Importantly, primary NM, even corrected for Breslow thickness and ulceration, is associated with lower overall survival and a reduced recurrence-free survival rate compared with primary SSM.[8,9,10] A retrospective study conducted by Lin et al. in melanoma research suggested that the aggressiveness of NM is attributed to a decreased presence of tumor-infiltrating lymphocytes and an upregulation of PD-L1 expression in neoplastic cells compared with SSM; however the exact mechanism of the aggressive behavior of NM has not yet been unraveled.

In the last decade, the advent of immune checkpoint inhibitors and targeted therapy has revolutionized the treatment landscape of metastatic cutaneous melanoma.[11,12,13] The efficacy of immunotherapy ought to be lower in patients with melanoma types with a lower mutation rate, such as acral melanoma, and immunotherapy is more effective in melanoma types with a higher mutation rate, which is the case in the histologic subtype desmoplastic melanoma.[14,15] Despite this, it is unclear whether the primary histologic subtype NM affects the efficacy of immunotherapy and targeted therapy, as the exact significance of the lower mutational profile of NM compared with SSM remains inconclusive.

To date, only two studies compared the efficacy of systemic immune checkpoint inhibitors in NM versus SSM patients and demonstrated contradictory results: Lattanzi et al. observed no difference in survival outcomes of NM versus SSM patients treated with immunotherapy (anti-PD-1 n = 29, anti-CTLA-4 n = 119), while Pala et al. displayed an improved survival of NM patients treated with immunotherapy compared with SSM patients (anti-PD-1 n = 35, anti PD-1/anti-CLTA-4 n = 7).[16,17] As previously conducted studies were small, unclarity remains regarding the efficacy of immunotherapy and targeted therapy in NM. Identifying the prognostic value of the melanoma subtype can be important in choosing the optimal systemic treatment for the individual patient.

Hence, we conducted an analysis using data from a nation-wide prospective registry for systemic treatment of melanoma (the Dutch Melanoma Treatment Registry) to assess survival outcomes of advanced SSM and NM treated with first-line immunotherapy or targeted therapy.

Methods

Study Design

The Dutch Melanoma Treatment Registry (DMTR) prospectively registers data of systemic therapy in advanced melanoma patients in the Netherlands since 2012 and of resectable stage III and IV melanoma since 2018. This registry and quality assurance has been described in detail by Jochems et al.[18] The medical ethics committee of each participating hospital approved research using DMTR data and this research was not deemed subject to the Medical Research Involving Human Subjects Act, in compliance with Dutch regulations.

Patients

Eligible patients were 18 years and older, had histologically confirmed advanced (irresectable stage III and IV) cutaneous superficial spreading or nodular melanoma, according to the eighth edition of the American Joint Committee on Cancer (AJCC) classification (including metastases to skin (M1a), lung (M1b), other visceral sites (M1c), and brain (M1d).[19] Included patients were naïve to treatment and received first-line systemic anti-CTLA-4 and/or anti-PD-1, or either first-line BRAF inhibitor monotherapy or the combination of BRAF inhibitors and MEK inhibitors. Adjuvant-treated patients were excluded from this study. Data on all patients were collected spanning the period January 2012 to January 2019, while the follow-up data cut off was set at 1 February 2020.

Clinical Variables

Demographic variables (age, gender, and WHO-status) and primary tumor characteristics (Breslow thickness (mm), presence of ulceration, dermal mitosis, satellites, mutation status, location, and histologic subtype were extracted from the DMTR database. Furthermore, clinical data on metastatic melanoma were collected, including site of metastasis, number of disease sites with metastasis, lactate dehydrogenase value (LDH), and details on the type and duration of systemic therapy.

Assessment

A comparative analysis, comparing demographic variables in the NM versus SSM groups based on treatment type, was conducted.

Primary Tumor

Distant metastasis-free survival (DMFS) was determined in both groups and was calculated from the diagnosis of primary melanoma until the occurrence of distant metastasis. Overall survival (OS) was calculated from the diagnosis of the primary tumor until death by any cause or the last moment of follow-up.

Advanced Disease

Progression-free survival (PFS) was calculated from the start of systemic therapy until disease progression or the last moment of follow-up. Furthermore, the response to therapy was assessed and included progressive disease (PD), stable disease (SD), partial response (PR), or complete response (CR). An objective response rate to therapy was calculated per treatment type by comparing the best overall response between NM and SSM patients. Lastly, OS was calculated from the start of systemic therapy until death by any cause or last moment of follow-up and compared between the NM versus SSM groups based on treatment type.

Statistical Analysis

Descriptive analysis was conducted to assess demographic variables, clinicopathological characteristics, and treatment type. Identified frequencies of variables were compared between the SSM and NM groups, using a Chi-square test or Wilcoxon rank test. Survival analyses were conducted with the Kaplan–Meier method and compared with the log rank test across each type of treatment group. Patients not reaching the endpoint were censored at the date of the last contact. Cox regression analysis was performed to correct for potential confounders. p-values were two-sided and p-values less than 0.05 were considered to be statistically significant. All statistical analyses were conducted using IBM SPSS Statistics version 24 (Armonk, New York, NY, USA).

Results

Between 2012 and 2021, a total of 2685 advanced (stadium IIIC or stadium IV) SSM and 1329 NM patients were identified (Table 1). Advanced SSM patients were significantly younger, with a median age of 58 (IQR 47–69) compared with the NM group 63 (IQR 52–72) ($p < 0.01$). Patients with NM had ulceration more often ($p < 0.01$), a higher median Breslow thickness ((3.9 mm (IQR 2.4–6.0) versus 1.9 mm in SSM patients (IQR 1.2–3.3) ($p < 0.01$)), and more frequently had dermal mitoses ($p < 0.01$) and satellite lesions ($p < 0.01$) (Table 1), compared with SSM patients. Considering mutation status, NM harbored NRAS-mutations more often (24% versus 16% than SSM patients ($p < 0.01$)), while SSM harbored BRAF mutations more frequently (61% compared with 49% in NM patients ($p < 0.01$)).

Variables	SSM (N=2685)	NM (N=1329)	P-value
Median age at moment of diagnosis (IQR)	58 (47 - 69)	63 (52 - 72)	
Gender - no. (%)			**<0.01**
Female	1133 (42)	457 (34)	
Male	1552 (58)	872 (66)	
WHO - no. (%)			**0.03**
0	1338 (50)	702 (53)	
1	762 (28)	345 (26)	
>1	290 (11)	125 (10)	
Not reported	288 (11)	157 (12)	
Location primary melanoma - no. (%)			**<0.01**
Head/neck	344 (13)	227 (17)	
Trunk	1405 (52)	589 (44)	
Extremities	903 (34)	497 (37)	
Acral	33 (1)	16 (1)	
Breslow thickness in mm (IQR)	1.9 (1.2 - 3.3)	3.9 (2.4 - 6.0)	
Ulceration - no. (%)			**<0.01**
Absent	1602 (60)	580 (44)	
Present	796 (30)	644 (49)	
Unknown	275 (10)	88 (7)	
Dermit - no. (%)			**<0.01**
None	287 (11)	98 (7)	
Any	1391 (52)	820 (62)	
Not reported	978 (36)	400 (30)	
Satellite lesions*			**<0.01**
None	3917 (80)	1748 (77)	
Any	380 (8)	295 (13)	
Not reported	574 (12)	231 (10)	
Mutation status - no. (%)**			
BRAF mutation	1629 (61)	655 (49)	**<0.01**
NRAS mutation	439 (16)	323 (24)	**<0.01**
KIT mutation	24 (0.01)	13 (0.01)	0.08

Table 1. Demographic and primary tumor characteristics.

**Satellite lesions and or in-transit metastasis*
***Total tested patients tested taken as denominator*

Distant Metastasis Free Survival between Primary Tumor and Advanced Disease

NM patients had a significantly shorter median DMFS compared with SSM patients when adjusting for Breslow thickness, BRAF-status, mitotic rate, and ulceration, respectively, that is, 1.9 years (95% CI 1.7–2.1) and 3.1 years (95% CI 2.9–3.3) ($p < 0.01$) (Kaplan Meier DMFS analysis, Figure S1 and Cox regression DMFS analysis, Table S1, are displayed in the Supplementary Materials). Overall survival calculated from primary tumor up to decease or last follow-up moment, corrected for

age, gender, Breslow thickness, BRAF-status, mitotic rate, and ulceration, demonstrated a median OS of 5.9 years (95% CI 2.7–13) and 8.0 years (95% CI 4.0–16) for NM and SSM, respectively (log-rank test $p < 0.05$).

Immunotherapy in Advanced Disease

A total of 747 advanced NM and 1357 SSM patients received first-line anti-CTLA-4, anti-PD-1 or anti-PD-1/anti-CTLA-4. The specific type of the immunotherapy did not differ significantly between NM and SSM patients ($p = 0.08$) (Table 2). Immunotherapy-treated NM patients were older with a median age of 67 years (IQR 55–74) versus 64 years (IQR 53–73) ($p = 0.01$) and the majority of NM patients were male, 521 patients (70%) versus 812 (60%) SSM patients ($p < 0.01$). No significant differences were observed between the two groups of patients with brain metastases, with metastases present in three or more organ sites, or with elevated LDH levels. Considering response to immunotherapy, NM and SSM patients had similar objective response rates of 47% and 46%, respectively (Table 3).

Progression-free survival demonstrated a median progressive-free survival of 16.2 months (95% CI 17.3–22.9) for NM patients and 18.1 months for SSM patients (95% CI 14–21) (logrank test $p = 0.72$) (Kaplan–Meier PFS analysis, Figures S2 and S3, and Cox regression PFS, Tables S2 and S3, in the Supplementary Materials). Overall survival analysis, calculated from the start of therapy up to death or last follow-up, showed a median overall survival of 36 months (95% CI 23–49) for NM patients and a median overall survival of 34 months (95% CI 28–41) for SSM patients (log-rank test $p = 0.53$) (Figure 1a).

Cox regression demonstrated that the histologic subtype NM was not associated with decreased survival (HR 0.90 (95% CI 0.76–1.08)) (Table 4). Factors associated with a decreased overall survival since the start of immunotherapy were the presence of brain metastasis (HR 1.05 95% CI 1.01–1.11), elevated LDH levels at the moment of metastasis detection/diagnosis (HR 1.27 (95% CI 1.17–1.38)), and the presence of NRAS mutation (HR 1.16 (95% CI 1.05–1.28), while BRAF mutation demonstrated a favorable effect with an HR of 0.69 (95% CI 0.58–0.83) (Table 4).

First line systemic immunotherapy				First line targeted therapy			
	SSM (N=1357)	NM (N=747)	p-value		SSM (N=889)	NM (N=339)	p-value
Treatment type			0.08				**0.02**
Anti-CTLA-4	277 (20)	185 (25)		BRAFi	411 (46)	182 (54)	
Anti-PD-1	865 (64)	464 (62)		BRAF/MEKi	478 (54)	157 (46)	
Anti-PD-1/anti-CTLA-4	215 (16)	98 (13)					
Median age (IQR)	64 (53 – 73)	65 (55 - 74)	**0.01**		60 (50 – 69)	64 (54 – 73)	**<0.01**
Gender - no. (%)			**<0.01**				0.22
Female	545 (40)	226 (30)			394 (44)	137 (40)	
Male	812 (60)	521 (70)			495 (56)	202 (60)	
WHO - no. (%)			0.22				0.34
0	863 (64)	479 (64)			324 (36)	143 (42)	
1	371 (27)	189 (25)			309 (35)	114 (34)	
>1	49 (4)	30 (4)			167 (19)	48 (14)	
Not reported	73 (5)	49 (7)			89 (10)	34 (10)	
Brain metastasis			0.37				0.99
Not present	1112 (82)	601 (80)			538 (61)	211 (62)	
Present	216 (16)	135 (18)			330 (37)	121 (36)	
Asymptomatic	133	84			112 (34)	44 (36)	
Symptomatic	83	51			208 (63)	77 (64)	
Not reported	29 (2)	11 (2)			21 (2)	7 (2)	
LDH			0.06				**0.03**
Normal	1018 (75)	591 (79)			424 (48)	176 (52)	
Elevated	311 (23)	147 (20)			443 (50)	146 (43)	
Not determined	24 (2)	9 (1)			19 (2)	17 (5)	
Organ sites with metastasis			0.35				0.97
<3	442 (33)	236 (32)			44 (5)	18 (5)	
>2	720 (53)	417 (56)			729 (82)	262 (77)	
Unknown	195 (14)	94 (13)			116 (14)	59 (18)	

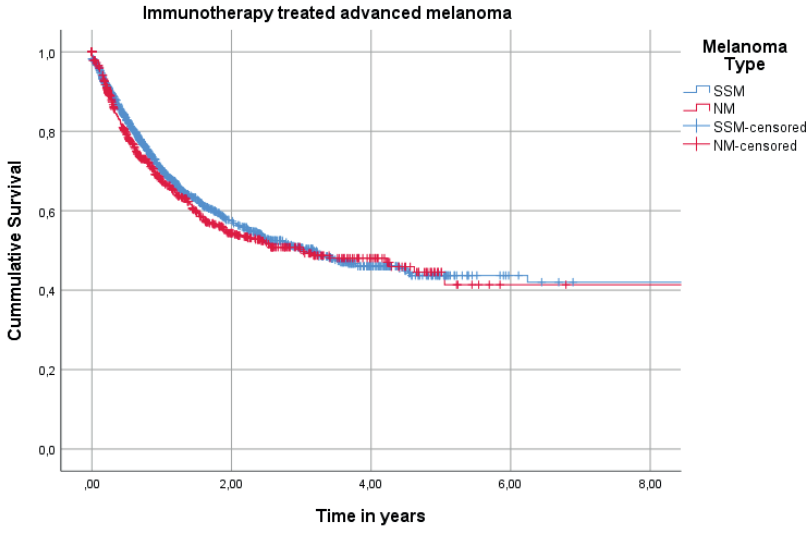
Table 2. First line systemic therapy

[¤] *Total brain metastasis taken as denominator*

	Immunotherapy		BRAF/MEKi	
	SSM	NM	SSM	NM
Complete response	196 (17)	108 (16)	17 (2)	16 (9)
Partial response	346 (30)	194 (30)	362 (43)	120 (37)
Stable disease	32 (3)	17 (3)	63 (7)	29 (9)
Progressive disease or death	596 (51)	336 (51)	401 (48)	162 (50)
Objective response rate	47%	46%	45%	46%

Table 3. Response rate stratified per treatment and melanoma type.

A.



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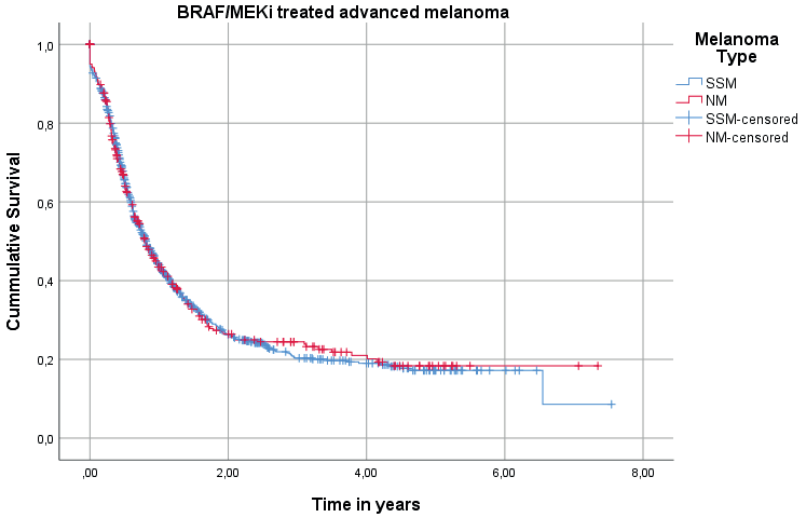


Figure 1. Kaplan–Meier curve survival analysis demonstrating the cumulative survival of NM (red) versus SSM (blue) patients treated with immune checkpoint inhibition (**A**) and Kaplan–Meier curve survival analysis demonstrating the cumulative survival of NM (red) versus SSM (blue) patients treated with immune checkpoint inhibition (**B**).

Variables		N	Hazard ratio - 95% CI	P-value
Age		2104	1.00 (0.99 - 1.01)	0.48
Gender	Male	1333	Reference	
	Female	771	0.89 (0.74 - 1.06)	0.19
WHO	0 - 1	1902	Reference	
	2 - 4	79	1.02 (0.99 - 1.06)	0.20
Treatment type	Anti-CTLA-4	462	Reference	
	Ant-PD-1 / Anti-CLTA-4	1642	0.64 (0.53 - 0.76)	**<0.01**
LDH	Not elevated	1609	Reference	
	Elevated	458	1.27 (1.17 - 1.38)	**<0.01**
Cerebral disease	Absent	1713	Reference	
	Present	351	1.05 (1.01 - 1.11)	**0.03**
Total organ sites	<3	678	Reference	
	>2	1137	1.03 (0.87 - 1.20)	0.76
Melanoma	SSM	1357	Reference	
	NM	747	0.90 0.76 - 1.08)	0.26
BRAF mutation	Absent	1053	Reference	
	Present	916	0.69 (0.58 - 0.83)	**<0.01**
NRAS mutation	Absent	1112	Reference	
	Present	574	1.16 (1.05 - 1.28)	**<0.01**

Table 4. Multivariable treatment-related Cox regression analysis in patients treated with immunotherapy. Significant values are highlighted in bold.

Targeted Therapy in Advanced Disease

In total, 339 advanced NM and 889 SSM patients were treated with BRAF inhibition monotherapy or BRAF/MEK combination therapy. NM patients received BRAF/MEKi combination therapy more frequently compared with SSM patients, 478 (54%) versus 157 (46%), respectively ($p = 0.02$). NM patients were significantly older with a median age of 64 (IQR 54–73) versus SSM patients with a median age of 60 years (IQR 50–69) ($p < 0.01$). Regarding characteristics of metastatic disease in the two groups, no difference was observed in elevated LDH levels at the moment of metastasis, brain metastasis, and total organ sites with metastatic lesions. As for treatment response, the objective response rate for NM patients was 46%, and 45% for SSM patients. Kaplan–Meier analysis demonstrated a PFS of 7.4 months (95% CI 6.2–8.6) for NM patients, while PFS was 7 months (95% CI 6.3–7.8) in SSM patients (log-rank test $p = 0.70$). Kaplan–Meier analysis for treatment-related overall survival demonstrated a median overall survival of 9.6 months (95% CI 7.9–11.0) and 9.6 months (95% CI 8.5–11.0) for NM and SSM patients, respectively (Figure 1b) (log-rank test $p = 0.31$). Cox regression analysis showed a hazard ratio of 0.92 (95% CI, 0.78–1.08) for the NM histologic subtype (Table 5). In addition, the presence of brain metastasis (HR 1.08, 95% CI 1.04–1.13), decreased WHO classification (HR 1.08, 95% CI 1.06–1.11), and elevated LDH levels (HR 1.24, 95% CI 1.12–1.32) were associated with a decreased overall survival.

Variable		N	Hazard ratio - 95% CI	P-value
Age		1228	1.004 (0.99 - 1.01)	0.08
Gender	Male	697	Reference	
	Female	531	0.98 (86 - 1.14)	0.87
WHO				
	0 - 1	890	Reference	
	2 - 4	123	1.08 (1.06 - 1.11)	**<0.01**
Treatment type	BRAF mono therapy	593	Reference	
	BRAF/MEKi	635	0.80 (0.74 - 0.86)	**<0.01**
LDH	Not elevated	600	Reference	
	Elevated	589	1.24 (1.12 - 1.32)	**<0.01**
Cerebral disease	Absent	749	Reference	
	Present	451	1.08 (1.04 - 1.13)	**<0.01**
Total organ sites	<3	62	Reference	
	>2	991	0.94 (0.78 - 1.13)	0.50
Melanoma	SSM	889	Reference	
	NM	339	0.92 (0.78 - 1.08)	0.30

Table 5. Multivariable treatment related cox regression analysis in patients treated with targeted therapy.

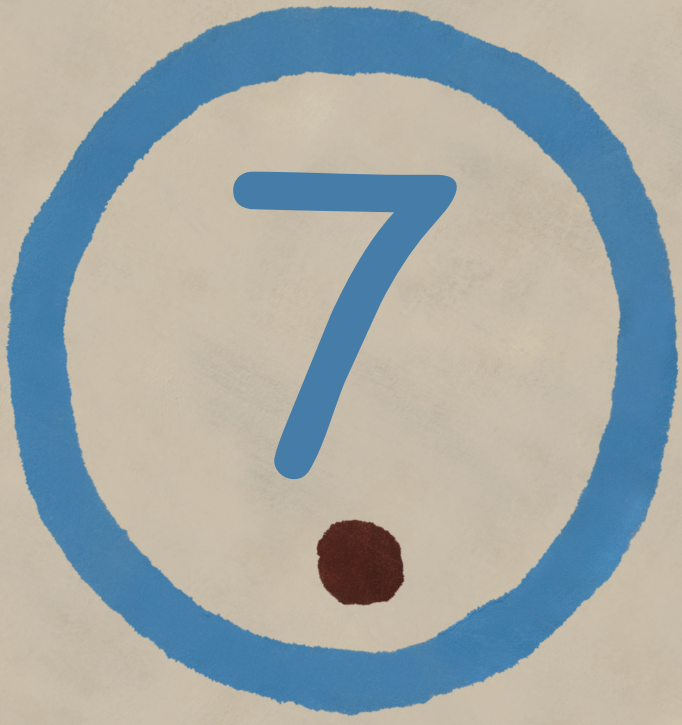
Discussion

To the best of our knowledge, this is the largest prospective cohort study investigating the efficacy of immune checkpoint inhibitors and targeted therapy in advanced NM compared with SSM patients. NM patients had a significantly shorter median DMFS compared with SSM patients when adjusting for Breslow thickness, BRAF-status, mitotic rate, and ulceration. No significant difference in terms of overall survival upon start of systemic therapy was observed in the NM versus SSM group: immune checkpoint inhibition-related survival analysis demonstrated similar survival outcomes. A multivariate analysis, corrected for metastatic and demographic variables, revealed that the histologic subtype NM was not independently associated with decreased treatment-related survival in immunotherapy patients. Considering patients treated with BRAF/MEKi, treatment-related survival analysis showed that survival outcomes did not differ between the NM and SSM group, and multivariate Cox regression analysis demonstrated that the histologic subtype NM was not associated with decreased survival in BRAF/MEKi-treated patients. Interestingly, we did not observe that gender was an independent risk factor for survival in this group, as, is in contrast to the recently published study conducted by Vellano et al. in Nature, it demonstrated that female patients treated with BRAF/MEKi neo-adjuvant treatment had significantly better relapse-free survival rates compared with male patients. Regarding the importance of histologic subtype of NM in the metastatic setting, only one study, in 21 NM patients, performed by Pala et al., analyzed the survival outcome in addition to the metastatic immunologic behavior of NM compared with SSM and demonstrated a prolonged survival of NM versus SSM.[16] The study attributes the improved survival in NM patients compared with SSM

patients to an overexpression of MHC-II molecules and IFN gamma signature, which are both involved in antigen processing and presentation mechanism, which play a significant role in tumor immunogenicity. Despite the improved survival outcome in NM patients, the study was limited in size (anti-PD-1 n = 35, anti-PD-1/anti-CTLA-4 = 7). In contrast, a study conducted by Lantazzi et al. demonstrated no improved survival for metastatic NM compared with SSM treated with immune checkpoint inhibition.[17] Nonetheless, this study was also limited by the fact that the most given treatment was anti-CTLA-4, with only 29 out of the 119 patients receiving anti-PD-1. In spite of the published results on advanced NM treated with immunotherapy, no large study has been performed investigating the efficacy of targeted therapy. Only the Cancers 2022, 14, 5694 10 of 12 study by Lantazi et al. analyzed the efficacy of targeted therapy in NM and SSM patients and demonstrated a decreased survival for BRAF-mutated NM as compared with BRAF-mutated SSM patients, and histologic subtype NM in the multivariate analysis was independently associated with a decreased survival. However, the power of this study was limited as only 52 patients were included. It is interesting that a large population-based cross-sectional analysis performed by Allais et al. showed that the diagnosis of primary detected histologic subtype NM, corrected for Breslow thickness and ulceration, was associated with a decreased 5-year relative survival compared with SSM, suggesting that the histologic subtype should be taken into consideration in making treatment decisions.[9] In addition, a large international multicenter study, conducted by Di Carlo and colleagues, demonstrated similar results, with an increased hazard ratio for death in patients with NM (N = 5375) compared with SSM patients (N = 19.592), adjusted for sex, age, and disease stage at diagnosis.[8] The reduced overall survival (measured from primary melanoma up to death), as mentioned in these studies, could be explained by the shorter distant-free metastasis survival for NM versus SSM, as we found in our analysis. Considering similar treatment-related survival outcomes in advanced SSM and NM patients, we hypothesize that decreased overall survival, measured as time from diagnosis of the primary tumor up to death or the last follow-up moment, in NM patients is mainly driven by primary tumor characteristics and primary tumoral genetic environment, leading to a shorter distant metastasis-free survival. Thus, if NM metastasizes earlier, this will ultimately lead to a worse prognosis. Yet, the histologic subtype NM has not been considered a prognostic metastatic variable, despite a shorter distant metastasis-free survival compared with SSM patients. This underlines the importance of reassessing the follow-up concerning NM patients, and the histologic subtype should be taken into consideration when a decision with regards to adjuvant immunotherapy is made, in order to prolong recurrence-free survival and distant metastasis-free survival.

Conclusions

Our study shows similar efficacy of immune checkpoint inhibition and BRAF/MEKi in advanced NM compared with SSM patients. However, overall survival, measured as the moment of primary diagnosis up to decease or the last follow-up moment, is shorter because of a shorter distant metastases-free interval in NM as compared with SSM. This might have implications for the follow-up from primary tumor diagnosis and for the consideration of (neo) adjuvant therapy. Future studies should focus on the biologic metastatic behavior.



Mixed Response to Immunotherapy in Patients with Metastatic Melanoma

Authors:
Rauwerdink DJW, Molina G, Frederick DT, Sharova T, van der Hage J, Cohen S, Boland GM. Mixed Response to Immunotherapy in Patients with Metastatic Melanoma.

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Abstract

Background

Immunotherapy has improved overall survival in metastatic melanoma. Response to therapy can be difficult to evaluate as the traditionally used RECIST 1.1 criteria do not capture heterogeneous responses. Here we describe the clinical characterization of melanoma patients with a clinically defined mixed response to immunotherapy.

Methods

This was a single institution, retrospective analysis of stage IV melanoma patients who received first-line anti-CTLA-4, anti-PD1, or combination anti-CTLA-4/anti-PD1. Therapy response was assessed via clinical definitions, which consisted of cross-sectional imaging combined with clinical exam. Course of disease, clinicopathological characteristics, and management in patients with a mixed clinical response were analyzed.

Results

In 292 patients (anti-CTLA4 = 63; anti-PD1 = 148, anti-CTLA4/anti-PD1 = 81), 103 were responders (35%), 64 mixed responders (22%), and 125 patients had progressive disease (43%). Of patients with a mixed response, 56% eventually had response to therapy (mixed response followed by response, MR–R), while 31% progressed on therapy (MR–NR). MR–NR patients had higher median LDH ($p < 0.01$), 3 or more organ sites with metastases ($p < 0.01$), and more frequently had M1d disease ($p < 0.01$). Mixed responders who underwent surgery ($n = 20$) had a significantly longer mean OS compared to patients who did not undergo surgery (6.9 years, 95% CI 6.2–7.6 vs. 6.0 years, 95% CI 4.6–7.3, $p = 0.02$).

Discussion

Mixed response to immunotherapy in metastatic melanoma was not uncommon in our cohort (22%). Clinical characteristics associated with progression of disease after initial mixed response included higher LDH, brain metastases, and ≥ 3 organ sites with metastases. Surgical treatment for highly selected patients with a mixed response was associated with improved outcomes.

Introduction

The advent of immune checkpoint inhibitors has revolutionized the therapeutic landscape of metastatic melanoma and has resulted in significant improvements in patient survival. Anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4, ipilimumab) enhances overall survival in metastatic melanoma patients, while anti-programmed cell death protein blocking antibodies (anti-PD-1) have demonstrated improved overall survival.[1,2] The combination of anti-PD-1 and anti-CLTA-4 therapy is associated with a higher response rate and a significantly longer survival in patients with metastatic melanoma.[3] Despite these improvements, the evaluation of individual patient responses to immunotherapy can be complex and unpredictable.[4,5] The kinetics and patterns of immunotherapy response are still being fully characterized, but there is a clearly defined subgroup, such as those with stable disease via RECIST (< 20% tumor progression and < 30% tumor regression) who have an intermediate survival.[6,7] Nuanced response patterns are poorly detected by current radiographic approaches, such as RECIST, which has led to other immunotherapy-specific radiographic assessments like immunotherapy response RECIST, which is useful but cumbersome to implement in clinical care.[8,9] Additionally, real-word clinical descriptions of these nuanced response patterns are still lacking. One current clinical challenge is pseudoprogression, a scenario in which tumors will increase in size but eventually regress.[10,11] Additionally, an individual can have simultaneous regression in some tumors with progression in others, termed a mixed response. In other patients, lesions may regress or remain stable for a long period of time (i.e., stable disease), while other patients progress in a single site or organ, termed oligometastatic progression.[12] These heterogeneous responses are challenging and clinical decisions for these situations are made on a case-by-case basis. Currently, only one study has explored the management of oligometastatic progression in metastatic melanoma, and the literature is comprised of a few individual case reports on patients with a mixed response.[13,14] Therefore, we conducted a single center retrospective study on patients with metastatic melanoma treated with first-line anti-CTLA-4 and/or anti-PD-1 therapy who developed a mixed response, defined as simultaneous tumor regression and progression, in order to identify clinicopathological characteristics, define high-risk subgroups, and assess subsequent management and outcomes.

Methods

Data Source and Study Design

We conducted a retrospective study of patients with unresectable stage IV melanoma treated at the Massachusetts General Hospital (MGH), spanning September 2011 to November 2019. Informed consent was obtained from all patients in accordance with the Institutional Review Board (IRB).

Patients

Eligible patients were 18 years of age or older, had histologically confirmed unresectable stage IV cutaneous melanoma according to the eighth edition of the American Joint Committee on Cancer (AJCC) classification [including metastases to skin (M1a), lung (M1b), other visceral sites (M1c), and brain (M1d)], and had an Eastern Cooperative Oncology Group performance-status score of 0 or 1.[15] Exclusion criteria included previously treated melanoma, ocular melanoma, and missing medical records. Staged patients were treated with first-line immune checkpoint inhibitors (anti-PD-1 or anti-CTLA-4 monotherapy or combined anti-PD-1/anti-CTLA-4 therapy,) according to standard therapeutic doses and cycles. All patients underwent standard of care follow-up at MGH, consisting of radiographic assessment every 12 weeks, clinical evaluation by the involved oncology team, and assessment by the treating medical oncologist in which physical exam and laboratory values were assessed.

Clinical Variables

Demographic variables (age, gender, race, and ECOG status) were extracted from the electronic medical record (EMR). Primary tumor characteristics were extracted from the dermatopathological report [Breslow thickness (mm), ulceration, location of primary tumor], pathological data were collected on metastatic melanoma lesions [number of sites of metastasis, metastatic mutational status (BRAF V600)]. Lactic acid dehydrogenase (LDH) values were collected from laboratory results. Information on timing of radiation and type/date of surgery was obtained from the EMR.

Assessment

Treatment response was assessed with computed tomography (CT scan) and, if suitable, magnetic resonance imaging (MRI) by a certified radiologist and included the evaluation of non-lymph node metastatic lesions $\geq$ 5 mm in the long axis, brain metastases $\geq$ 2 mm in the long axis, and lymph nodes with $\geq$ 15 mm in the short axis. Tumor burden was defined as the total sum of all measured lesions. We classified response to treatment into three groups: responders (metastatic lesions regressing and no presence of recurrences or new lesions), mixed responders (simultaneously regressing and progressing metastatic lesions) and non-responders (progressive metastatic lesions without any sites of tumor regression). Mixed response to first-line immunotherapy was measured during the first three follow-up scans. The course of disease in patients with a mixed response was divided into three cohorts: (1) mixed response followed by response (MR–R), (2) stable mixed responder (SMR), and (3) mixed response followed by progression (MR–NR). Subsequent analysis was undertaken for the MR–R and MR–NR groups. The SMR group was excluded due to small cohort size (n = 6 patients). An overall survival (OS) analysis was performed on the MR–R and MR–NR groups, defined as the time between metastatic disease confirmation

and the date of last follow-up or date of death. An additional survival analysis was performed for the entire mixed response cohort treated with or without subsequent surgery to assess survival outcomes.

To compare our clinical response categories with tumor response measurements according to standard guidelines, we performed treatment response evaluation in a subset of patients with available Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1: complete response (CR, 100% disappearance of target lesion), partial response (PR, ≥ 30% decrease in tumor size), progressive disease (PD, ≥ 20% increase of lesion size), and stable disease (SD, < 30% tumor decrease and < 20% increase in tumor size).

Statistical Analysis

Descriptive analysis was performed to identify frequencies of demographic variables, clinicopathological variables, and recurrence events for selected patients. Observed frequencies of characteristics were compared between the mixed response groups using a Chi square test, Fisher's exact test, or Wilcoxon rank test when appropriate. Overall survival curves (OS) were estimated with the Kaplan–Meier method and compared with the log rank test for each response to therapy group. *p* values were two-sided and a *p* value less than 0.05 was considered to be statistically significant. All statistical analyses were conducted using IBM SPSS Statistics version 24 (Armonk, New York) and Stata/IC 13.1 (College Station, TX).

Results

Between 2011 and 2019, a total of 292 patients were diagnosed with unresectable stage IV melanoma and enrolled into our translational protocol. Of these patients, 148 received anti-PD-1 monotherapy (51%), 63 were treated with anti-CTLA-4 monotherapy (22%), and 81 were treated with anti-PD-1/anti-CTLA-4 combination therapy (28%). During treatment, 103 patients were classified as responders (35%), 64 patients demonstrated a mixed response (22%), and 125 patients progressed on therapy (43%, Figure 1). For patients who had a mixed response, a total of 38 patients subsequently responded to therapy (MR–R), 22 patients eventually progressed on therapy (MR–NR), and 6 patients had a stable mixed responder (SMR). In a subset of patients with RECIST 1.1 data available, a comparison of our clinical categories to RECIST was conducted (n = 101 patients). Defined clinical response definitions closely mirrored RECIST findings, as all clinical responders (n = 33) correlated to RECIST responders (CR 27%, PR 73%), and similar findings were observed with clinical non-responders (n = 41) (SD 7%, PD 93%). Clinical mixed responders (MR–R n = 14; MR–SMR n = 4; MR–NR n = 9) were most commonly categorized into the RECIST 1.1 stable disease group (SD = 63%).

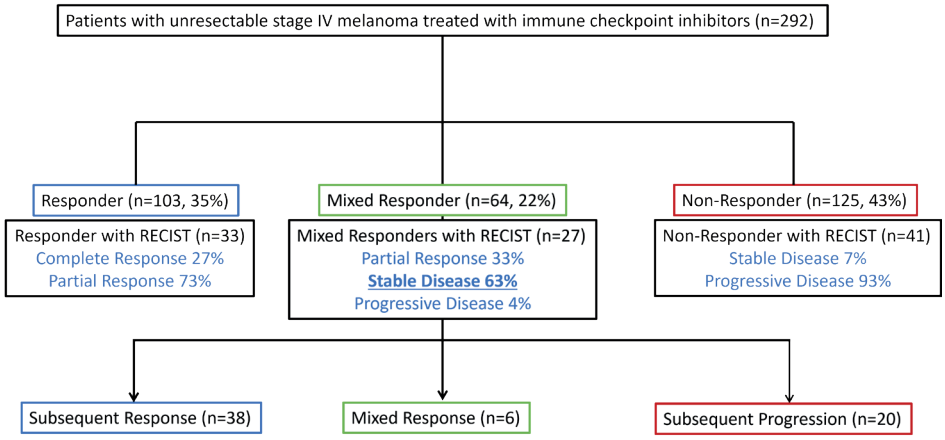


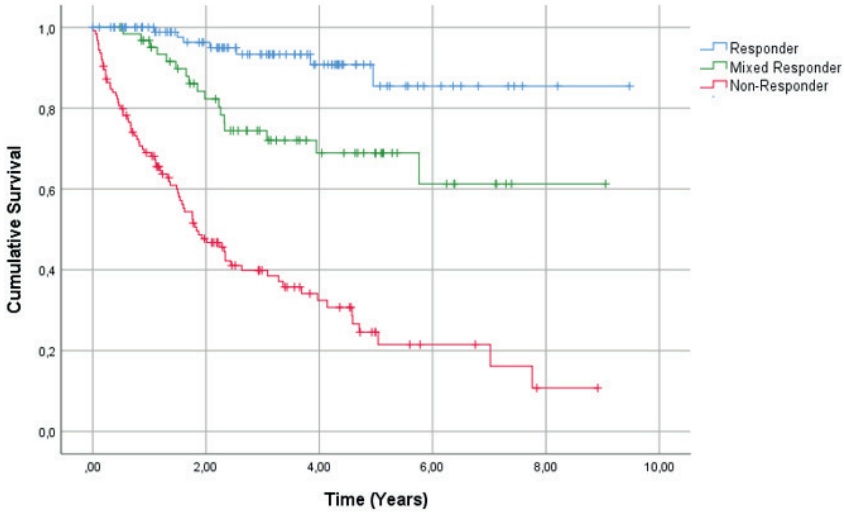
Figure 1. Experimental selection scheme according to clinical classification and RECIST 1.1. Two hundred ninety-two patients with metastatic melanoma were classified by combined clinical/radiographic findings into responder categories: responder (blue), mixed responder (green), and non-responder (red). The black boxes compare RECIST 1.1 categories in a subset of the clinically defined cohorts and demonstrate that the majority of the mixed responders fall into the stable disease (SD) category according to RECIST 1.1. Within the mixed responder cohort, we categorized subsequent response from the time of clinical mixed response.

Unsurprisingly, overall survival analysis demonstrated best outcomes for clinical responders (mean OS of 8.6 years; 95% CI 7.9–9.3) and worst outcomes for clinical non-responders (mean OS 3.2 years; 95% CI 2.5–3.8). There was an intermediate OS for the clinical mixed response group (mean OS 6.6 years; 95% CI 5.6–7.6) (Figure 2A). Overall survival according to RECIST 1.1 demonstrated the best mean OS of PR in 24 patients (7.5 years; 95% CI 6.8–8.3) median follow-up 3.8 years (IQR 3.1–5.4) and for 9 patients with CR (4.8 years; 95% CI 4.1–5.5) median follow-up 2.7 years (IQR 2.2–4.2). An intermediate mean OS was seen in 20 patients with stable disease (4.5 years; 95%, 3.8–5.7) with median follow-up of 3.5 years (IQR 2.0–4.9), and the worst mean OS was seen in 38 patients with PD (3.5 years; 95% CI 2.5–4.5) and a median follow-up of 3.5 years (2.5–4.5) (Figure 2B). The observation of an intermediate OS for RECIST 1.1 stable disease and for clinically defined mixed responders supports the previously demonstrated overlap between RECIST 1.1 SD and our mixed responder patients (63% overlap).

A total of 27 patients who developed a mixed response had RECIST data available at the moment of mixed response confirmation. Of these, 14 patients with MR–R were categorized as SD (43%) versus PR (57%) (Figure 3). In the sustained mixed response category, 4 (67%) patients had RECIST values for comparison and all (100%) were classified as SD, while 9 patients (41%) in the MR–NR group showed either SD (78%) or PD (22%). Regarding treatments in the mixed response group, patients with an initial mixed response and subsequent progression (MR–NR) were more frequently treated with anti-PD-1 monotherapy (73%) as compared to mixed responders with subsequent response (MR–R, 39%) although this was not statistically significant ($p = 0.16$). On

the other hand, MR–R were more often treated with anti-PD-1/anti-CTLA-4 combination therapy (25% vs. 9%), but this was also not significantly different ($p = 0.06$).

A.



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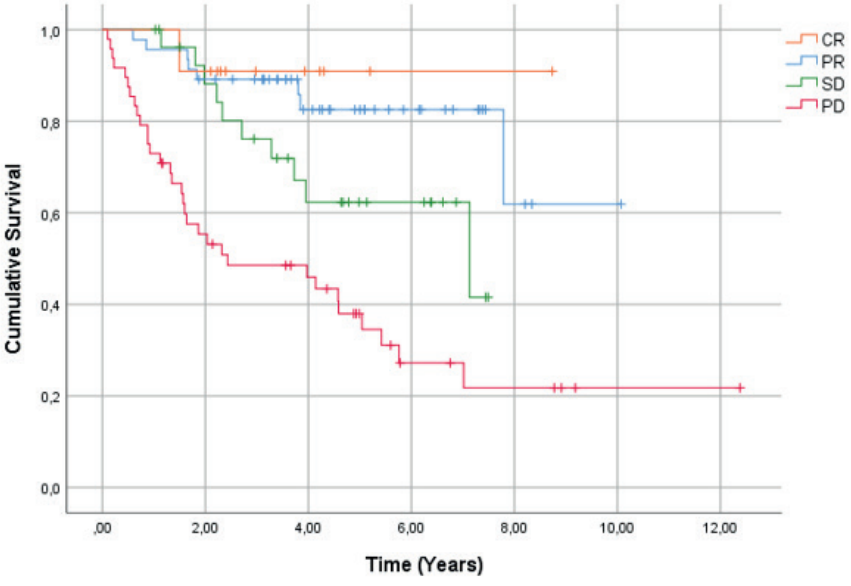

Figure 2. Kaplan–Meier overall survival per clinical response categories, and by RECIST 1.1. **A.** Utilizing our clinical categories, the mixed responders (green) have an intermediate response as compared to responders (blue) and non-responders (red). **B.** The patients with a partial response (PR) and stable disease (SD) by RECIST 1.1 have an intermediate survival as compared to those with a complete response (CR) or progressive disease (PD).

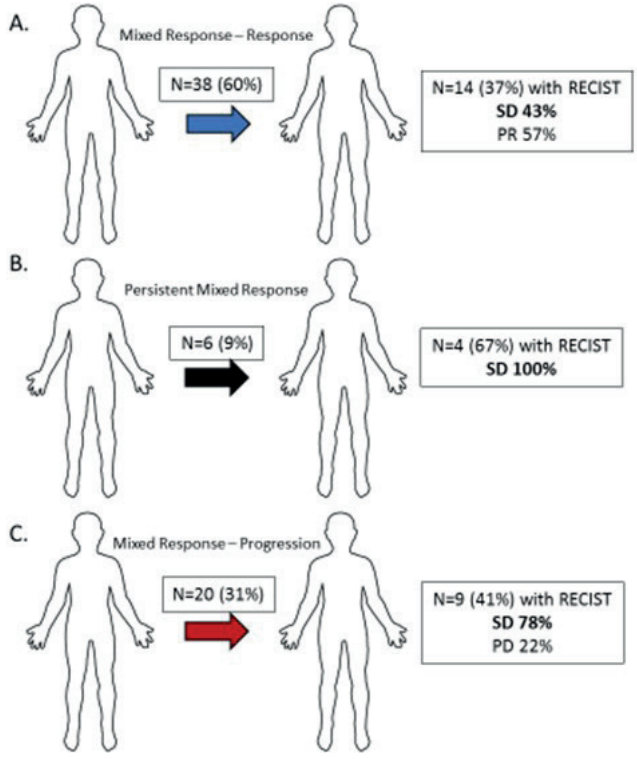

Figure 3. Clinical mixed response group. **A.** Within the mixed response clinical group, those with a mixed response and subsequent response (MR–R) comprised 59% of the cohort. A subset of these patients had RECIST 1.1 data for comparison: 43% had SD and 57% had a PR by RECIST. **B.** In the subset (9%) of mixed responders with a persistent mixed response, RECIST 1.1 evaluation categorized them as SD (100%). **C.** In the mixed responder subset with eventual progression (MR–NR) (31%), RECIST 1.1 data was available in a subset. These patients were characterized as either SD (78%) or PD (22%).

Demographic variables, gender, race, ECOG performance status, location of disease, tumor histology, tumor ulceration, and Breslow thickness, were similar between MR–R versus MR–NR (Table 1). Patients in the MR–NR group as compared to the MR–R group, were older ($p = 0.03$) with a median age of 75 years (IQR 67.5–81.3), had a higher median LDH at mixed response confirmation ($p < 0.01$), more often had brain metastases (i.e., stage M1d; $p < 0.01$), had a higher number of total disease sites ($p < 0.01$), and a trend was seen in BRAF wild-type involvement ($p = 0.05$). In addition, the estimated tumor burden at mixed response was not significantly different between the groups ($p = 0.11$). Furthermore, the median time between mixed response and new response was shorter in MR–NR (141 days IQR 74–265) as compared with MR–R (260 days, IQR 98–434), but was not statistically significant ($p = 0.09$). A schematic of the mixed responder categories is displayed (Figure 4A). Kaplan–Meier survival curves showed a mean OS of 8.6 years (95% CI 7.9–9.2) for patients with MR–R, 4.0 years (95% CI 2.0–6.0) for patients with a sustained mixed response (not shown), and 3.9 years (95% 2.8–4.9) for MR–NR ($p < 0.01$) (Figure 4B).

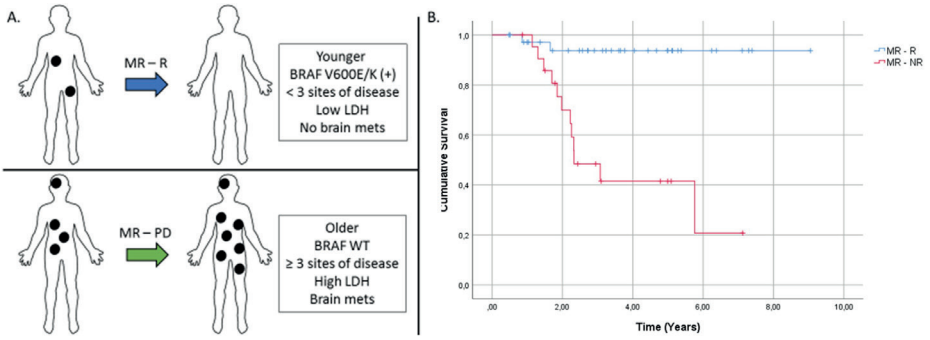

Figure 4. Description of mixed responder categories. **A.** Schematic of mixed responders—mixed responders with subsequent responses were more likely to be younger, have BRAF V600E/K mutations, have fewer than 3 sites of disease, low LDH, and no brain metastases. **B.** Kaplan–Meier overall survival curves for mixed responders with subsequent response (MR–R) versus those who subsequently progress (MR–NR).

Patients with a subsequent response tended to be younger ($p = 0.03$), were less likely to have M1d disease ($p < 0.01$), had fewer than 3 sites of metastases ($p < 0.01$), were more likely to have a BRAF V600E/K mutation ($p = 0.05$), and were less likely to have an elevated LDH ($p < 0.01$).

Between the onset of mixed response and a new clinical response, a total of 10 (28%) patients with MR–R received radiotherapy versus 6 (27%) patients with MR–NR (Figure 5A). Significantly more patients received surgery in the MR–R group ($n = 16$) compared to the MR–NR group ($n = 4$; p value < 0.01). The majority of patients with a mixed response who received surgery underwent visceral metastasectomy (50%), while the remainder underwent subcutaneous metastasectomy (25%), lymph node dissection (20%), or craniotomy (15%) (Figure 5B). A univariate comparison between mixed responders who subsequently had surgery versus mixed responders who did not receive surgical treatment showed similar demographic variables including age, gender, and ECOG status (Table 2). No differences were seen amongst patients who underwent surgery in terms of disease stage, number of organ sites with metastases, or overall tumor burden. However, mixed responders who did not have surgery had a significantly higher median LDH at mixed response (207 U/l IQR 180–237) versus 165 U/l (IQR 141–205) $p < 0.01$) and a significantly shorter time to new response [3.9 months (IQR 2.6–8.9) versus 11.6 months (IQR 6.7–19.3; $p < 0.01$)]. Finally, survival analysis demonstrated a mean OS of 6.0 years (95% CI 4.6–7.3) for mixed responders without surgery and a longer mean OS of 6.9 years (95% CI 6.15–7.6) for mixed responders who subsequently had surgery (log-rank test $p = 0.02$).

		MR – R (n=36)	MR – NR (n=22)	P-value
Median age		67 (29 - 85)	75 (48 - 85)	**0.03**
ECOG	<1	28 (78)	12 (55)	0.06
	≥1	8 (22)	10 (45)	
Gender	Female	10 (28)	4 (18)	0.53
	Male	26 (72)	18 (82)	
Location Primary	Trunk	8 (22)	6 (27)	0.55
	Lower ex	9 (28)	7 (32)	
	Head/neck	10 (28)	3 (14)	
	Penis	NA	1 (5)	
	Vulva/vaginal	1 (3)	2 (9)	
	Unknown	7 (19)	3 (14)	
Tumor Histology	SSM	7 (19)	5 (23)	0.76
	Nodular	9 (25)	4 (18)	
	Acral	NA	1 (5)	
	Mucosal	1 (3)	NA	
	Desmoplastic	1 (3)	NA	
	Unknown	18 (50)	12 (54)	
Ulceration	No	12 (33)	8 (36)	0.99
	Yes	13 (36)	8 (36)	
	Unknown	11 (31)	6 (27)	
Median Breslow (mm)		4.1 (0.4 - 8.3)	3.2 (0.7 - 42)	0.64
M stage	M1a	5 (14)	1 (5)	0.39
	M1b	11 (31)	2 (9)	0.1
	M1c	12 (33)	6 (27)	0.77
	M1d	7 (19)	13 (59)	**<0.01**
No. Sites Mets	No	25 (69)	6 (27)	**<0.01**
	Yes	11 (31)	16 (73)	
Mutational Status	BRAF V600E/K	14 (39)	4 (18)	**0.05**
	NRAS	7 (19)	8 (36)	0.54
	WT	7 (19)	8 (36)	0.37
	Not tested	3 (8)	NA	0.27
	Total mutations ≥ 5	7 (19)	3 (14)	0.31
Median LDH		183	212	**<0.01**

Table 1. Clinical characteristics of mixed response group.

MR–R mixed response to response, *MR–NR* mixed response to non-response, *ECOG* Eastern Cooperative Oncology Group, *SSM* sustained mixed response, *BRAF V600E/K* B-Raf proto-oncogene V600E/K mutation, *NRAS* NRAS proto-oncogene, *WT* wild type, *LDH* lactate dehydrogenase. Bold values indicate $p < 0.05$.

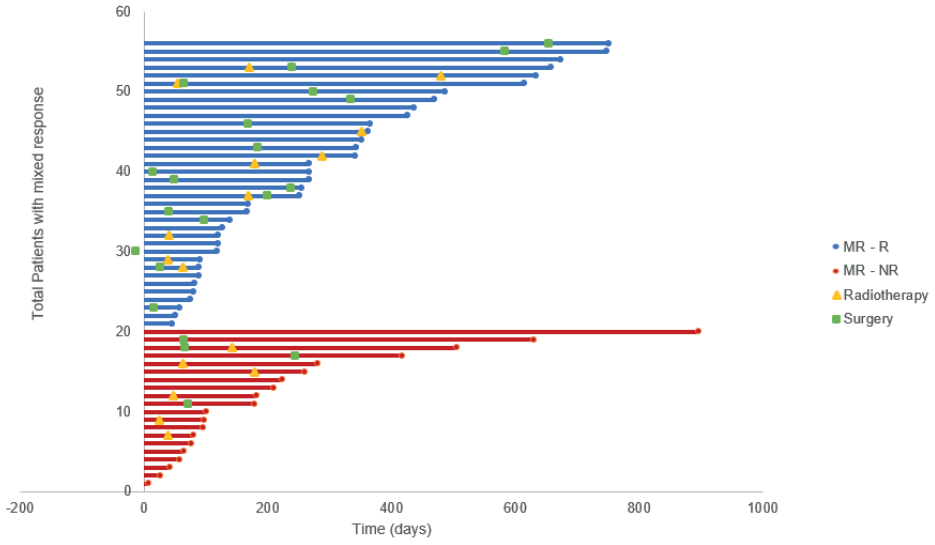

Figure 5A. Management of mixed responders**.**
Swimmer plot showing subsequent treatments in mixed responders. MR–R (blue), MR–NR (red), radiation (yellow triangle), surgery (green square).

Surgery No. (%)	MR later R / NR (N=58)
Any surgery ł	20 (36) ᵗ
Visceral Metastectomy ǂ	10 (50)
Bowel resection	2 (10)
Bladder resection	1 (5)
Adrenalectomy	2 (10)
Pulmonar Lobectomy	3 (15)
Gastrectomy	2 (10)
Subcutaneous Metastectomy	5 (25)
Lymph node dissection	5 (20)
Craniotomy	3 (15)
Radiation Therapy	11 (55)

Figure 5B. Surgery in patients with a mixed response. In our cohort, 36% of patients had surgery after being categorized as mixed responders. Of these patients, 50% had visceral metastasectomy, 25% had a subcutaneous metastasectomy, 20% had a lymph node dissection, and 15% had a craniotomy.

***ł** Any surgery listed as total and percentage () of all mixed responders.*
***ǂ** Types of surgery listed as numbers and percentage (%) calculated from any surgery patients.*
ᵗ Total number of surgery exceeds 20 (3 patients had multiple surgeries)

		No Surgery between MR - NR n=38	Surgery between MR - NR n=20	P-value
Median age (range) - yr				
		72 (48 - 85)	67 (29 - 86)	0.1
ECOG				
	<1	24	16	
	≥1	14	4	0.94
Gender				
	Female	7 (18)	7 (35)	0.14
	Male	31 (82)	13 (65)	
Metastasis status in stage IV				
	M1a	3 (8)	3 (15)	0.41
	M1b	11 (29)	2 (10)	0.18
	M1c	11 (29)	8 (40)	0.77
	M1d	13 (34)	7 (35)	1
No. of organ sites w metastasis ≥ 3				
	No	19 (50)	12 (60)	0.33
	Yes	19 (50)	8 (40)	
Median LDH at MR confirmation				
		33.8	21.3	**<0.01**
Total tumor burden at MR confirmation				
		29.6	29.4	0.96
Time between MR and new response (months)				
		24.5	39.1	**<0.01**

Table 4. Clinical characteristics of mixed responders who had subsequent surgery.

MR mixed response (or mixed responder)
Bold values indicate p < 0.05
There was no statistical difference between mixed responder groups who subsequently had surgery, except a higher median LDH in the MR–NR group (p < 0.01) and a shorter time to next response (i.e., progression) in the MR–NR group (p < 0.01).

Discussion

In this retrospective study we analyzed response to immune checkpoint inhibitors in advanced metastatic melanoma patients, and categorized response to therapy into 3 clinical categories: (1) clinical responders, (2) mixed responders, and (3) clinical non-responders. In our cohort, a mixed response to immunotherapy was not uncommon, 22% ($n = 64$), while responses and non-responses were seen in 35% ($n = 103$) and 43% ($n = 125$) of patients, respectively. Direct comparison of our clinical response categories with RECIST 1.1 suggested that our mixed responder cohort aligned most closely with the RECIST stable disease category (63%) and was associated with intermediate survival outcomes.

The mixed responder state was not definitive, as most of the patients with a mixed response eventually developed either a response to therapy (MR–R; 59%) or progression (MR–NR; 31%). Clinical variables associated with MR–NR were a higher median age, higher median LDH at mixed response confirmation, stage M1d, BRAF wild-type tumoral status, and 3 or more organ

sites with metastasis. Regarding management in the mixed responder category, patients with a mixed response who went on to respond to therapy (MR–R), were significantly more likely to be treated with surgery as compared to patients in the MR–NR group ($p < 0.01$), likely due to a more favorable phenotype. The types of surgery included visceral metastasectomy (50%), subcutaneous metastasectomy (25%), lymph node dissection (20%), or craniotomy (15%). Unsurprisingly, patients who received surgical treatment had an improved OS as compared to patients who did not undergo surgery ($p = 0.02$). Patients who underwent surgery tended to have a less aggressive disease (i.e., lower LDH, longer period to new disease development). Other studies have shown that surgical treatment in patients with less aggressive heterogeneous responses, such as oligometastatic progression and mixed response to therapy can render patients disease free.[13,16,18] This potentially supports the added value of our clinical classification system in identifying patients who might benefit from surgical treatment in our mixed response cohort. While surgical decision-making is nuanced, our clinical practice generally supports an observation period for patients with stable disease or mixed response with re-assessment with serial imaging (usually at 3 months). Surgery is favored in patients with no systemic therapy options (i.e., BRAF WT, ongoing immunotherapy toxicity) or those with progressive symptoms. In other settings, surgery is considered on a case by case basis in the context of their overall disease stability/progression.

Clearly the kinetics and heterogeneity of immune checkpoint inhibitor responses are insufficiently captured by RECIST 1.1, which is cumbersome to use in real-world clinical management outside of clinical trials. However, we found that our mixed responder cohort was enriched for RECIST stable disease, with an intermediate survival outcome, and we show that these responses are dynamic and can evolve over time. Interestingly, our work suggests that the mixed responder state is dynamic (ranging from 2.6 to 19.2 months) with the majority of patients transitioning into a definitive response category (R or NR) with associated differences in outcomes. The aim of this current work was to describe the characteristics of the low- versus high-risk groups to assist in risk assessment and clinical decision-making in real-world practice, particularly as it relates to selecting surgical candidates. Our study was limited by the retrospective nature of analysis and small sample size. Despite this, the clinical mixed responder group aligns with the stable disease group according to the RECIST classification, a group in which nuanced clinical decision-making remains a challenge.

Conclusion

A heterogeneous or mixed tumoral response to immunotherapy in advanced melanoma is not uncommon and represents a dynamic and often transient state, correlating with RECIST 1.1 stable disease. Clinical variables associated with mixed response and subsequent progression of disease were higher median LDH, brain metastases, BRAF wild-type status, and 3 or more organ sites with metastases. In our cohort surgical treatment appeared beneficial for a highly selected group of patients.

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8

Management of Heterogeneous Tumor Response Patterns to Immunotherapy in Patients with Metastatic Melanoma

Authors:
D.J.W. Rauwerdink, van Persijn van Meerten E, van der Hage J, Kapiteijn E.

Published in Melanoma research

Abstract

Introduction

The advent of immunotherapy has revolutionized treatment outcomes of metastatic melanoma. Response to therapy, however, can be complex to evaluate, as Response Evaluation Criteria in Solid Tumor (RECIST) does not capture heterogeneous responses.

Methods

In this retrospective single-institution analysis, we describe the management, clinicopathological characteristics, RECIST and disease course of metastatic melanoma patients with a heterogeneous response to first-line anti-CLTA-4 and/or anti-PD-1 between September 2011 and September 2020.

Results

In 196 patients, 37 had a heterogeneous response to immunotherapy (19%). Distinct identified responses included a mixed response (MR) (15%), pseudoprogressive disease (PP) (3%), and a sarcoid-like reaction (2%). Patients with a MR and possibly no response to therapy (MR-NR) had a higher median lactic acid dehydrogenase (LDH) ($P = 0.01$), were more often male ($P = 0.04$), had more involved disease sites ($P = 0.01$), and had brain metastasis more frequently ($P = 0.02$). MR patients with later response to therapy (MR-R) and PP patients had a longer overall survival of 1.7 [95% confidence interval (CI), 1.1–2.7] and 1.6 years (95% CI, 1.3–2.0) versus MR-NR 1.2 (0.7–1.7) ($P < 0.01$).

Discussion

In this cohort study, we identified prognostic clinical characteristics that can contribute to clinical decision-making for patients with a MR. Additionally, patients with pseudoprogression had benefited from therapy continuation, suggesting the importance of not halting therapy early in case of suspected PP. Male sex, a greater number of disease sites, the presence of brain metastases, and higher median LDH levels were associated with poorer survival in patients with mixed responses (MR). These clinical variables may serve as potential predictors for determining whether a mixed responder is likely to benefit from therapy.

Introduction

The emergence of immunotherapy has transformed the treatment landscape of metastatic melanoma. Approval and implementation of systemic immunotherapy, anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab/pembrolizumab) have significantly improved overall survival in metastatic melanoma patients.[1,2] Concurrently, the combination of anti-PD-1/anti-CTLA-4 has resulted in even higher response rates and prolonged overall survival.[3] To optimize these current treatment strategies in individual patients, careful and meticulous monitoring of tumor response is essential. Accurate determination of clinical response to therapy can be complex, as heterogeneous responses may occur.[4,5] Responding tumor sites may initially increase in size, due to edema and immunological infiltration, prior to shrinkage, so-called pseudoprogression (PP).[6] Adequate and precise radiological imaging and clinical assessment are needed in suspected PP, in order to differentiate immunotherapy-induced inflammation from disease progression, so that optimal therapeutic options can be applied in these patients. Furthermore, a mixed response (MR) can occur, in which some tumor lesions simultaneously decrease and increase in size.[7,8]

Accurate radiological and clinicopathological assessment in patients with a MR can potentially be of aid in determining subsequent response or progression of disease and is urgently needed to optimize therapeutic management in these patients. In addition, a sarcoid-like reaction (SR) in lymph nodes or in the lungs during immunotherapy can appear and can be challenging in assessing whether the patient is responding to therapy.[9] Moreover, the progression of solely one tumor lesion during a long-lasting tumor response is termed oligoprogression.[10]

Radiological evaluation of these distinct patterns can be difficult, since the traditionally used Response Evaluation Criteria in Solid Tumor (RECIST) v1.1. does not adequately capture these heterogeneous responses.[11,12] Partially to overcome this problem, immune-related response criteria have been introduced, with iRECIST in 2017 being the most recent version.[13] However, these criteria have not yet been validated. In clinical practice, the interpretation of heterogeneous response patterns remains a clinical decision-based issue, especially because of the shortcomings of RECIST v.1.1 for these distinct responses.

The intertumor heterogenic mechanisms are still being studied to identify potential novel routes for treatment options.[14] Conversely, therapeutic outcomes of these heterogeneous response patterns have not been studied in detail. Only one study on mixed responders has been published, describing the management, but without exact RECIST information, details on tumor measurements and tumor site information.[7] While the incidence of PP has been explored, therapeutic management has not been elaborated upon yet.[15] Correct clinicoradiological interpretation is warranted for these patients, since continuation or change of treatment is dependent on radiological and clinical information.

In this retrospective, single-center study, we analyzed metastatic melanoma patients who developed a heterogeneous response during first-line anti-CTLA-4 and or anti-PD-1 treatment. The incidence, management, clinicopathological characteristics, (1) RECIST evaluations and survival outcomes were assessed per heterogeneous response type.

Methods

Data source and study design

In this retrospective single-center study, patients with unresectable metastatic melanoma treated with checkpoint inhibitor therapy between September 2011 and November 2020 at the Leiden University Medical Center were analyzed. This study was performed in accordance with and approved by the medical ethical committee.

Patients

Selected patients were 18 years of age or older, had histologically confirmed unresectable metastatic cutaneous melanoma, were classified as stage IV according to the eighth edition of the American Joint Committee on Cancer (AJCC) classification [including metastases to skin (M1a), lung (M1b), other visceral sites (M1c), and brain (M1d)] and had a WHO performance score of 0 or 1 [16,17]. All patients were treated with first-line systemic immunotherapy (anti-CTLA-4, ipilimumab and/or anti-PD-1, nivolumab/pembrolizumab) or BRAF/MEKi initiation therapy followed by systemic immunotherapy, and received standard therapeutic doses and cycles.

Exclusion criteria consisted of ocular melanoma and missing or incomplete medical records. Follow-up included standard of care radiologic response evaluation every twelve weeks and clinical examination every 3 to 6 weeks in which physical exam and laboratory values were evaluated.

Patients were selected for analysis if a heterogeneous response during immunotherapy in the first or second treatment cycle was noted in the original report. A central revision of all performed imaging modalities was performed by a specialized radiologist with ample experience in the assessing tumor response using the RECIST version 1.1.[12] Patients were excluded if a heterogeneous response to therapy was due to other causes than metastatic melanoma disease.

Clinical variables

Demographic variables (age, sex and WHO performance status) were obtained from the electronic medical record (EMR). Tumor characteristics (histology, Breslow thickness, ulceration, location of primary tumor and mutational status) were extracted from the dermatopathological report. Lactic acid dehydrogenase (LDH) values were obtained from laboratory results. Detailed information on immunotherapy (duration time, type, cycles), the timing of radiotherapy, and type/date of

surgery were collected from the EMR.

Assessment

Response to immunotherapy was assessed with computed tomography (CT-scan), MRI and/or FDG-PET–CT. Patients in whom a heterogeneous response during immunotherapy in the first or second follow-up CT-scan was reported, were analyzed in more detail by a radiologist experienced in applying RECIST version 1.1 criteria.[12]

Additional response assessment using iRECIST was performed in case PD, according to RECIST v1.1., was present. The locations of heterogeneous responses to therapy included: a single (target or non-target) lesion, multiple lesions within the same organ or across different organs, and to the appearance of new tumor lesions in combination with nonprogressive existing target or non-target lesions. Radiated tumor lesions were assessed according to RECIST v1.1. criteria, and patients with increasing tumor lesions and shrinkage of tumor lesions due to radiation therapy, were not regarded as a heterogeneous response, a heterogeneous response was based on true tumoral response to immunotherapy only.

We classified a heterogeneous response into three groups: mixed response (MR), pseudoprogression (PP) and sarcoid like response (SR) (Figure 1).

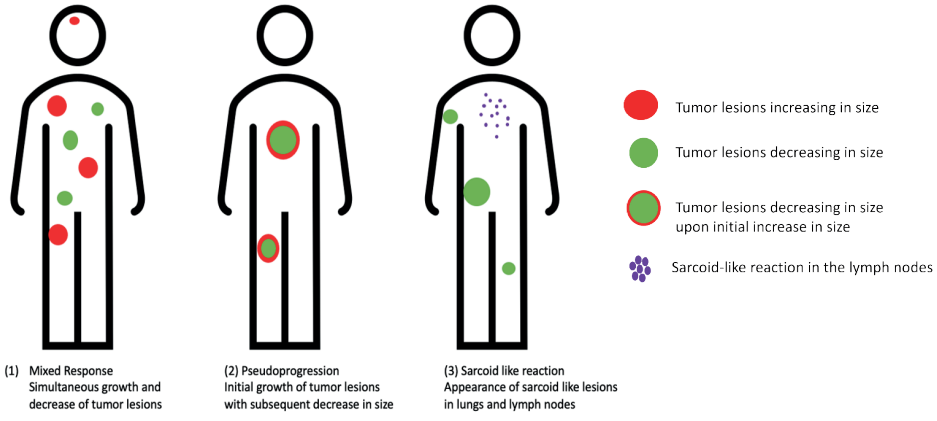


Figure 1. Visual display of different types of heterogeneous response.
(1) Mixed response: simultaneous growth of some tumor lesions and shrinkage of others; stable tumor lesions and growing lesions; stable or responding tumor lesions and the appearance of new tumor lesions. (2) Pseudoprogression: initially growth of tumor lesions with subsequent decrease in tumor lesion size. (3) Sarcoid-like reaction: simultaneously appearance of lymph nodes and/or lung nodules with a sarcoid-like pattern.

We defined a MR as simultaneous growth of some and shrinkage of other existing tumor lesions, presence of stable lesions and increasing tumor lesions or responding lesions with simultaneously appearance of new lesions. PP was defined as an increase in tumor size or the occurrence of new

tumor lesions, subsequently followed by a decrease in tumor size on a later scan. The occurrence of PP was confirmed retrospectively by a certified radiologist, in order to separate patients with a PP from the MR group. In addition, PP was differentiated from patients with progression and subsequent response based on radiological findings. A sarcoid like reaction was noted, the moment a scan was performed, in case of responding lesions with simultaneously appearance of lymph nodes and or lung nodules with a sarcoid-like pattern.

The details of RECIST evaluation, involved organs, size of target lesions and response of individual target lesions and non-target lesions per location, and the location of new lesions (if present) were analyzed. The course of disease of the heterogeneous responses was divided into clinical responders and clinical non-responders, based on physical, radiological and clinical evaluation. Patients with a MR were divided into two groups: MR and later response (MR-R) and patients with a MR and later no response (MR-NR). Subsequent comparative analysis was performed for the MR-R and MR-NR groups. An overall survival analysis was conducted for the PP, SR and MR group, and overall survival was defined as the time between metastatic disease detection up to the last follow-up date or death.

Statistical analysis

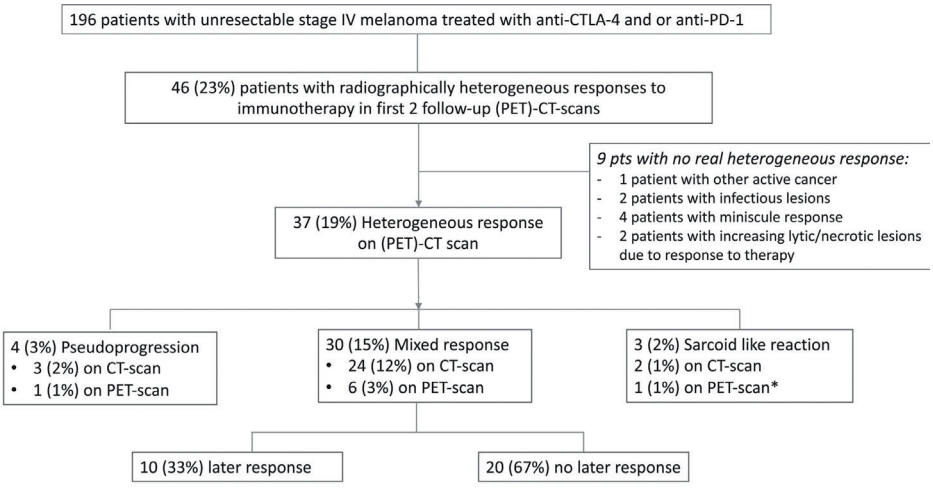
A descriptive analysis was performed to assess frequencies of demographic-, clinicopathological variables, therapy information and tumor response according to RECIST v1.1 and iRECIST (if applicable). The incidence of variables was compared between the three different heterogeneous response groups (MR, PP and SR) and a separate comparative analysis was undertaken between the MR-R and MR-NR groups, using a Chi-square test, Fisher's exact test and or Wilcoxon Rank test when suitable. Survival analysis was performed with the Kaplan–Meier procedure and survival rates were compared using the Log Rank test for each individual response group. P-values were two-sided and a P-value greater than 0.05 was considered to be statistically significant. All statistical analyses were conducted using IBM SPSS Statistics version 24 (Armonk, New York, USA).

Results

Heterogeneous response

Between September 2011 and September 2020, a total of 196 WHO 0-1 performance score patients with unresectable stage IV melanoma were treated with first-line systemic immune checkpoint inhibitors and were included in this study. During treatment, a total of 46 (23%) patients had a heterogeneous tumor response to immunotherapy on the first or second follow-up scan (after 3 or 6 months) according to the original radiologic report. Clinicoradiological examination revealed that 37 patients (19%) had a heterogeneous response pattern on either CT-scan or PET-scan, and nine patients were excluded for analysis, as they had no objectifiable heterogeneous melanoma tumor response, but had a second active cancer, infectious disease, minimal changes in measurements

within the range of normal variation in measurements (1 mm) or delayed lytic response (increasing lytic lesions due to response to therapy) (figure 2). Out of 37 patients with a heterogeneous response, 30 patients (15%) were classified as mixed responders, four (3%) as pseudoprogressive disease and three patients (2%) with a SR. Each individual distinct response group will be addressed in separate text subdivisions. For the included patients with a heterogeneous response, the median age was 64 [interquartile range (IQR) 53–73], 23 were male (62%), 17 patients had brain metastases (46%), and 21 patients had increased LDH value upon treatment initiation (57%) (Table 1). Detailed RECIST v1.1. assessment could be performed in 29 patients (78%), and eight patients (22%) received clinicoradiological assessment as they underwent baseline and/or follow-up with PET-CT scans and RECIST v1.1 criteria could not be applied in these patients. Of the patients who received a CT-scan, a heterogenous response was observed on the first response time point scan in 18 cases (62%), and the moment of heterogeneous response occurrences was classified as PD in 22 patients (76%) and measured as PR and SR in five patients (17%) and two patients (7%), respectively. Additional radiologic analysis revealed that a heterogeneous response occurred most frequently within specific disease sites, including lymph nodes in seven patients (24%), lungs in five patients (17%) and brain in six patients (21%). Other heterogeneous response locations included multiple disease sites/organs, for example, progressive lesions in the brain with concurrent responding lesions in the lymph nodes. As for individual lesions, brain progression (38%), lung response (41%) and lymph node response (59%) occurred most frequently (Table 2).



Number has been rounded up

Figure 2. Scheme displaying type of responses 196 WHO 0–1 patients with metastatic melanoma treated with immunotherapy were analyzed. A total of 37 patients had a true heterogeneous response, and nine patients were excluded. A heterogeneous response pattern was subdivided into mixed responders, pseudoprogression and sarcoid-like reaction. Additionally, the figure shows the type of radiological scan that has been used to capture the distinct response. Lastly, the mixed response group was further divided into mixed response with a later response, and mixed response with no later response.

	Total patients n=37
Median age (range) years)	64 (53 - 73)
ECOG, no. (%)	
0	19 (51)
≥1	18 (49)
Sex, no. (%)	
Female	14 (38)
Male	23 (62)
Location primary, no. (%)	
Trunk	11 (30)
Lower extremity	6 (16)
Upper extremity	3 (8)
Head/neck	6 (16)
Anogentinal	1 (3)
Unknown	10 (27)
Tumor Histology, no. (%)	
SSM	12 (32)
Nodular	6 (16)
LM	1 (3)
Unknown	18 (49)
Ulceration, no. (%)	
No	16 (43)
Yes	6 (16)
Unknown	15 (41)
Median Breslow (range) in mm	2.8 (1.1 - 4.2)
Metastasis stage, no. (%)	
M1a, M1b, M1c	20 (54)
M1d	17 (46)
Mutational status, no. (%)	
Wildtype	9 (24)
BRAFV600E	14 (38)
NRAS	11 (30)
KIT	1 (3)
CDKN2a	2 (5)
LDH moment of metastatic disease, no. (%)	
≤2× ULN	16 (43)
>2× ULN	21 (57)
Total disease sites ≥3, no. (%)	
No	16 (43)
Yes	21 (57)

Table 1. Baseline characteristics of the heterogeneous response group. Demographic characteristics showed that patients were mostly male, had a median age of 64 (IQR 53–73), had mainly an elevated LDH value (57%) and metastatic disease was present in 57% of the patients with three or more disease sites. ECOG, Eastern Cooperative Oncology Group score; IQR, interquartile range.

Disease sites	Progressive lesion(s), no. (%)	Responding lesion(s), no. (%)
Lung	8 (28)	12 (41)
Lymph node	10 (34)	17 (59)
Subcutaneous	4 (14)	4 (14)
Liver	7 (24)	10 (35)
Brain	13 (45)	6 (21)
Adrenal	3 (10)	NA
Spleen	3 (10)	1 (3)
Renal	1 (3)	NA

Table 2. Disease site response at the moment of heterogeneous response. Patients with a heterogeneous response had most frequently disease progression in the brain (38%) and lymph nodes (34%), while a disease response was seen in the lymph nodes (59%), lung(s) (41%) and the liver in most cases.

Mixed responders

Of the total 30 patients with a MR, the median age was 64 years (IQR 53–73), 20 were male (67%) and had a median LDH of 212 (IQR 176–338). A total of 20 patients (66%) with MR had three or more involved organs with metastatic disease involvement. Anti-PD-1 monotherapy was administered to 21 patients (70%) with a MR, and the median received treatment duration prior to MR development was 11 weeks (IQR 8.9–11) (Table 3). In addition, detailed RECIST information was available in 24 MR patients (80%) who received CT-scan surveillance. The moment of MR observation was classified as PD in 18 patients (75%) and as PR/CR in five (21%) cases (Table 4). The course of disease with accessory RECIST findings are displayed in figure 3a and b, respectively. A total of 20 patients with a MR had later no response to therapy (67%), and 10 patients were later objectified as responders to therapy (MR-R) (33%). The majority of patients with a later response to therapy continued immunotherapy and three patients discontinued therapy early due to immunotherapy-related adverse events. MR-NR patients received immunotherapy for a shorter period, and 13 patients went on to receive targeted therapy (BRAF/MEKi), and survival analysis demonstrated a mean survival time of 5.7 months (range 3.9–8.1), calculated from the moment of therapy change, up to the last moment alive.

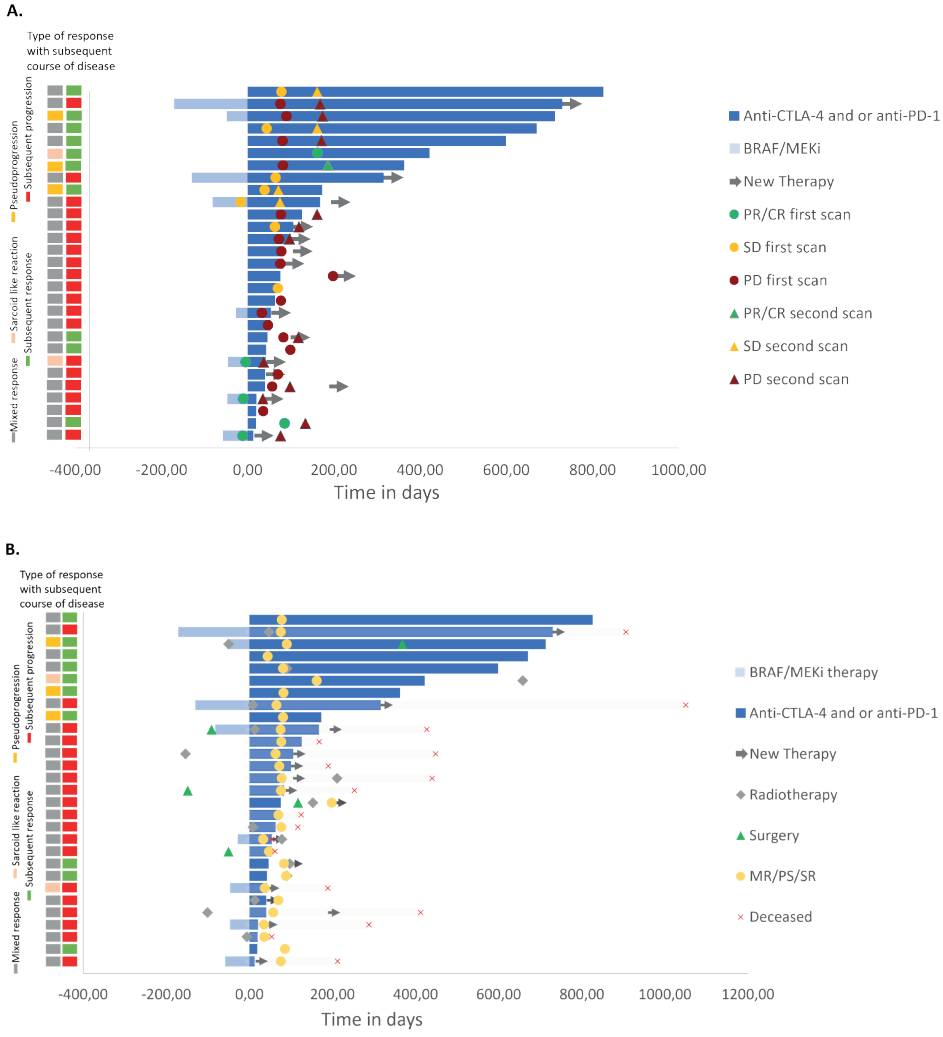


Figure 3. Swimmerplots displaying the type of heterogeneous response (mixed response, sarcoid-like reaction and pseudoprogression) and subsequent response to therapy on the Y axis. The duration of immunotherapy is displayed on the x axis, together with the moment of new therapy initiation.
(A) Swimmerplot displaying the RECIST v1.1 timepoint measurements. The majority of patients had a fixed, that is, similar RECIST value on the first and second scan, 79% versus 21%. Patients with a subsequent response to therapy were assessed as PD on the first follow-up scan in the majority of cases (50%). Patients with no later response to therapy were classified mainly as PD, 84% of the cases
(B) Swimmerplot showing the management for systemic therapy, radiotherapy, surgery and, if the case, time to decease. Notably, all three patients with pseudoprogressive disease (yellow bar), continued to receive immunotherapy. Sixteen patients with a mixed response and no later response, discontinued immunotherapy and were treated with targeted therapy most frequently, while three patients with a later clinical response after initial mixed response continued therapy. RECIST, Response Evaluation Criteria in Solid Tumor.

	Mixed Response	Pseudoprogression	Sarcoid like reaction
Age	64 (53 - 73)	64 (55 - 70)	54 (42 - 54)
Sex, no. (%)			
Female	10 (33)	2 (50)	2 (67)
Male	20 (67)	2 (50)	1 (33)
M stage, no. (%)			
M1a	2 (7)	1 (25)	2 (67)
M1b	6 (20)	NA	NA
M1c	9 (30)	NA	NA
M1d	13 (43)	3 (75)	1 (33)
Total disease sites ≥ 3, no. (%)			
No	10 (34)	1 (25)	2 (67)
Yes	19 (66)	3 (75)	1 (33)
Type immunotherapy, no. (%)			
Anti-PD-1	21 (70)	NA	2 (67)
Anti-CLTA-4	4 (13)	NA	NA
Anti-PD-1/Anti-CTLA-4	5 (17)	4 (100)	1 (33)
Total treatment weeks to response (range)	11 (8.9–11)	12 (11–20)	12 (6.0–23)
LDH at moment of response, no. (%)	212 (176 - 338)	189 (163 - 255)	179 (177 - 179)

Table 3. Clinical characteristics per response group.

Mixed responders were more often male (67%), had three or more organ sites with metastatic disease, had a median LDH of 212 (IQR 176–338), and were treated most often with anti-PD-1. Patients with pseudoprogressive disease had fewer involved organ sites with metastatic disease (75%) and were all treated with anti-PD1/anti-CTLA-4 combination therapy. Patients who had a sarcoid-like reaction received anti-PD-1 only, were more often female and were most frequently staged with M1a disease and had a median LDH of 270 (IQR 197–743). IQR, interquartile range.

[a]Visceral metastases in mixed response group contained three adrenal, three spleens, one renal and one peritoneal lesion.

[b]Visceral metastases in the sarcoid-like reaction group contained one spleen lesion.

A comparative analysis between the MR-R and MR-NR groups showed similar demographic variables such as age and WHO performance score (Table 5). In contrast, a univariate analysis demonstrated clinicopathological differences between the MR-NR and MR-R group and demonstrated that MR-NR patients were mostly male, 15 patients (75%) versus five patients (50%) (P = 0.04), had brain metastases more often 11 (55%) versus two (20%)

(P = 0.02), had a higher median LDH at the moment of MR, had three or more organ sites involved with metastases 15 (75%) versus five (50%) (P = 0.01), had cerebral disease progression more often 12 patients (71%) versus one patient (10%), while patients with MR-R had cutaneous disease progression more frequently on the moment of MR confirmation, compared to MR-NR patients, five (50%) versus three (14%) (P = 0.04). One-year landmark survival analysis demonstrated an intermediate median survival of 3.4 years [95% confidence interval (CI), not reached −1.4]. Interestingly, survival analysis revealed an prolonged mean overall survival of 1.7 years (95% CI, 1.1–2.7) (median overall survival and 1-year land mark analysis not reached) for the MR-R group compared to a median overall survival of 1.0 years (95% CI, 0.5–1.4) (1-year land mark analysis not reached) of the MR-NR group (log rank-test P < 0.01) (Figure 4).

	Mixed response	Pseudoprogression	Sarcoid like reaction
Sum lesions	84 (47–118)	74 (44–74)	77 (38–77)
Median total lesions, IQR	5 (4.0–7.0)	6 (3.0–7.0)	5 (4.0–5.0)
Disease site, no. (%)			
Subcutaneous	9 (38)	NA	NA
Lymph nodes	18 (75)	2 (50)	2 (100)
Lung	14 (58)	1 (25)	1 (50)
Liver	13 (44)	1 (25)	1 (50)
Brain	10 (42)	2 (50)	NA
Visceral[a]	8 (33)[a]	NA	1 (50)[b]
RECIST at MR/PR/SR, no. (%)			
PR/CR	5 (21)	NA	NA
SD	1 (4)	NA	1 (50)
PD	18 (75)	4 (100)	1 (50)
Clinical response upon MR/PR/SR, no. (%)			
Response	6 (25)	4 (100)	1 (50)
No response	18 (75)	NA	1 (50)

Table 4. Radiographical disease assessment.

Baseline disease assessment according to RECIST version 1.1 showed no clear differences in the total sum of tumor lesions and size. Patients with pseudoprogressive and sarcoid-like reaction had no subcutaneous disease sites involved, in contrast to patients with mixed response (38%). Patients with mixed response and pseudoprogressive disease were classified as PD most frequently in 75 and 100% of the cases, respectively.
MR, mixed response; RECIST, Response Evaluation Criteria in Solid Tumor.

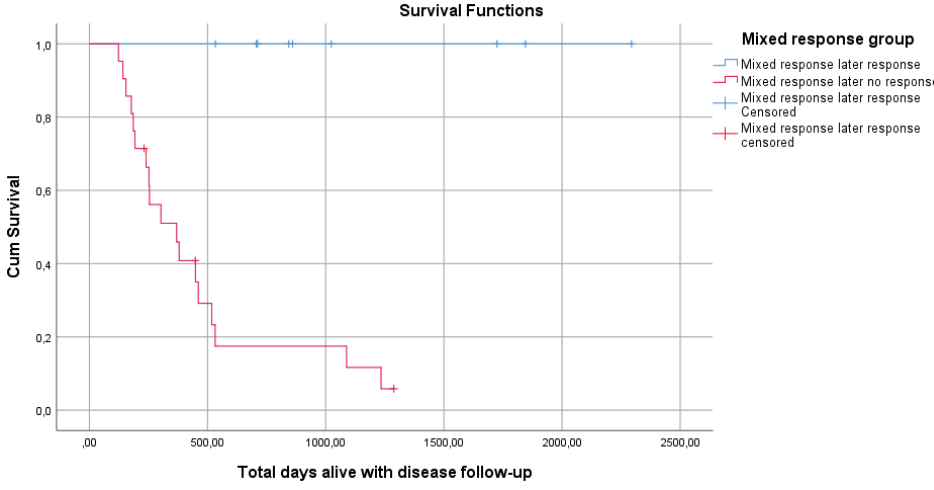


Figure 4. Kaplan–Meier curve showing the overall survival of patients with Mixed response and later response (MR-R) and survival for mixed response mpatients with subsequent no response (MR-NR). Patients with a mixed response and subsequent response had a prolonged overall survival compared to patients who went on to have progressive disease after an initial mixed response ($P < 0.01$).

	MR later R	MR later NR	*P*-value
Median age (range) (years)	65 (52–73)	64 (53–73)	0.9
Sex, no. (%)			0.04
Female	5 (50)	5 (25)	
Male	5 (50)	15 (75)	
Metastasis stage, no. (%)			0.02
M1a	2 (20)	NA	
M1b	3 (30)	3 (15)	
M1c	3 (30)	6 (30)	
M1d	2 (20)	11 (55)	
Total disease sites ≥3, no. (%)			0.01
No	5 (50)	5 (25)	
Yes	5 (50)	15 (75)	
Median LDH at MR moment, no. (%)	178 (162–209)	281 (188–387)	**0.01**
RECIST at MR confirmation, no. (%)			0.12
PR/CR	2 (20)	3 (20)	
SD	1 (10)	NA	
PD	3 (30)	15 (75)	
Progression site at MR, no. (%)			
Subcutaneous progression	5 (50)	3 (14)	**0.04**
Lymph node progression	4 (40)	5 (22)	0.43
Lung progression	2 (20)	6 (27)	0.68
Liver progression	2 (20)	4 (18)	0.35
Brain progression	1 (10)	12 (71)	**0.02**

Table 5. Demographic and clinicoradiological characteristics for the mixed response later no response and mixed response later response groups. MR-NR patients were staged m1d more frequently, had a higher median LDH at the moment of MR confirmation, were staged as PD more frequently and had cerebral disease progression in most of the cases.

Significant values are highlighted in bold.
MR, mixed response; MR-NR, mixed response later no response; MR-R, mixed response later response group; RECIST, Response Evaluation Criteria in Solid Tumor.

Pseudoprogression

Out of four patients with PP, three patients (25%) had three or more involved disease sites. The median LDH was 189 (IQR 163–255) at PP observation. Of the four patients, two patients had brain metastases (50%) (Table 3). All PP patients were treated with anti-CTLA-4/anti-PD-1 combination therapy and received therapy during a period of 12 weeks (IQR 11–20) prior to the onset of PP. Detailed CT-scanning was performed in three patients, demonstrating that PP detection was classified as PD in all cases according to RECIST v1.1 (Table 4). Conducted iRECIST evaluations demonstrated that patients with PD were obviously all classified as iUPD (unconfirmed progressive disease) on that time point, and second-time point evaluation revealed iPR (partial response) in 67% of the cases and iCPD in 33% (confirmed progressive disease). Regarding treatment, these patients went on to receive immunotherapy, despite observed PD and, all patients had later

clinical response to therapy (Fig. 2). Survival analysis demonstrated a mean overall survival of 1.6 years (95% CI, 1.3–2.0), (median overall survival and landmark time point of 1-year survival was not reached) (Fig. 5).

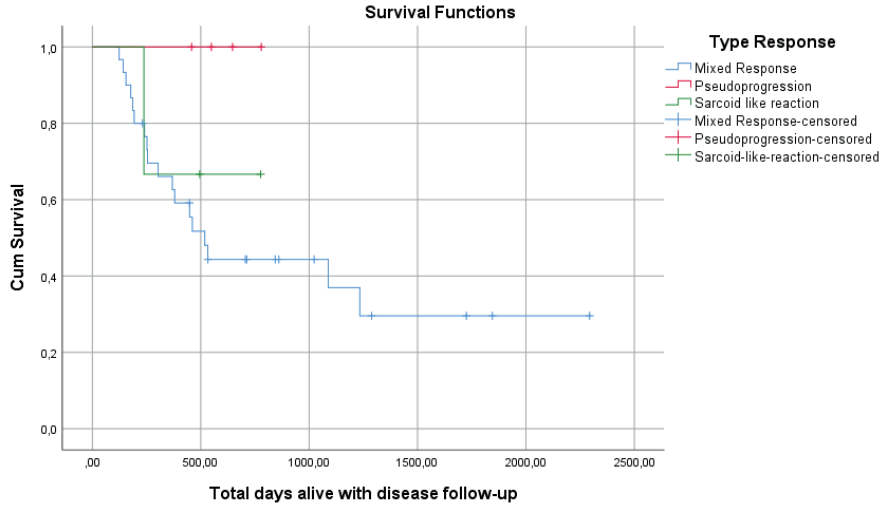


Figure 5. Overall survival per response group. Survival per heterogeneous response group showed that patients with pseudoprogressive disease had an improved overall survival of 1.6 years (95% CI, 1.3–2.0) and patients with a sarcoid-like reaction had an intermediate mean survival of 1.4 years (95% CI 0.5–3.2).
CI, confidence interval.

Sarcoid like reaction

Of the three patients with a SR, median age was 54 years (IQR 42–54), two were female (67%) were staged most frequently M1a (67%) and had less than three organ sites with disease involvement in the majority of the cases (67%) (Table 3). Two patients received anti-PD-1 monotherapy (67%) and one patient (33%) received anti-ctla4/anti-PD-1 combination therapy (33%). Two out of three patients underwent follow-up with CT scans, which demonstrated that the moment of sarcoid-like response observation was classified either as PD (50%) or SD (50%) (Table 4). Two of the three patients had later response to therapy, while one patient had no later response. Regarding survival, a conducted survival analysis demonstrated an intermediate mean survival of 1.4 years (0.5–3.2) (median overall survival and landmark time point of 1-year survival was not reached) (figure 5).

Discussion

In this retrospective single-institution study, we analyzed heterogeneous responses to immunotherapy on the first and/or second follow-up scan combined with clinical assessment

in patients with metastatic melanoma. Between 2011 and 2020, a total of 196 patients with unresectable metastatic melanoma were treated with first-line systemic immunotherapy. A heterogeneous response was not uncommon and occurred in 19% of the patients and was seen on the first response evaluation scan in most of the patients (62%). Baseline radiologic assessment demonstrated that the majority of patients had a high burden of disease, with three or more disease sites with metastatic lesions (57%) and an increased median LDH value (57%). With regard to the high burden of disease, previously published studies have demonstrated the association of multiple number of tumor clones that can potentially harbor various mutations which contribute to a high level of tumor heterogeneity.[14,18] A recently published study demonstrated that highly heterogeneous melanoma tumors are associated with a decreased survival, implying the importance and need for detailed disease assessment of patients with a heterogeneous response, next to genomic analysis.[19]

As for the exact location of a disease progression, this was observed in the brain in most of the cases and responding lesions were seen more frequently in the lymph nodes, suggesting that the location of disease does matter. In fact, the progression of brain metastases could presumably be caused by the inequal distribution of the immunotherapy agents in the blood, due to semi-permeable blood-brain barrier and distinct brain tumor microenvironment.[20,21] Frequent disease response evaluation in the brain is necessary and important in these patients, since early detected brain metastases can potentially be managed surgically or with radiation therapy, according to previously published case reports describing the management of mixed cerebral responses.[22]

Considering radiological response evaluation in patients with a heterogeneous response, 76% of the patients were classified as PD according to RECIST v1.1., despite that 40% of the patients later had a response to therapy, hence unfolding the rationale for not halting therapy the moment PD is observed in patients with no clear progression or response to therapy. The indistinct response should be followed up in addition to clinical assessment, up to the next RECIST timepoint measurement in order to determine the 'true' response to therapy. Additionally, a shorter scan interval next to the application of iRECIST should be considered with a low threshold in these complex cases. Furthermore, performed PET-CT scans should be analyzed in more detail to assess the avidity of individual tumor lesions to predict potential response or progression.

To provide potentially prognostic indicators, we analyzed three distinct response groups (MR, PP and sarcoid-like reaction). A MR was not uncommon and was noted in 15% of the cases, which is in line with previously conducted studies.[8,3,24] This relatively high incidence should raise the awareness of the existence of this clinicoradiological phenomenon, and suspected patients with a MR should be evaluated in more detail so that most optimal clinical-based decision can be made. A MR to therapy was a dynamic disease state, as patients eventually had response (MR-

R) or no response to therapy (MR-NR), and no patients remained their MR status. Identified clinical characteristics associated with later no response included male sex, brain metastases, higher median LDH on the moment of MR confirmation and cerebral disease progression. These identified clinical characteristics associated with later no response, have been established as prognostic factors in metastatic melanoma, in previously published studies.[25,27] Our results suggest, that these clinical characteristics are also applicable as a prognostic tool in this patient group and have been suggested as a prognostic tool in a previously conducted study.[7]

Considering PP, this specific response occurred less frequently, in 4 (3%) out of 196 patients treated with immunotherapy, which is in accordance with incidence rates of this phenomenon in other published studies.[6,15] The moment of PP detection was insufficiently captured by RECIST; with PD in 100% of the cases. Despite evaluation of PD according to RECIST, clinical-based decision making and additionally used iRECIST criteria resulted in continuation of immunotherapy in these patients. Patients with PP good survival outcomes, which is in line with previously published survival rates of patients with PP.[6,14] The management and results of our cohort justify the continuation of immunotherapy in presumed PP in patients with metastatic melanoma. It is important to emphasize this finding, since not all clinical trials and compassionate care situations are designed to re-consenting patients for treatment beyond progression after removing them from experimental regimen(s) at the first sign of disease progression. Meticulous clinicoradiological measurements should be performed in order to rule out PP before halting therapy too early and withholding a beneficial effect of the therapy.

Considering a SR, this was less common, as only three (2%) patients in our series had a SR. Since the number of patients with SR was little and heterogeneous, additional research is needed to identify potential prognostic clinical characteristics and therapeutic management for these patients.

Limitations and strengths

Our study was limited by the retrospective single-institution nature of the analysis. Despite this, we performed in depth RECIST analysis and iRECIST by an experienced radiologist in addition to already available radiological data. To our knowledge, this is the first study, describing real-world outcomes in management and detailed radiographically description of MR, PP and sarcoid-like reaction during treatment with immunotherapy. In addition, our results are in line with previously published less detailed studies investigating PP and MR. Lastly, by showing the occurrence of this clinicoradiological phenomenon, hopefully, national databases will notify and or register these unique responses in the future, so that the further requested research can be performed.

Conclusion

A heterogeneous response in our cohort was not uncommon, and consisted mainly of a MR, while PP and sarcoid-like reaction were observed less frequently. A MR represented a dynamic disease state, as patients possibly had response or no response to therapy during follow up. Identified clinical characteristics associated with later no response to therapy upon initial MR included the male sex, increased LDH, brain metastasis, cerebral progression, and more involved disease sites the moment a MR was noted. These clinical characteristics can aid in clinical decision-making for cases of mixed response (MR). Notably, patients with pseudoprogression (PP) benefited from continued therapy, underscoring the importance of avoiding premature discontinuation of treatment in cases of suspected pseudoprogressive disease.

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General Discussion and Future Perspectives

Resectable melanoma

Surveillance of melanoma

The rising incidence of cutaneous melanoma poses an important challenge for cancer control and public health globally, specifically in populations with fair skin and of Caucasian descent.[1,2] National guidelines recommend surveillance of patients with familial melanoma. Another risk factor for the development of melanoma is multiple (atypical) naevi. However, whether patients with multiple common naevi and or atypical naevi should receive thoroughly skin surveillance is less clear.[3,4] Shedding light on this unclarity is important, as this can provide new insights in potential novel clinical managements in patients with multiple (atypical) naevi.

In chapter 2 we assessed the incidence of melanoma in patients with multiple atypical and or common naevi. We identified specific risk factors (multiple actinic keratoses, history of melanoma, sunburns in childhood, fair skin, blue eyes) for developing melanoma in patients with more than 100 naevi and or 5 atypical naevi.[5] Moreover, we found that the majority of melanoma cases in patients with multiple atypical nevi were detected by a dermatologist, without having been noted by the patient. Additionally, we observed that melanoma originated more frequently from pre-existing naevi in this patient population, while sporadic melanomas developed more frequently from normal appearing skin in the general population.[6] Considering these results, we recommend that patients with multiple atypical naevi (>5) and or numerous common naevi (>100) and additional identified risk factors multiple actinic keratosis, history of melanoma, sunburn in childhood, fair skin and or blue eyes should be monitored on an annual basis by a dermatologist.

Concurrently, patients with a *CDKN2A* germline mutation are already seen by a dermatologist on a biannual basis, as these patients have an estimated risk of melanoma development of 70%.[7,8] It is important to assess whether melanoma develops from a preexistent naevus or de novo in this patient group, as these findings might alter surveillance and or clinical management. In **chapter 3**, we analyzed melanoma cases in HMel^{CDKN2A} patients, and examined total body photography to determine whether melanoma arose from a preexistent nevus of normal appearing skin. Our findings demonstrate that the majority of melanoma cases arose from a preexistent naevus (78%). This result supports the routine use of total body photography and sequential dermoscopy to determine alterations in melanocytic naevi. Additionally, since melanoma emerged from a preexistent naevus in a relatively high proportion of cases, this suggesting that HMel^{CDKN2A} patients might benefit from more frequent removal of melanocytic naevi.

Regarding patients who develop melanoma, no concrete consensus in follow-up guidelines for cutaneous melanoma exists.[4,9] The conventional follow-up schedule suggests biannually follow-up for patients diagnosed with stage IB (T1bN0), IIA (T1BN0) melanoma for a total of 5 years.[10,11] Since

existing lack of consensus, the MELFO study was performed in 2022, a multicenter randomized clinical trial in which conventional follow-up care was compared with an experimental follow-up interval.[12] Interestingly, no differences were observed in recurrence free survival between the conventional and experimental follow-up scheme. Seemingly the support for an appropriate, safe and cost-effective follow-up regimen. As a consequence, the MELFO follow-up scheme has been implemented into the Dutch Melanoma Guideline and has been adopted into clinical care practice.

The current follow-up schedule is based on the TNM melanoma classification. Important is to identify additional prognostic factors next to the traditional T,N,M variables. For instance histologic subtype. To explain the importance of this, the subtype nodular melanoma is associated with an increased local and distant recurrence rate, regardless of Breslow thickness.[13] Additionally, melanoma subtypes such as acral melanoma and mucosal melanoma are also associated with lower recurrence-free survival rates and should therefore also be regarded as a prognostic marker for melanoma recurrence.[14,15] These identified subtypes should have their own specific follow-up regimen, in order to detect melanoma recurrence in early stage and thereby improving melanoma-specific survival.

Lymph nodal management for stage III melanoma

In addition to changes in follow-up for melanoma patients, the standard of care for sentinel node-positive status has also evolved. In 2017, the MSLT-II trial demonstrated no improvement in melanoma-specific survival for patients with micro metastatic lymph node disease in the sentinel node who underwent complete lymph node dissection compared to those who had only ultrasonic lymph node observation.[16] As a result, immediate complete lymph node dissection for sentinel lymph node-positive patients has been omitted and this has been adopted in clinical practice since 2018. A real-world study published in 2021 confirmed the adoption and implementation of active nodal surveillance instead of immediate complete lymph node dissection in sentinel node-positive patients, demonstrating similar melanoma-specific survival rates in both patient groups.[17,18,19]

Regarding patients with macroscopic lymph node disease, there is currently no clear consensus on the optimal surgical management.[20] Historically, the standard of care involved complete lymph node dissection. However, influenced by the findings of the MSLT-II study and the emergence of (neo)adjuvant therapies, complete lymph node dissection is omitted more frequently and surgical management is moving towards dissection of affected lymph nodes only, so-called lymph node picking.

The PRADO study, published in 2022 by Reijers and colleagues, assessed the efficacy of neoadjuvant ipilimumab/nivolumab in clinical stage IIIB-D melanoma patients.[21] The study found that therapeutic lymph node dissection could be safely omitted in patients who achieved a major pathological response (<10% viable tumor) to neoadjuvant immunotherapy. In addition, the study showed that patients who omitted therapeutic lymph node dissection reported significantly higher scores in specific health-related quality of life domains, including physical functioning, role functioning, global functioning, and social functioning, compared to those who underwent lymph node dissection.

Currently, a Dutch study is underway to investigate the outcomes of performing only a therapeutic index lymph node dissection in resectable high risk stage III melanoma patients, to assess the response to neoadjuvant treatment, thereby omitting complete therapeutic lymph node dissection. If this study confirms the safety of this approach, it will likely lead to a further shift in the management of lymph nodal melanoma disease towards less extensive surgical interventions.

Adjuvant therapy for stage II-III melanoma

These alterations in surgical management have significantly changed the treatment landscape, reducing potential surgery-related side effects and decreasing associated costs. Beyond these adjustments in follow-up care and lymph node management, the introduction of anti-PD-1 and *BRAF/MEKi* therapies in the adjuvant setting has further transformed the treatment paradigm.[22-27] The FDA has approved adjuvant anti-PD-1 therapy for stages IIB, IIC, IIIA, IIIC, and IIID melanoma. In the Netherlands, adjuvant anti-PD-1 therapy has received EMA approval for stage IIIA-D disease. Similarly, adjuvant *BRAF/MEKi* therapy has been approved for stage IIIA-D disease, and its efficacy for stage II disease is currently under investigation.

Clinical trials exploring the effectiveness of adjuvant therapy required complete lymph node dissection for stage IIIA-D melanoma patients. As a result, the effectiveness of adjuvant therapy in stage III melanoma patients who did not undergo complete lymph node dissection is unclear.

In Chapter 4, we describe that resected stage III melanoma patients who omitted complete lymph node dissection and were treated with adjuvant anti-PD-1 and adjuvant *BRAF/MEKi* had an improved progression free survival, compared to patients treated with surgery only. This suggests that adjuvant therapy may also be effective in eradicating occult regional disease. Regarding the timing of recurrence, patients treated with anti-PD-1 therapy experienced an earlier onset of disease recurrence compared to those in the *BRAF/MEKi* group. Although, our study was limited by a relatively short follow-up period, a larger study with an extended follow-up period, published by Broman and colleagues in 2021, demonstrated similar results.[18]

Despite reported improvement in local disease control, no improved overall survival has been observed in stage II/III melanoma patients treated with adjuvant anti-PD-1 therapy and *BRAF/MEKi*.[28] This raises the question whether it is more effective to treat with adjuvant therapy or to perform regular follow-ups consisting of clinical-radiological and blood assessments until disease recurrence is detected. As of now, there is no clear answer to this question.

While adjuvant treatment enhances local and distant disease control, it is important to note that this benefit can come at a cost. Approximately 30% of patients undergoing adjuvant therapy may experience immune therapy-related adverse events (irAEs).[29-31] Our study in **Chapter 4** highlights that immune therapy and *BRAF/MEKi* related adverse events were not uncommon and occurred more frequently in *BRAF/MEKi*-treated patients. This aligns with adjuvant trial data showing a higher frequency of any grade adverse event with targeted therapy compared to anti-PD-1 treatment.[25,27,32]

Regarding irAEs, these can include adrenal insufficiency, hypophysitis, colitis, hepatitis, thyroiditis, arthritis, diarrhea, pneumonitis, and toxic epidermal necrolysis.[33] These adverse events can be severe, long-lasting, and require additional treatment, significantly impacting the quality of life.[29,31] Similarly, adverse events related to *BRAF/MEK* inhibitors can have a serious impact on the quality of life, however, these side effects often resolve within a few days after discontinuation of therapy.[34,35]

Adjuvant therapy patient selection

Given the potential impact of treatment-related adverse events, patient selection must be conducted carefully to identify individuals at higher risk of developing irAEs, considering factors like comorbidities (including autoimmune diseases), age, and ECOG performance status.[30,36-39] While these risk factors for adverse event development are well established in the advanced treatment setting, it remains unclear whether this hold true in the adjuvant setting. Additionally, it is unclear whether patients treated with adjuvant therapy experience adverse events more frequently than advanced melanoma patients treated with systemic immune checkpoint inhibitors.

To address this, we analyzed the incidence and severity of adverse events in both adjuvant-treated and systemically treated advanced melanoma patients in **Chapter 5**, and assessed prognostic factors associated with adverse event development. Our results show that patients receiving adjuvant therapy had fewer comorbidities and pre-existing autoimmune conditions compared to those with advanced melanoma, indicating that relatively healthier patients are selected for adjuvant therapy This seemingly logical, as the importance of immunotherapy might be less essential in the adjuvant setting compared to the advanced setting.

Our study found that ECOG performance status and any type of comorbidity were independently associated with the development of adverse events in patients receiving adjuvant therapy. Therefore, these factors should be taken into consideration when advising patients adjuvant anti-PD-1 therapy. Additionally, we did not observe a significant difference in the incidence and severity of adverse events between adjuvant-treated and systemically treated advanced melanoma patients. This may be due to the low absolute number of adverse events captured in our study, as we only recorded grade III-IV irAEs. Therapy cessation due to adverse events was observed more frequently in the adjuvant group than in the systemic therapy group, suggesting a potential less therapy compliance, as treatment is less crucial, in adjuvant treated patients.

Further research is needed to include all grades of adverse events to determine if there is a significant difference across the full spectrum of adverse events between these patient groups.

In addition to identifying risk factors associated with adverse event development, it is important to pinpoint patients who can benefit most from adjuvant therapy. Patients with a relatively good prognosis, such as those with stage IIB and IIIA melanoma, can likely forego additional adjuvant treatment. In contrast, those with a less favorable prognosis, such as resected stage IIIC-D and stage IV-M1c-d disease, should be prioritized for adjuvant therapy consideration.[40]

Identifying patients at risk for disease recurrence can be complex, and biomarkers like circulating tumor DNA (ctDNA) may aid in therapy selection. Recent studies have shown that stage III melanoma patients with detectable ctDNA have an increased risk of relapse, suggesting that ctDNA, in conjunction with the AJCC classification scheme, could be valuable in making adjuvant therapy decisions for stage III melanoma patients.[41,42]

Another emerging biomarker is tumor mutational burden (TMB), which quantifies the number of somatic mutations per megabase of genomic DNA. Studies have suggested a correlation between TMB and the number of neo-antigens generated by the tumor, as well as the response of melanoma patients to immune checkpoint inhibitors. However, a significant proportion of patients with high TMB do not respond to anti-CTLA-4 or anti-PD-1 therapies, leaving the clinical utility of TMB uncertain.[43]

Interferon gamma (IFN-γ), which influences immune response and PD-1 expression, may also play a role in predicting response to adjuvant anti-PD-1 therapy. Several studies have demonstrated that upregulated IFN-γ expression correlates with a higher response to neoadjuvant therapy in stage III melanoma patients.[44,46] However, it is unclear whether the presence of an IFN-γ signature could aid in monitoring therapy response in adjuvant-treated patients, especially since a portion of these patients are rendered disease-free. The lack of tumor-related neo-antigens in patients who are disease free might result in a less potent immune response.

In summary, while identifying patients who would benefit most from adjuvant therapy remains challenging, advancements in biomarkers like ctDNA, TMB, and IFN-γ offer promising approaches for improving patient selection and treatment efficacy.

Critical view on adjuvant therapy

Despite the booked advancements in adjuvant immune checkpoint inhibition and targeted therapy for melanoma, the Dutch Society for Medical Oncology and Lung Oncology has recently updated its criteria for recommending adjuvant treatments. The Dutch healthcare association is currently developing a new framework for assessing and making reimbursement decisions regarding these therapies. There is growing criticism against the indiscriminate use of immune-checkpoint inhibitors in the adjuvant setting, with a preference emerging for more targeted, personalized neoadjuvant or adjuvant therapy approaches. Additionally, there is a trend towards deferring immunotherapy until a later stage, particularly in cases of disease relapse.[28] This approach is praised for its efficient resource utilization, prevention of potential therapy-induced drug resistance, avoidance of overtreatment, and preservation of the quality of life for patients without cancer-related symptoms who are susceptible to side effects from immune checkpoint inhibitors.

In conclusion, while adjuvant therapy can play a pivotal role in preventing local and distant disease recurrence, it is crucial to weigh the potential benefits and drawbacks for each individual patient. Since no overall survival benefit has been demonstrated yet for adjuvant therapy, clinical decisions should be made on a case-by-case basis to optimize patient selection for adjuvant treatment.

Neo-adjuvant therapy for stage III melanoma

Although the revolutionary progress that has been made with the introduction of adjuvant therapy, the outcomes of adjuvant therapy treated stage IIIC-D melanoma patients remain poor.[40] The relapse rate for these patients at 2 year follow-up has been estimated to be 40% for adjuvant immunotherapy and 40% at 3 years with adjuvant targeted therapy.[26,32,47] Our study in **chapter 4**, confirms indeed that adjuvant treated patients with macroscopic disease (stage IIIC-D) had a worse prognosis compared to stage IIIB melanoma in the post-MSLT-II era.

The advent of neo-adjuvant therapy has the potential to further improve outcomes for melanoma patients with resectable high risk stage III disease. The rationale for neo-adjuvant therapy stems from the observation of an increased immune response in patients exhibiting tumor neo-antigens, whereas those with completely resected melanoma might not show such potent immune responses due to the absence of tumor neo-antigens.[44,48]

Several studies have been conducted to analyze the efficacy of neo-adjuvant therapy. A pooled analysis of six melanoma neo-adjuvant trials in 2021 demonstrated that, at a 12-month follow-up, neo-adjuvant anti-CTLA-4/anti-PD-1 combination therapy had the highest response rate compared to anti-PD-1 monotherapy in patients with clinical stage III melanoma, with recurrence-free survival rates of 84% and 64%, respectively.[49] In addition, the SWOG trial in 2023 comparing neo-adjuvant pembrolizumab versus adjuvant pembrolizumab in patients with clinically detectable stage IIIB to IVc melanoma, demonstrated greater benefits from neo-adjuvant pembrolizumab, with a two-year recurrence-free survival of 72% in the neo-adjuvant group compared to 49% in the adjuvant-only group.[50]

Moreover, the results from the recently published NADINA study underscores the efficacy of neo-adjuvant immunotherapy. In this phase 3 trial, patients with resectable macroscopic stage III melanoma were randomly assigned to receive neo-adjuvant ipilimumab plus nivolumab followed by surgery or surgery followed by 12 cycles of adjuvant nivolumab. Patients who developed a partial or no response response to immunotherapy received adjuvant nivolumab therapy, while patients with a major pathological response received neoadjuvant treatment only.

During a follow period of 9.9 months, the estimated 12 month event free survival was 84% in the neoadjuvant group and 57% in the adjuvant group. In addition, in patients with a major pathological response to immunotherapy, the recurrence free survival was 95%, suggesting that solely neoadjuvant therapy might be sufficient for this response group.

In light of these results, neo-adjuvant therapy seems more effective in disease recurrence reduction compared to adjuvant treated patients in patients with clinical stage III melanoma. If studies with a longer follow-up period confirms these findings, then neo adjuvant therapy should be selected as preferred treatment choice in melanoma patients with macroscopic nodal disease.

Regarding neo-adjuvant *BRAF/MEK* inhibitors for clinical stage III melanoma patients, a pooled analysis showed a recurrence-free survival rate of 75% at 12 months and 47% at 24 months in treated high risk stage III melanoma patients.[49] Interestingly, in resectable stage IIIB/C melanoma patients with a partial response, the two-year recurrence-free survival was significantly higher in the neo-adjuvant immunotherapy group compared to the neo-adjuvant *BRAF/MEK* cohort, with rates of 64% versus 13%, respectively.[49] This highlights the importance of achieving a pathological (near) complete response in patients treated with targeted therapy, whereas a partial response to immunotherapy might be sufficient to improve recurrence-free survival.

Interestingly, the DOMINI trial showed that an upregulation of IFN-Y signature is associated with an improved response to neo-adjuvant ipilimumab/nivolumab therapy.[51] The IFN-Y signature could aid in optimizing the neo-adjuvant and adjuvant treatment scheme and potentially alter

surgical management in these patients. For example, patients who develop a partial response to neo-adjuvant therapy with a low IFN-Y signature, should be selected and or guided for additional surgery and or adjuvant therapy.

Potential disadvantages of neo-adjuvant treatment include delay in surgery, risk of disease progression to unresectable disease state and immune therapy related adverse events which could potentially impact surgical resection and outcomes. Despite these drawbacks, the potential benefits of neo-adjuvant treatment appear to outweigh the disadvantages. Hopefully, neo-adjuvant immune checkpoint inhibition will soon be approved for clinical use in patients with stage III resectable high-risk melanoma.

Prognostic factors for systemic therapy response in advanced melanoma

The emergence of systemic immune checkpoint inhibition has significantly shifted the treatment paradigm for metastatic melanoma.[24,52-54] Despite the progress made in improving overall survival, several knowledge gaps regarding prognostic factors in advanced melanoma still exists. Established prognostic indicators for cutaneous metastatic melanoma include LDH value, cerebral involvement, ECOG status, and the overall number of affected organ sites with metastases.[55] Our study in **Chapter 6** confirmed that increased LDH values, the presence of cerebral disease, multiple affected organ sites, and decreased ECOG status are all associated with poorer survival outcomes in patients treated with systemic anti-PD-1 or *BRAF/MEKi* therapies.

However, there is limited knowledge regarding other prognostic factors related to treatment response, such as histologic subtype, age, mutation status, gender, PD-L1 status, comorbidities, therapy-related adverse events, and ethnicity. Identifying and understanding these factors are crucial for optimizing treatment strategies and improving patient outcomes in advanced melanoma.

Histologic subtype

Melanoma subtypes characterized by a higher tumor burden tend to exhibit a more favorable response to therapy compared to those with a lower tumor burden.[56,57] Specifically, metastatic acral and metastatic mucosal melanomas, which harbor a lower tumor mutation burden, have been shown in retrospective studies to have worse treatment-related survival outcomes compared to advanced superficial spreading melanomas. Given that the histologic subtype nodular melanoma is associated with worse recurrence-free survival than superficial spreading melanoma, regardless of Breslow thickness, we conducted a study (**Chapter 6**) to assess the efficacy of immunotherapy and targeted therapy in advanced nodular- and superficial spreading melanoma patients.[13,58]

This study in **Chapter 6** demonstrates that treatment-related survival outcomes for nodular melanoma were similar to those for superficial spreading melanoma. However, despite similar treatment-related survival, overall survival was worse for nodular melanoma patients. This can be explained by the shorter time to metastasis for nodular melanoma compared to superficial spreading melanoma. Earlier metastasis in nodular melanoma ultimately leads to a worse prognosis. Traditionally, the histologic subtype NM has not yet been regarded as a prognostic variable for metastasis, highlighting the need to reevaluate follow-up protocols for nodular melanoma patients.

Given these findings, it is important to consider the histologic subtype for both melanoma specific follow-up scheme and the histologic subtype should be assessed when deciding on adjuvant or advanced immunotherapy for melanoma patients. By doing so, we can aim to improve the outcomes in melanoma patients.

Age and comorbidity

Since clinical trial participants typically consist of relatively healthy individuals, it was uncertain whether older patients with advanced melanoma were suitable for systemic therapy. However, a large population-based retrospective study conducted by Glas and colleagues demonstrated that the response rates and toxicity of checkpoint inhibitors did not vary with increasing age or comorbidity.[59] Despite similar grade III-IV toxicity rates, elderly patients discontinued therapy more frequently than their younger counterparts. This could be due to older patients experiencing a higher frequency of immune therapy-related adverse events of any grade, which may lead to a deterioration in quality of life and physical functioning. Additionally, therapy might be discontinued more frequently as its clinical utility potentially has less impact on the life span of elderly patients nearing the end of life.

Mutation status

The prognostic role of pathogenic mutations in treated advanced melanoma is complex. A recently published meta-analysis demonstrated that *NRAS* mutated melanoma is associated with a higher likelihood of partial or complete tumor response compared to *NRAS*-wildtype melanoma.[60] Therefore, the presence of a *NRAS* mutation status might be considered a prognostic marker for response to immune checkpoint inhibition therapy. Albeit this, advanced *NRAS*-mutated melanoma can only be treated with immune checkpoint inhibitors and no *BRAF/MEK* inhibitors, the prognostic value of the *NRAS* mutation remains uncertain.

In parallel, a study conducted by van Not and colleagues, investigating the efficacy of anti-PD-1 in advanced melanoma patients, reported no survival difference between *BRAF* or *NRAS* mutated

melanoma patients.[61] Interestingly, the study observed an improved survival for *BRAF*-mutated melanoma patients treated with the ipilimumab/nivolumab combination therapy compared to patients with *NRAS*-mutant and wild-type melanoma. Suggesting that BRAF mutation status is a prognostic factor to consider when deciding between mono- and dual-checkpoint inhibition therapies.

Gender

The prognostic role of gender in response to immune checkpoint inhibition therapy is not fully clarified. It has been suggested that response to immune checkpoint inhibition may differ based on gender due to differences in autoimmune comorbidities: women have a higher susceptibility to autoimmune disorders. A large meta-analysis published in 2018 in the Lancet, which included more than 110,000 patients with melanoma (32%) and non-small-cell lung cancer (31%) treated with immune checkpoint inhibitors, reported a pooled overall survival hazard ratio of 0.72 (95% CI 0.65-0.79) for females and 0.86 (95% CI 0.79-0.93) for males.[62] This suggests that the magnitude of response to immune checkpoint inhibition might be sex-dependent. However, this analysis might be biased due to the relatively low proportion of melanoma patients and the exclusion of patients with a history of autoimmune disorders in clinical trials, which is more prevalent in women. The presence of autoimmune comorbidities might lead to more frequent adverse events and therapy cessation. On the other hand, the occurrence of immune-induced adverse events could also potentially be associated with a good prognostic value.

A study published in 2021 by Jang et al. in JAMA Oncology observed a higher mortality hazard ratio for women with melanoma treated with ipilimumab/nivolumab compared to men, though no difference was seen in patients treated with anti-PD-1 monotherapy.[63] Conversely, a more recent study by van der Kooij and colleagues demonstrated no gender related differences in immune checkpoint inhibitor-related survival overall.[64] However, they did observe a survival advantage in female patients aged 60 years and older with *BRAF* V600 mutant melanoma compared to their male counterparts. This finding was in line with a study published in 2022 in Nature, which reported improved survival for females versus males in *BRAF/MEKi*-treated advanced melanoma patients, even after adjusting for age, LDH, disease load, affected organ sites, and ECOG performance status.[65] Given these inconsistent results, additional research is needed to understand the specific effects of gender on the effectiveness of immune checkpoint inhibitors and targeted therapy.

Ethnicity

A more recent focus in advanced melanoma research is the role of ethnicity. An international multicenter observational study involving 1135 patients examined the outcomes of anti-PD-1

monotherapy across different ethnic groups.[66] Interestingly, white patients with non-acral cutaneous melanoma had significantly higher objective response rates and longer progression-free survival compared to East Asian, Hispanic, and African patients. This was after adjusting for age, gender, anatomical location, metastasis stage, baseline lactate dehydrogenase levels, mutational status, and prior systemic treatment. In contrast, no survival benefit related to ethnicity was observed in patients with advanced acral/mucosal/uveal melanoma treated with anti-PD-1 therapy.

This finding challenges the prevailing notion that the connections between the effectiveness of PD-1 blockade and ethnicity are solely due to differences in melanoma histologic subtype. Other factors, such as sociodemographic variables and access to healthcare, may contribute to these outcome differences, particularly in regions with rural areas and healthcare disparities. However, the exact reasons behind the variations in treatment benefits remain unclear.

Additional research is needed to explore the effect of therapy response across different demographic groups and to assess biomolecular factors, including genomic assessments and immune activation and exhaustion status assays, across different ethnicities. As such, no firm conclusions can be drawn based on current study results.

Immune therapy related adverse events

Recently, a study published in JAMA Open in 2022 demonstrated that patients experiencing immune therapy-related adverse events had better survival compared to those without such events.[67] Additionally, a study by Eggermont and colleagues reported an association between immune-related adverse events and improved recurrence-free survival in stage III melanoma patients treated with adjuvant anti-PD-1 therapy.[68]

Despite these findings, to date only immune therapy induced vitiligo has been directly linked with improved survival. Additional research is needed to identify other specific adverse events associated with improved outcomes, in order to enhance response prediction in immunotherapy treated patients.

Radiological response

Mixed response

Immunotherapy has significantly improved overall survival in metastatic melanoma. However, evaluating individual patient responses to immunotherapy can be complex.[69-71] The dynamics and configurations of immunotherapy response are still being comprehensively characterized. A distinct subgroup of patients with stable disease according to RECIST criteria (less than 20% tumor

progression and less than 30% tumor regression) display intermediate survival rates.[72] Nuanced response patterns often elude detection with current radiographic methods like RECIST, prompting the development of alternative immunotherapy-specific assessments, such as iRECIST.[73] While iRECIST can be beneficial, its implementation in clinical care can be complex and cumbersome.

A specific radiological response is pseudoprogression, in which tumors initially increase in size but eventually regress.[74,75] Additionally, patients may exhibit regression in some tumors while others progress, known as a mixed response. Alternatively, some patients experience progression in a solitary site or organ, termed oligometastatic progression.

The management of these specific responses is less clear. In **Chapter 7**, we describe the occurrence and management of mixed responses to immunotherapy in patients with advanced melanoma. Mixed response most closely aligned with stable disease, demonstrating a dynamic state as mixed responders eventually continued to partial response or disease progression. Notably, patients with a mixed response who eventually responded to therapy underwent surgery more frequently than those who did not respond. Unsurprisingly, patients who underwent surgical treatment exhibited an enhanced overall survival compared to those who did not.

Our study supports an observation period of approximately three months for advanced melanoma patients with a mixed response to immune checkpoint inhibition, with re-assessment using serial imaging. We identified prognostic factors related to therapy response in this specific group, including higher median LDH levels, the presence of metastases in three or more organ sites, and brain metastases. Surgery could be suitable for mixed responders with later no response, if progressive symptoms are present and no other systemic therapy options are available.

Further refinement of clinical classification systems and identification of features associated with better or worse outcomes may guide clinical decision-making in these challenging situations. The use of consolidative surgery in mixed response patients should be considered on a case to case basis.

Heterogeneous response

In **Chapter 8**, we reported the clinical outcomes and management of mixed response, pseudoprogression, and sarcoid-like reaction in advanced melanoma patients treated with immune checkpoint inhibition therapy. We found that mixed response occurred most frequently, while pseudoprogression and sarcoid-like reactions were less common. We emphasize the importance of recognizing mixed response, as it is not uncommon, and clinicians should be aware of this clinicoradiological phenomenon.

Patients who develop a mixed response should be evaluated in more detail, with attention to previously identified prognostic factors to potentially predict further therapy response. Since therapy response can still occur in these complex cases, it is important not to halt therapy too early.

For patients who develop pseudoprogression, these were all classified as having progressive disease (PD) according to RECIST criteria. Despite PD classification, these patients had good overall survival outcomes, consistent with findings from several other studies. These findings support continuing immune checkpoint inhibition when pseudoprogression is suspected, and underlines the importance of assessing response clinically next to radiological evaluation.

Given the complexity of radiological disease assessment in patients with heterogeneous responses, additional biomarkers may be helpful in identifying those who will eventually respond to therapy. By documenting and reporting the occurrences of these specific radiological phenomena, we hope that national databases will begin to register these unique responses in the coming years. This could significantly improve clinical management for this patient group.

Conclusion

Early detection of melanoma is crucial for enhancing melanoma-specific survival. Personalized surveillance for who are at risk for melanoma development can aid in improving early melanoma detection. We identified specific phenotypic clinical risk factors associated with melanoma development in patients with multiple (atypical) naevi, and these should be taken into consideration when determining whether follow-up is necessary in this patient group.

Patients with a *CDKN2A* germline mutation have a 70% lifetime risk of developing melanoma, necessitating regular skin examinations for early detection. Understanding whether melanoma arises from pre-existing nevi or normal appearing skin in these patients can enhance clinical management. Our study using total body photography (TBP) found that most melanomas developed from pre-existing nevi, supporting the use of TBP and sequential dermoscopy. Surgical removal of melanocytic nevi in patients with *CDKN2A* germline mutations might help prevent melanoma development.

The introduction of immune checkpoint inhibitors has significantly improved outcomes for stage III/IV melanoma in both adjuvant and systemic settings. Our study found that adjuvant anti-PD-1 or *BRAF/MEKi* therapies improves locoregional disease control in resected stage III/IV melanoma patients who did not undergo standard complete lymph node dissection.

Despite improved outcomes in the adjuvant setting, immunotherapy can induce severe long lasting adverse events. Therefore, it is important to identify clinical prognostic variables

associated with adverse event development. In our study, we identified that the presence of any type of comorbidity and decreased ECOG performance status were associated with an increased risk on adverse event development in adjuvant treated stage III/IV melanoma patients. We observed no significant difference in adverse event occurrence rates in adjuvant versus advanced immunotherapy treated patients, suggesting that the extent of disease is of little influence in adverse event development.

Currently, the advent of neo-adjuvant immunotherapy revolutionized the treatment for stage III melanoma even further, as it has demonstrated greater benefits than adjuvant therapy alone, suggesting it may become the primary treatment for high risk stage III melanoma patients.

For advanced melanoma, predictive factors for systemic therapy response include age, LDH levels, cerebral disease, the number of affected organ sites, ECOG status, mutation status, immune therapy-related adverse events, and histologic subtype. We observed similar treatment-specific survival in advanced nodular melanoma patients treated with anti-PD-1, compared to stage IV superficial spreading melanoma patients. However, despite this, we observed a shorter overall survival in advanced nodular melanoma due to its tendency to metastasize earlier than superficial spreading melanoma. Therefore, histologic subtype should be assessed when determining optimal follow-up for primary melanoma patients. Additionally, histologic subtype could potentially play a role in adjuvant therapy considerations.

Clearly the kinetics and heterogeneity of immune checkpoint inhibitor responses are insufficiently captured by RECIST 1.1, which is cumbersome to use in real-world clinical management outside of clinical trials. We identified that a heterogeneous response was not uncommon to immunotherapy and we observed specific radiological patterns; mixed response, pseudoprogression and a sarcoid like reaction. Mied response patients had an intermediate survival outcome, and we show that these responses are dynamic and can evolve over time. Clinical characteristics associated with progression of disease after initial mixed response included higher LDH, brain metastases, and ≥ 3 organ sites with metastases. Surgical treatment for highly selected patients with a mixed response was associated with improved outcomes. Patients with pseudogrogression were all staged as progressive disease (PD), underlining the shortcoming of RECIST criteria for this patient group, and all had benefit from therapy continuation.

We recommend to perform serial imaging with a low threshold in patients with a heterogeneous response to immunotherapy and not to halt therapy too early, as response to treatment may still occur. In-depth analysis of immunogenic and tumoral responses is required in these complex cases and could potentially shed light on this phenomenon in the future.

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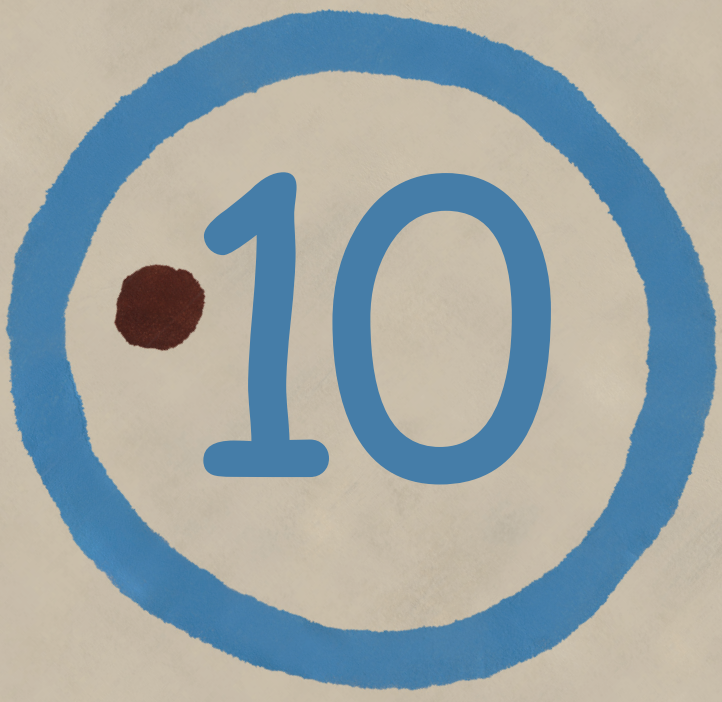
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Summary

Summary

Effective screening is crucial for early melanoma detection, particularly in high-risk patients with genetic predispositions or multiple atypical naevi. Optimal surveillance strategies can significantly improve outcomes and reduce melanoma-related mortality.

In **Chapter 2**, we evaluated the effectiveness of dermatological surveillance in detecting melanoma among Dutch patients with multiple (atypical) naevi. Over a follow-up period totaling 3,268 years, 1,131 patients were monitored, and melanoma was diagnosed in 39 patients, with an annual incidence rate of 1.1%. Most melanomas (72%) originated from preexisting naevi, a higher proportion than the general population (30%). Key risk factors included red or blonde hair, over 100 solar lentigines, and childhood sunburns. Notably, 79% of melanomas were detected during routine dermatologist examinations, emphasizing the importance of regular surveillance. These findings highlight the necessity of periodic dermatological examinations for early melanoma detection in patients with multiple (atypical) naevi.

Individuals with the CDKN2A pathogenic variant, also known as hereditary melanoma (FAMMM syndrome), have an estimated 70% lifetime risk of developing melanoma. These patients are recommended to undergo biannual skin examinations to detect melanoma at an early stage. FAMMM syndrome, associated with an increased number of atypical naevi, complicates the distinction between atypical naevi and melanoma. Total body photography (TBP) can be beneficial for identifying alterations in melanocytic naevi and the emergence of new lesions. In **Chapter 3**, we assessed TBP to identify naevi alterations and melanoma development in patients with a pathogenic CDKN2a germline mutation. Among 60 melanoma cases, 78% developed from preexisting naevi, significantly higher than the estimated 30% in the sporadic melanoma population. These results support the routine use of TBP in these high-risk patients, and surgical excision of atypical naevi might be beneficial in preventing melanoma development.

The advent of immune checkpoint inhibitors and targeted therapies (*BRAF*/*MEKi*) has revolutionized the treatment of metastatic melanoma. In 2010, the FDA approved ipilimumab (anti-CTLA-4) for patients with metastatic melanoma. Further, the FDA approved anti-PD-1 therapy, an immune-checkpoint-inhibitor that selectively blocks the PD-1 receptor. Anti-PD-1 therapy demonstrated a significantly improved overall survival and fewer treatment-related adverse events in treated metastatic melanoma patients compared to ipilimumab. In addition, anti-PD-1 nivolumab was approved in the adjuvant setting in 2017, improving recurrence free survival in resected stage III/IV melanoma.

Additionally, trials investigating *BRAF* plus *MEK* inhibitors (*BRAF*/*MEKi*) in the advanced and adjuvant setting demonstrated an improved overall survival and RFS for melanoma patients

with a BRAF V600 mutation. These advancements have transformed the treatment paradigm for adjuvant and advanced therapy in patients with resected stage III and IV melanoma.

Concurrently, the Multicenter Selective Lymphadenectomy Trial 2 (MSLT-2) demonstrated that lymph node observation in patients with resected melanoma and low-burden stage III disease had similar melanoma-specific survival rates compared to patients who underwent immediate completion lymph node dissection (CLND). This strategy is now preferred in the National Comprehensive Cancer Network (NCCN) guidelines. However, clinical trials investigating immunotherapy and *BRAF*/*MEKi* required complete lymphadenectomy in patients with positive sentinel lymph node biopsy. To address adjuvant outcomes under current MSLT-2 guidelines, we investigated real-world outcomes in stage III and resected stage IV melanoma patients treated with adjuvant anti-PD-1 or *BRAF*/*MEKi* after adopting the MSLT-2 nodal management guidelines, in **chapter 4**.

We conducted an analysis of real-world data from patients with resected stage III-IV melanoma who received treatment at Massachusetts General Hospital in the post MSLT-II era. Patients who had a complete lymph node dissection upon a positive sentinel lymph node biopsy were excluded. Among 137 treated patients, 46 received adjuvant anti-PD-1, 30 received adjuvant *BRAF*/*MEKi*, and 26 patients had surgery only. During a median follow-up period of 17 months, patients treated with adjuvant anti-PD-1 and adjuvant *BRAF*/*MEKi* showed significantly longer RFS compared to those who received surgery only. Prognostic factors associated with decreased adjuvant therapy outcomes included stage IIIC/IIID disease and macroscopic lymph node involvement. Patients treated with anti-PD-1 experienced earlier disease recurrence compared to those treated with *BRAF*/*MEKi*, suggesting that *BRAF*/*MEKi* might induce a more swift response, although long-term follow-up is needed. *BRAF*/*MEKi*-treated patients had a higher incidence of any grade adverse events compared to anti-PD-1-treated patients, aligning with previous trial data. Our findings support the efficacy of adjuvant systemic therapy in resected high-risk stage III and oligometastatic stage IV melanoma, with adjuvant therapy potentially eradicating occult regional disease.

While adjuvant and systemic immunotherapy enhances outcomes, it is imperative to note that this benefit can come at a cost. Approximately 30% of patients undergoing systemic therapy may experience immune therapy-related adverse events (irAEs). Serious irAEs include colitis, hypophysitis, adrenalitis, hepatitis, toxic epidermal necrolysis, and Guillain-Barré, and these irAE can occur during or even after treatment. Most adverse events resolve with immune suppression, but some, particularly the endocrine side effects, can persist. Due to the significant impact on the quality of life, the initiation of immune checkpoint inhibitors is decided on a case-by-case basis by medical oncologists, considering clinical condition, comorbidities, overall disease load, stage, metastasis location, and *BRAF* mutation status.

It is unclear whether the incidence and severity of anti-PD-1 therapy-related adverse events (irAEs) vary in adjuvant and advanced treated melanoma patients, according to the safety analysis of clinical trials. In addition, to date no head-to-head study has been conducted to assess adverse events in anti-PD-1 adjuvant and advanced melanoma patients. Shedding light on this topic might aid in therapy decision making.

In **Chapter 5** we assessed melanoma patients treated with first-line anti-PD-1 in adjuvant or advanced setting between 2015 and 2021 from the Dutch Melanoma Treatment Registry (DMTR). Comorbidities and ECOG performance score were assessed before treatment, and grade III-IV irAEs were monitored during treatment. Univariate and multivariate regression analysis was conducted to calculate factors associated with irAE development. In total 1,499 advanced melanoma patients and 1,071 adjuvant patients received anti-PD-1 therapy. The latter group consisted of younger patients (median age 63 years vs 69 years, $p<0.01$), who had a better ECOG performance status ($p<0.01$). Patients treated in the advanced setting for advanced melanoma more often had comorbidities than adjuvant treated patients, 76% versus 68%, respectively ($p<0.01$). Grade III-IV irAEs occurred in 212 (14%) advanced treated patients and in 130 (12%) adjuvant treated patients ($p=0.14$). Grade III-IV endocrine side effects were seen more often in adjuvant patients, 25 (2%) compared to 19 (1%) in advanced melanoma patients ($p=0.04$). Multivariate analysis demonstrated no increased risk for Grade III-IV irAE development in adjuvant treated patients, (adjusted OR 1.01 (95% CI 0.79 – 1.29), compared to advanced patients, corrected for comorbidities and ECOG performance score. Our results demonstrate that a higher ECOG performance status and presence of any comorbidity were independently associated with an increased risk for Grade III-IV irAE in all patients. Patients treated in the adjuvant setting did not have an increased risk for developing severe irAEs compared to advanced melanoma patients. These findings are of clinical significance in consulting patients for adjuvant anti-PD-1 treatment.

Despite the improved survival associated with systemic therapy, a significant proportion of patients will ultimately not respond. Identifying prognostic variables associated with therapy response could optimize therapy decision-making. For instance, the presence of cerebral metastasis is associated with a more aggressive disease course and should therefore, if suited, be treated more aggressively. Furthermore, the *BRAF* status, age, ECOG performance status, increased LDH, and total organ sites with metastasis are of clinical significance. Additionally, the histologic subtype can affect systemic therapy efficacy, with anti-PD-1 being less effective in metastatic acral and mucosal melanoma compared to metastatic superficial spreading melanoma. The histologic subtype primary nodular melanoma is associated with a shorter overall survival compared to superficial spreading melanoma, regardless of important prognostic factor Breslow thickness. Whether the histologic subtype nodular melanoma affects the efficacy of immunotherapy and *BRAF*/*MEKi* has not been investigated yet.

Therefore, we assessed the efficacy of systemic anti-PD-1 and *BRAF*/*MEKi* in advanced melanoma patients in the DMTR, in **Chapter 6**. Our study included 1,086 advanced nodular melanoma (NM) patients and 2,246 metastatic superficial spreading melanoma (SSM) patients. Distant metastatic free survival (DMFS) was significantly shorter for patients with advanced NM, suggesting a higher propensity for metastatic outgrowth compared to SSM. Multivariate survival analysis showed no significant difference in efficacy between NM and SSM patients for both immunotherapy and *BRAF*/*MEKi*. These findings indicate that while NM has a higher potential for metastatic outgrowth, the efficacy of systemic therapy does not differ significantly between NM and SSM patients. The histologic subtype nodular melanoma could potentially be considered when determining follow-up periods for patients with primary resected melanoma in addition to conventional TNM classification.

Response to anti-PD-1/anti-CTLA-4 can be complex to evaluate as the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) may not sufficiently capture the heterogeneous tumor responses observed with immunotherapy, leading to the development of iRECIST, a guideline that standardizes radiographic approaches to tumor measurements and changes during immunotherapy. A subset of patients exhibits a mixed response, where synchronous regression in some tumors occurs alongside progression in others, which is distinct from pseudoprogression—where initial tumor enlargement due to immune infiltration is followed by regression. Additionally, some patients experience long-term stability (RECIST stable disease), with the biological significance of these tumors remaining unclear. Others may show oligoprogression, where progression is limited to a single site or organ after a good initial response. These varied responses complicate clinical decisions, which are currently made on a case-by-case basis.

In **Chapter 7**, we describe real-world outcomes in a cohort of stage IV unresectable cutaneous melanoma patients treated with first-line immunotherapy at a high-volume institution (Massachusetts General hospital). Response to treatment was assessed radiographically using clinical response categories, integrating both radiographic and clinical responses, and compared with RECIST 1.1 criteria when available. Mixed responses were defined as patients exhibiting synchronous regressing and progressing metastatic lesions. Among 292 patients, 103 (35%) were responders, 64 (22%) exhibited mixed responses (MR), and 125 (43%) had progressive disease. Of the patients with mixed responses, 64% would be classified as having stable disease according to RECIST 1.1. Furthermore, 56% of mixed responders eventually responded to therapy (MR-R), while 31% progressed (MR-NR). MR-NR patients had significantly worse overall survival compared to MR-R patients, with clinical variables associated with poorer survival, such as higher median lactate dehydrogenase (LDH), metastases in three or more organ sites, a lack of *BRAF* V600E mutations, and more frequent M1d disease (brain metastases). Mixed responders who underwent surgery had significantly longer survival and time to new response compared to those who did not undergo surgery.

Overall, mixed response represented a dynamic state, lasting on average from 141 to 260 days, with 59% eventually responding to treatment. Surgical treatment appeared beneficial for highly selected patients in the MR category. Our data indicate that mixed tumoral responses to immunotherapy are common in metastatic melanoma and represent a dynamic, often transient state, correlating most closely with RECIST 1.1 stable disease. Risk stratification in these patients is critical for effective clinical decision-making. Our study identified several features in patients with a clinical MR that are associated with subsequent progression, including higher median LDH, metastases in three or more organ sites at the time of MR, and the presence of brain metastases. Refining clinical classification systems and identifying prognostic features may guide decision-making in these complex cases. Additionally, surgery was associated with improved survival in a subset of MR patients where it was feasible. Therefore, consolidative surgery should be considered in this population. Further research is needed to optimize treatment strategies and improve outcomes for patients with a mixed responses to immunotherapy.

Lastly, in **Chapter 8** we assessed different types of heterogeneous response to immunotherapy in patients treated at Leiden University Medical Hospital. Out of 196 patients, 37 (19%) exhibited a heterogeneous response to immunotherapy. These responses were categorized into mixed response (MR) (15%), pseudoprogressive disease (PP) (3%), and sarcoid-like reaction (2%). Patients with a mixed response but no subsequent response to therapy (MR-NR) were characterized by higher median lactic acid dehydrogenase (LDH) levels ($p = 0.01$), a higher prevalence of male patients ($p = 0.04$), more involved metastatic organ sites ($p = 0.01$), and a higher incidence of brain metastases ($p = 0.02$). Patients exhibiting a mixed response with eventual therapeutic response (MR-R) and those with pseudoprogression (PP) demonstrated longer overall survival, with medians of 1.7 years (95% CI: 1.1-2.7) and 1.6 years (95% CI: 1.3-2.0), respectively, compared to MR-NR patients who had a median overall survival of 1.2 years (95% CI: 0.7-1.7) ($p < 0.01$). Patients with pseudoprogression benefited from continued therapy, highlighting the importance of not prematurely discontinuing treatment in cases of suspected pseudoprogression. The findings of our studies in **Chapter 7 and 8** suggest that male sex, more involved disease sites, brain metastases, and higher median LDH levels are associated with poorer survival outcomes in patients with a mixed response, indicating that these variables could be predictive of whether a mixed responder will ultimately benefit from therapy.


11

Nederlandse samenvatting

Effectieve screening is cruciaal voor vroege detectie van melanoom, met name bij hoog risico patiënten met een genetische aanleg of meerdere atypische naevi. Optimalisatie van surveillancestrategieën kan de uitkomsten significant verbeteren en de sterfte door melanoom verminderen.

In **Hoofdstuk 2** hebben we de effectiviteit van dermatologische surveillance geëvalueerd bij Nederlandse patiënten met meerdere (atypische) naevi. Gedurende een totale follow-up periode van 3.268 jaren werden 1.131 patiënten gevolgd, waarbij bij 39 patiënten melanoom werd vastgesteld, met een jaarlijkse incidentie van 1,1%. De meeste melanomen (72%) ontstonden uit pre-existente naevi, een hoger percentage dan in de algemene populatie (30%). Belangrijke risicofactoren waren rood of blond haar, meer dan 100 solar lentigines en zonnebrand in de kindertijd. Opmerkelijk is dat 79% van de melanomen werd gedetecteerd tijdens routineonderzoeken door een dermatoloog, wat het belang van regelmatige controle onderstreept. Deze bevindingen benadrukken het belang van aanvullende risicofactoren bij de noodzaak van periodieke dermatologische controles voor vroege melanoomdetectie bij patiënten met meerdere (atypische) naevi.

Individuen met een pathogene CDKN2A-variant, ook bekend als familiaire atypische multiple mole-melanoomsyndroom (FAMMM-syndroom), hebben een geschatte levenslange kans van 70% op het ontwikkelen van melanoom. Voor deze patiënten wordt aanbevolen tweemaal per jaar huidonderzoek te ondergaan om melanoom in een vroeg stadium te detecteren. Het FAMMM-syndroom, dat gepaard gaat met een verhoogd aantal atypische naevi, bemoeilijkt de onderscheidingsdiagnose tussen atypische naevi en melanoom. Total body photography (TBP) kan nuttig zijn bij het identificeren van veranderingen in melanocytaire naevi en het ontstaan van nieuwe laesies. **In Hoofdstuk 3** hebben we het gebruik van TBP onderzocht om veranderingen in naevi en melanoomontwikkeling te identificeren bij patiënten met een pathogene CDKN2A-genmutatie. Van de 60 melanoomgevallen ontwikkelde 78% zich vanuit pre-existente naevi, significant hoger dan de geschatte 30% in de sporadische melanoompopulatie. Deze resultaten ondersteunen het routinematig gebruik van TBP bij deze hoogrisicopatiënten, en chirurgische excisie van atypische naevi zou nuttig kunnen zijn om melanoomontwikkeling te voorkomen.

De introductie van immune checkpoint inhibitors (ICI) en gerichte therapieën (BRAF/MEKi) heeft de behandeling van gemetastaseerd melanoom significant veranderd. In 2010 keurde de FDA ipilimumab (anti-CTLA-4) goed voor patiënten met gemetastaseerd melanoom. Later volgde de goedkeuring van anti-PD-1-therapie, die selectief de PD-1-receptor blokkeert. Anti-PD-1-therapie verbeterde de algehele overleving aanzienlijk en ging gepaard met minder bijwerkingen in vergelijking met ipilimumab. Daarnaast werd adjuvante anti-PD-1 (nivolumab) in 2017

goedgekeurd, wat leidde tot een verbeterde ziektevrije overleving bij gereseceerd stadium III/IV melanoom.

In **Hoofdstuk 4** hebben we uitkomsten onderzocht bij patiënten met stadium III/IV melanoom die werden behandeld volgens de richtlijnen van de MSLT-2-studie, waarbij lymfeklierdissectie alleen werd uitgevoerd bij duidelijke klinische noodzaak. Onze resultaten tonen aan dat adjuvante anti-PD-1-therapie en BRAF/MEKi geassocieerd zijn met een betere ziektevrije overleving in vergelijking met alleen chirurgie. Desalniettemin bleef stadium IIIC/IIID-ziekte geassocieerd met een slechtere prognose, wat wijst op de noodzaak van geavanceerde behandelstrategieën voor deze subgroepen.

Immunotherapie geassocieerde bijwerkingen kunnen hevig zijn en lang aanhouden, zelfs nog na het staken van de therapie. Het is onbekend of de mate van bijwerkingen verschilt in patiënten die systemisch worden behandeld versus patiënten die adjuvant worden behandeld. Het is belangrijk om dit uit te zoeken, daar inzicht hierin mogelijk kan bijdragen aan het selecteren van de juiste patiënt voor de juiste behandeling. In **Hoofdstuk 5** onderzochten we de incidentie en ernst van immuungerelateerde bijwerkingen (irAEs) bij patiënten die adjuvante of systemische anti-PD-1-therapie ontvingen. Bijwerkingen zoals endocriene stoornissen kwamen vaker voor bij adjuvant behandelde patiënten, maar de totale incidentie van irAEs was vergelijkbaar tussen beide groepen. Belangrijke prognostische factoren voor irAE-ontwikkeling waren een verminderde ECOG-status en de aanwezigheid van comorbiditeiten. Onze bevindingen benadrukken dat adjuvante therapie veilig kan worden toegepast bij geschikte patiënten.

Het histologische subtype van het melanoom kan een invloed hebben op de effectiviteit van de behandeling. Het subtype acraal melanoom is geassocieerd met een slechtere respons op immunotherapie, terwijl het desmoplastisch melanoom betere uitkomsten laat zien op immunotherapie. In **Hoofdstuk 6** hebben we de effectiviteit van systemische anti-PD-1- en BRAF/MEKi-therapieën bij patiënten met gevorderd melanoom onderzocht. Onze studie omvatte 1.086 patiënten met gevorderd nodulair melanoom (NM) en 2.246 patiënten met gemetastaseerd superficieel spreidend melanoom (SSM). De afstandsmetastasevrije overleving was significant korter bij patiënten met gevorderd NM, wat wijst op een hogere neiging tot metastatische uitgroei in vergelijking met SSM. Multivariate overlevingsanalyse toonde geen significant verschil in effectiviteit tussen NM- en SSM-patiënten, zowel bij immunotherapie als bij BRAF/MEKi. Deze bevindingen suggereren dat hoewel NM een hoger potentieel heeft voor metastatische uitgroei, de effectiviteit van systemische therapie niet significant verschilt tussen NM- en SSM-patiënten. Het histologische subtype nodulair melanoom zou mogelijk kunnen worden overwogen bij het bepalen van follow-up periodes voor patiënten met primair gereseceerd melanoom, naast de conventionele TNM-classificatie.

Ondanks de verbeterde overleving door immunotherapie blijft de respons op anti-PD-1/anti-CTLA-4-behandeling moeilijk te meten. De bestaande RECIST 1.1-criteria schieten tekort bij het vastleggen van de complexe tumorreacties die optreden, wat leidde tot de ontwikkeling van iRECIST. Heterogene responsen, zoals een mixed respons met gelijktijdige regressie en progressie, pseudoprogressie door immuuncelinfiltratie gevolgd door regressie en oligoprogressie beperkt tot één locatie, maken klinische besluitvorming uitdagend en vereisen een individuele benadering.

In **Hoofdstuk 7 en 8** bespreken we de resultaten van stadium IV melanoom patiënten, behandeld met eerstelijns immunotherapie die een heterogene response ontwikkelen op immunotherapie (anti-CTLA-4 en of anti-PD-1). De respons werd beoordeeld aan de hand van klinische en radiografische criteria en vergeleken met RECIST 1.1. De heterogene respons omvatte mixed respons (MR), pseudoprogressie (PP) en sarcoïd-like respons.

Patiënten met MR zonder latere respons op therapie (MR-NR) hadden hogere LDH-waarden, meer dan drie verschillende orgaan metastasen, hersenmetastasen en waren vaker man. Daarentegen hadden patiënten met MR die wel reageerden (MR-R) en patiënten met pseudoprogressie een langere mediane overleving van respectievelijk 1,7 en 1,6 jaar, vergeleken met 1,2 jaar voor MR-NR-patiënten. Pseudoprogressie bleek te profiteren van voortgezette behandeling, wat benadrukt dat therapie niet te vroeg moet worden gestaakt bij vermoeden van progressie.

De bevindingen uit onze studies in **Hoofdstuk 7 en 8** suggereren dat mannelijk geslacht, meer betrokken ziekteplaatsen, hersenmetastasen en hogere mediane LDH-waarden geassocieerd zijn met slechtere overlevingsuitkomsten bij patiënten met een mixed respons. Deze variabele kunnen mogelijk voorspellend zijn voor de vraag of een mixed respons uiteindelijk baat zal hebben bij therapie. Het is belangrijk om niet te vroeg met de behandeling te staken, daar de respons nog kan optreden. Het laagdrempelig herhalen van radiologisch en of klinisch onderzoek kan bijdragen aan het vast stellen van de definitieve therapie respons.

11

List of Abbreviations

ABCDE	Asymmetry, Border, Color, Diameter, Evolution
ACT	Adoptive Cell Therapy
AJCC	American Joint Committee on Cancer
AN	Atypical Nevi
Anti-TNF-a	Anti-Tumor Necrosis Factor alpha
BAP1	BRCA1 Associated Protein 1
BRAF	B-Raf proto-oncogene
BRAF/MEKi	BRAF and MEK inhibitors
CDK4	Cyclin-Dependent Kinase 4
CDKN2A	Cyclin-Dependent Kinase Inhibitor 2A
CLND	Completion Lymph Node Dissection
CR	Complete Response
CTLA-4	Cytotoxic T-Lymphocyte Associated Protein 4
ctDNA	Circulating tumor DNA
ECOG	Eastern Cooperative Oncology Group performance score
ENT	Ear, Nose, and Throat
EMA	European Medical Agency
FDA	Food and Drug Administration
$HMel^{CDKN2A}$	Hereditary Melanoma due to CDKN2A mutation
HR	Hazard Ratio
IFNy	Interferon Gamma
iRECIST	Immune Response Evaluation Criteria in Solid Tumors
irAE	Immune-Related Adverse Events
KIT	tyrosine-protein kinase proto-oncogene
LAG-3	Lymphocyte-Activation Gene 3
LDH	Lactate Dehydrogenase
MAPK	Mitogen-Activated Protein Kinase
M	M-stadium in the TNM-classification that stands for Metastasis involvement
MELFO	Melanoma Follow-up study
MIT	Melanocyte Inducing Transcription factor – gen
MSLT-I/MSLT-II	Multicenter Selective Lymphadenectomy Trial-I/II
NF1	Neurofibromin 1 - gen
N	N-stadium in the TNM-classification that stands for Lymph Node Involvement
NM	Nodular Melanoma
NRAS	Neuroblastoma RAS Viral - Oncogene Homolog

PD	Progressive Disease
PD-1	Programmed Death 1 protein
PD-L1	Programmed Death-Ligand 1
POT1	Protection of Telomeres 1 - gene
PR	Partial Response
PTEN	Phosphatase and Tensin Homolog - gene
RECIST	Response Evaluation Criteria in Solid Tumors
RFS	Recurrence-Free Survival
SD	Stable Disease
SSM	Superficial Spreading Melanoma
TERF21P	Telomeric Repeat Binding Factor 2, Interacting Protein
TERT	Telomerase Reverse Transcriptase - gene
T	T-stadium in the TNM-classification that stands for primary tumor involvement
TIL	Tumor-Infiltrating Lymphocytes
TMB	Tumor Mutation Burden
T-VEC	Talimogene Laherparepvec
TP53	Tumor Protein p53

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Curriculum vitae

Daan Jan Willem Rauwerdink was born on November 30, 1993, in Almelo, the Netherlands. After completing his secondary education at OSG Erasmus in Almelo in 2013, he began studying Medicine at Leiden University Medical Center (LUMC). During his studies, he pursued a minor on the molecular targets of cancer therapies and participated in the Medical Business Masterclass on healthcare innovation and technology. In 2020, he obtained his Master of Medicine degree, completing his senior surgical internship at Alrijne Hospital.

During his medical training, he developed a strong interest in scientific research, undertaking research internships at LUMC, Alrijne, and Massachusetts General Hospital/Harvard Medical School in Boston. His research primarily focused on melanoma treatment and diagnostics, leading to multiple publications in peer-reviewed journals and presentations at international conferences.

Following his graduation, he worked as a dermatology resident at Maasstad Hospital before commencing his dermatology residency at LUMC in 2022. Alongside his clinical work, he remains actively engaged in research and innovation within the dermatologic field.

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tot nu zijn jullie altijd belangrijk voor mij geweest. Maandagavond chestday, een avond petanquen in een te kleine zwembroek, een foto op een paard, discussiëren over Israël, talloze avonden in De Keyzer, een avond stiften, een week zeilen in Kroatië, een weekend Porto, een zaterdagmiddag in een Brauhaus in Köln, of het raden van het juiste smaakpalet bij het wijnproeven – stuk voor stuk momenten die onze studententijd onvergetelijk maakten. Hoewel het leven continu verandert, blijft de vriendschap hetzelfde, en daar ben ik enorm dankbaar voor.

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Lieve **Paulien en Anneloek**, de jeugd is de basis voor de sociale en persoonlijke ontwikkeling en jullie hebben daar een belangrijk aandeel in gehad. De creatieve spellen die Paulien bedacht en de meer competitieve uitdagingen van Anneloek maakten mijn jeugd zorgeloos en vol plezier. Samen maakten de verplichte musea-bezoeken en culturele bezienswaardigheden op vakantie veel dragelijker. Het is mooi om te zien hoe jullie nu de volgende stap in het leven al hebben gezet en een gezin zijn begonnen. Een drukke baan combineren met het moederschap vraagt veel en ik heb ontzettend veel respect voor hoe jullie dat doen. Heel veel dank voor alle wijze lessen en praktische hulp die jullie mij hebben gegeven.

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