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Risk factors for developing brain metastases in cutaneous malignant melanoma

Degree Project in Medicine

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List of abbreviations

AJCC	American joint committee on cancer
CI	Confidence interval
CMM	Cutaneous malignant melanoma
CTLA-4	Cytotoxic T-lymphocyte-associated antigen 4
ECOG	Eastern Cooperative Oncology Group
HR	Hazard ratio
ILP	Isolated limb perfusion
LDH	Lactate dehydrogenase
M-stage	Metastatic stage
MBM	Melanoma brain metastases
MRI	Magnetic resonance imaging
OR	Odds ratio
OS	Overall survival
PD-1	Programmed cell death protein 1
PT	Primary tumour
RT	Radiotherapy
SD	Standard deviation
SE	Standard error
SRS	Stereotactic radiosurgery
SSM	Superficial spreading melanoma
SU	Sahlgrenska University Hospital
Tis	Tumour in situ
ULN	Upper limit of normal
WT	Wild type

Abstract

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Introduction: Cutaneous malignant melanoma (CMM) is with an increasing incidence one of the most common cancer forms in Sweden. With the highest propensity of all cancers to metastasise to the brain, brain metastases are a common cause of death in CMM. Current guidelines only describe screening and follow up programmes for CMM brain metastases to a limited extent. To improve the management of this challenging disease, risk factors must be discerned to optimise treatment of high-risk individuals.

Aim: The primary purpose of this study is to identify potential risk factors associated with the development of brain metastases in CMM.

Methods: Case-control study of patients with inoperable or metastatic CMM, handled directly or in consultation with the Department of Oncology, Sahlgrenska University Hospital, Gothenburg, between 1st of January 2013 and 30th of June 2019. Potential risk factors were evaluated by comparing data from patients who developed brain metastases, with patients who presented with extracranial disseminated disease only.

Results: 402 cases were included in the study. 94 developed brain metastases. Variables found to be associated with the development of brain metastases included younger age, unknown primary tumour (PT), PT located on the torso, BRAF-V600 mutation and elevated levels of S-100 at time of inoperable or metastatic CMM. Conversely acral histological type and systemic

treatment, particularly immunotherapy, were associated with lower risk of developing brain metastases. No differences were found in tumour thickness or presence of ulceration.

Conclusions: The obtained data suggest that high risk individuals for development of brain metastases in CMM may benefit from more intense follow up including e.g., frequent MRI brain scans. The observation that patients receiving systemic treatment with immunotherapy are associated with having a lower risk of developing brain metastases than patients receiving other treatment warrant further investigation.

Key words: Malignant Melanoma, Brain Metastases, Risk Factors, Predicting Variables

Introduction

Cutaneous malignant melanoma (CMM) is one of the most common cancer forms in Sweden (1). With an increasing incidence there has been over 4500 new cases reported annually during the past few years (2). This is a cancer that affect all ages but is most common in patients over 60 years old (3). Moreover it is the third most common cause of brain metastases, with the highest propensity of all cancers to metastasise to the brain (4). Therefore brain metastases is one common cause of death in CMM (5). Clinical studies show that 10-40 % of patients with metastatic or unresectable CMM develop brain metastases (6-9). Although, in autopsy series there has been reported up to 73 % of CNS involvement (5, 10). However, Budman et al (1978) shows only half of these cases were the cause of death, suggesting that all brain metastases will not clinically present nor affect the outcome (5).

Historically there has been a very poor prognosis for patients with brain metastasised CMM, with a median overall survival (OS) of approximately four months (8, 9, 11, 12), and treatment options have been limited. However, apart from the conventional treatment with surgery, whole brain radiotherapy and chemotherapy, we have in recent years seen a development in treatment strategies that have had a revolutionary effect. Recent data suggest that the new systemic treatments, in particular BRAF inhibitors +/- MEK inhibitors (targeted therapy) and immune checkpoint inhibitors (immunotherapy), such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) inhibitors and programmed cell death protein 1 (PD-1) inhibitors, have a promising effect on brain metastases as well as extracranial disease. In a systemic review, published in 2020 by van Opijnen and co-workers, 95 studies were examined. They concluded that immunotherapy and targeted therapy were superior to chemotherapy when comparing OS in patients with melanoma brain metastases (MBM) (9.0,

7.6 and 5.6 months respectively). An even greater effect was seen when combining two different types of immunotherapy or targeted therapy (14.1 and 11.5 months, respectively), as well as combining immunotherapy with targeted therapy (13.9 months). Furthermore, stereotactic surgery (SRS) has been emphasised to play an important key roll, both on its own and together with systemic treatment, in the management of MBM. The combination of targeted therapy and SRS resulted in a median OS of 11.7 months, whereas combining immunotherapy and SRS increased OS to 17.4 months (13). Data might be biased considering the criteria to be offered the more modern treatments. For instance, SRS have previously only been given to patients with a limited number of brain metastases, who already have a better prognosis compared to those with generally disseminated brain metastases (14). Recent data suggest that patients with up to 10 metastases may benefit similarly from this treatment (15).

The recommendation of combining multiple systemic treatments with or without radiotherapy (RT), including SRS, has raised concerns regarding possible toxicity including radiation necrosis, intracranial haemorrhage and cerebral oedema. Van Opijnen et al continues to explain in their study how immunotherapy on its own is prone to mild and moderate toxicity, whereas severe toxicity is rather uncommon. Though in combination with RT, an increase in brain toxicity was observed and the combination of two immunotherapies (CTLA-4 inhibitors + PD-1 inhibitors) led to an increase in severe general toxicity, such as increased alanine aminotransferase or aspartate aminotransferase. When examining targeted therapy severe toxicity, especially skin lesions/rash, is more common compared to treatment with immunotherapy. However, combining targeted therapy with RT showed a brain toxicity rate similar to that of RT alone (13). In 2017 a systematic review was published on the subject of severe toxicity in the treatment of brain metastases (not solely originating from malignant

melanoma) which concluded that the combination of cranial SRS and immunotherapy was tolerated well, bearing in mind that the studies are few and the populations were small. Data concerning combination of SRS with BRAF inhibitors were more uncertain, with conflicting results from the included studies (17). Consensuses state that more research is needed, and practitioners should be cautious and aware of radiation necrosis, intracranial haemorrhage and cerebral oedema, quickly treating them as they would other side effects (13, 17).

Table 1
Clinical staging in Malignant melanoma*

Clinical stage	
0	**Malignant melanoma in situ, no metastases**
I-II	**Invasive malignant melanoma, no metastases**
III	**Invasive malignant melanoma, nodal, satellite or in-transit metastases**
IV	**Invasive malignant melanoma, distant metastases**
M1A	***Distant metastases to skin/muscles/nonregional lymph nodes***
M1B	***Distant metastases to lung***
M1C	***Distant metastases to visceral organs***
M1D	***Distant metastases to brain***

*Based on the clinical staging by AJCC eight edition (16)

Historically, surveillance of patients with CMM regarding potential development of brain metastases could not be justified with the limited treatment options and its insufficient response. Conversely, in this new era of promising therapies, surveillance with MRI of the brain should be considered in an effort to detect early metastases still treatable with quickly deployed treatment, given the potential of prolonged life expectancy (18-20). As mentioned earlier, the survival after a MBM diagnosis is inversely related to the number of brain metastases, which Bottoni et al has shown (2013) (14).

Internationally, different follow up schemes are followed. In Sweden there is currently no guidelines on standardised screening for brain metastasis in advanced CMM (21). At the same time, in the European consensus-based interdisciplinary guideline for melanoma, follow up with MRI of the brain even during the first year after AJCC seventh edition (American joint committee on cancer) stage IIC and III melanoma (See Table 1) is shown to be

cost-effective (22). To optimise the screening process of brain metastases there is a need to know who and why they are affected.

Risk factors for developing brain metastases in CMM is a subject that has been well researched in previous studies. Factors often shown correlated with MBM development are thickness, ulceration and location of primary tumour (PT) (head and neck, scalp in particular) (9, 12, 20, 23-29), as well as male gender (9, 27, 28), bearing in mind that men are more likely to have a PT located on the torso and peripheral area of head and neck (including the scalp) (30, 31). Nevertheless, it seems to be uncertain whether these factors predict brain metastases in particular or systemic disease as a whole, as discussed by Sampson et al (1998) (9). Few studies can be found where the control group consists solely of patients with extracranial disseminated CMM, and these studies generate rather conflicting results. Frankel et al (2014) shows by using data from prospectively maintained melanoma databases that the only predictive factors on developing MBM as part of initial recurrence is thinner primary lesion and younger age, however with a median Breslow thickness of 3.4 mm compared to 4.5 mm and a median age of 55 years (32). Additionally, in a retrospective study including patients with inoperable nodal, satellite or in-transit metastases (inoperable stage III) or distant metastases (stage IV), Bedikian et al (2011) confirms earlier findings of thicker lesions, male gender and PTs located in the head and neck area as predictors of MBM. However, they also state that patients with PTs located on the trunk/abdomen has an equal risk of developing MBM, and an even higher risk is seen when the site of the PT is unknown. They also present new findings of elevated levels of lactate dehydrogenase (LDH) at time of inoperable stage III or stage IV diagnosis associated with an increased risk of developing brain metastases, and when assessing clinical stage (see Table 1) a higher risk is seen in patients at stage M1B or M1C

compared to stage III or M1A (27). The lack of significant difference in ulceration in these two studies and the inconsistent results regarding PT thickness between patients with MBM and patients with extracranial metastases only may indicate that the hypothesis made by Sampson et al (1998), suggesting these variables as indicators for generally disseminated CMM, may be proven true (9). Since only few studies is to be found on the subject, where the control group consists exclusively of patients with extracranial inoperable stage III or stage IV disease, further research in this area is warranted.

Aim

The aim of this study was to identify risk factors of brain metastases in patients with CMM in an effort to optimise and personalise surveillance and treatment of such high-risk individuals.

Material and Methods

Study Design

Case-control study

Study Population

Data were obtained from electronic medical records of patients who developed inoperable stage III or stage IV CMM, based on clinical staging by AJCC eight edition (16), (Table 1), and were handled directly or in consultation with the Department of Oncology, Sahlgrenska University Hospital (SU), Gothenburg, between 1st of January 2013 and 30th of June 2019. Data cut-off was set to 31st of December 2019 which offered a follow up-time of a minimum of six months.

This project is a sub-study of an ongoing project at the Department of Oncology, SU, Gothenburg, where our study population were earlier identified by using the search terms ICD-10 diagnoses codes C43.1-9 (i.e., malignant melanoma) and C79.3 (i.e., brain metastases). This generated 102 patients, where eight patients were excluded; two did not develop brain metastases, five developed brain metastases before the set time frame and one developed brain metastases after the set time frame. We then distinguished the control group by using the search terms C43.1-9 (i.e., malignant melanoma) and removed the previously identified patients in the study population. Of the 1084 patients enrolled in the control group, 778 were excluded; 159 developed brain metastases, 54 developed inoperable stage III or stage IV disease before the set time frame, 295 did not meet the diagnosis criteria (e.g., malignant melanoma other than cutaneous; non metastatic or operable stage III CMM; familial malignant melanoma without metastases; no malignant melanoma diagnosis), 136 were treated after the set time frame, many who would have been otherwise excluded due to not meeting the diagnosis criteria or developing MBM, and 134 had no medical records at the Department of Oncology, SU, Gothenburg. In the control group two patients had multiple PTs that could be the cause of their disseminated disease. For each of these patients, two cases were reported. This generated a total population of 402 cases who were subsequently divided into two groups; the study population; patients with development of brain metastases BM(+), (n=94), and the control group; patients without development of brain metastases BM(-), (n=308). The process of inclusion and exclusion can be seen in Figure 1.

Data Collection

Data were collected as follows: gender and age, at diagnosis of PT as well as at diagnosis of inoperable stage III or stage IV CMM, of the patient; time of PT resection, site, tumour

thickness according to Breslow (21, 33), presence of ulceration and histological characteristics; clinical stage at diagnosis of PT according to AJCC eight edition (16); mutation status; time interval between diagnosis of PT and inoperable stage III or stage IV CMM; M-stage according to AJCC eight edition (16), LDH levels, S-100 levels, ECOG performance status, first-line of treatment, first-line of systemic treatment, first-line of local treatment, time to start of first-line systemic or local treatment at diagnosis of inoperable stage III or stage IV CMM; duration of follow up; OS; time and cause of death. When full date was missing the data was imputed as shown in Table 5.

Statistical Analysis

All analysis was made using the statistical software program SPSS (version 27). Descriptive analyses were performed on the study group (BM+) as well as the control group (BM-). Separate descriptive analyses were made after dividing the control group into two sections: patients with inoperable stage III disease and patients with stage IV disease.

Predicting variables for development of brain metastases were sought by comparing data from the BM(+) group with the BM(-) group. Separate analyses were made for variables related to the PT and variables associated with the time of inoperable stage III or stage IV diagnosis. Where data related to the development of inoperable stage III or stage IV disease only a smaller part of the study population were included in the analysis, such that patients who developed M1D disease (i.e., brain metastases) as part of first recurrence were excluded. In the explorative analysis age was categorised as patients ≥ 65 years old and patients <65 years old. First-line of systemic treatment were divided into the subcategories immunotherapy (CTLA-4

inhibitors; PD-1 inhibitors), targeted therapy (BRAF inhibitors -/- MEK inhibitors) and chemotherapy. Local treatment was analysed as one unit.

Differences in PT characteristics and data associated with time of inoperable stage III or stage IV diagnosis were evaluated using contingency tables and Chi square test was used to determine significance. When >25% of cells contained an expected count of less than five Fishers test was used instead. Significant categorical variables were then evaluated on multivariate analyses using binary logistic regression.

The Kaplan Meier method was used to assess time from diagnosis of PT to inoperable stage III or stage IV disease, time from inoperable stage III or stage IV disease to first-line of systemic or local treatment, follow up duration, as well as OS from both diagnosis of PT and inoperable stage III or stage IV disease. Log Rank (Mantel-Cox) test was used when comparing the plots.

When totals for some variables are less than the total population it is because data were not available for all patients. A p-value of less than 0.05 was considered to be significant.

Ethical considerations

Data were handled unidentified and coded. Only the author and supervisors had access to the code key. Ethical approval expedited 2018-06-27, registration number 477-18. Access was given to the patients' journals after approval by the Head of Department, Department of Oncology, SU.

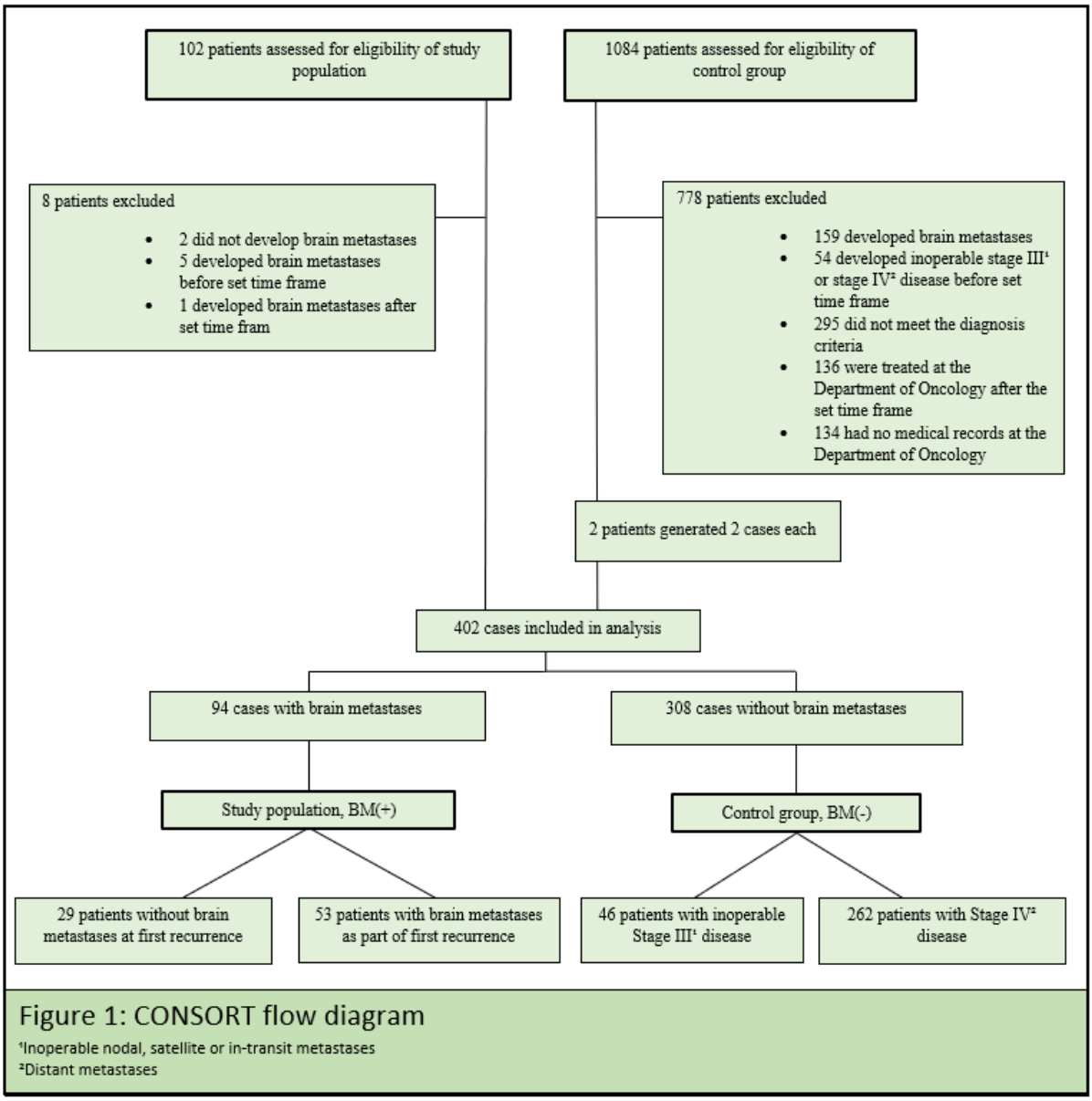


Figure 1: CONSORT flow diagram

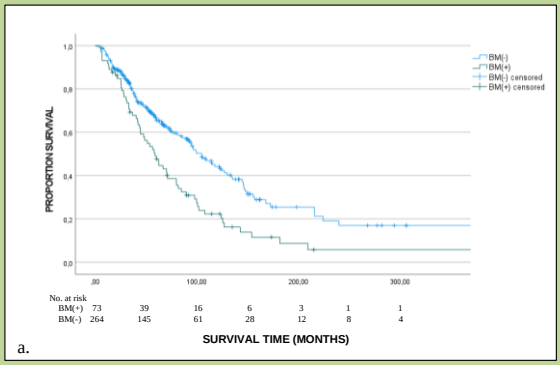
[1]Inoperable nodal, satellite or in-transit metastases
[2]Distant metastases

Results

We identified 94 patients for our study group (BM+) and 308 patients for our control group (BM-). Dividing the control group into two categories generated 46 patients with inoperable stage III disease (INOP ST III BM(-)) and 262 patients with stage IV disease (ST IV BM(-)).

Their demographics are summarized in Table 2. The median follow up duration were 12.5 months. 220 patients were deceased at data cut off: 181 cases (82 %) due to metastasised CMM.

The results from analysis of OS are shown in Fig. 2. BM(+) had an estimated median OS from diagnosis of PT of 58.9 months (CI 44.9-72.8), compared to BM(-) who had an estimated OS of 104.8 months (CI 87.8-121.9), with a hazard ratio (HR) of 0.56 (p-value<0.001). Median OS from inoperable stage III or stage IV disease for BM(+) was estimated at 13.2 months (CI 8.9-17.5), with 95%, 78%, 50% and 23% patients remaining alive at 3, 6, 12 and 24 months. Median OS for BM(-) was 28.1 months (CI 10.6-45.7) with 94%, 84%, 57 % and 33% patients remaining alive at 3, 6, 12 and 24 months. Comparing BM(+) to BM(-) gave a HR of 0.47 (p-value<0.001).



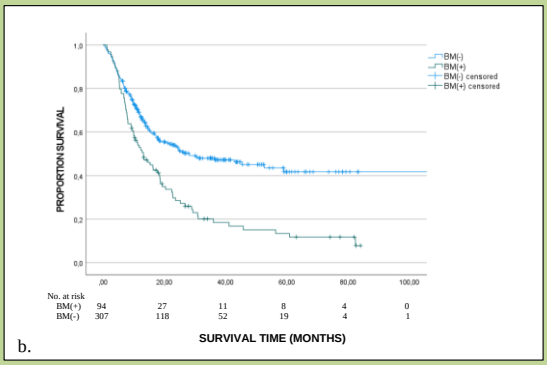


Figure 2: Overall survival from a) time of PT diagnosis and b) time of inoperable stage III or stage IV CMM
Abbreviations: PT, primary tumour; inoperable stage III, inoperable nodal, satellite or in-transit metastasis; stage IV, distant metastases; CMM, cutaneous malignant melanoma; BM(+), patients with brain metastases; BM(-) patients without brain metastases

Table 2
Baseline characteristics in patients with disseminated cutaneous malignant melanoma

Variables	INOP ST III BM(-)[1]	ST IV BM(-)[2]	BM(-)[3]	BM(+)[4]	ALL
Number of cases, N(%)	46(11.4)	262(65.2)	308(76.6)	94(23.4)	402(100.0)
Age at diagnosis of PT, median(range)	73(66)	64(69)	66(69)	58(57)	64(69)
Sex, N(%)					
Female	20(43.5)	117(44.7)	137(44.5)	39(41.5)	176(43.8)
Male	26(56.5)	145(55.3)	171(55.5)	55(58.5)	226(56.2)
PT unknown, N(%)					
Yes	3(6.5)	40(15.3)	43(14.0)	22(23.4)	65(16.2)
No	43(93.5)	222(84.7)	265(86.0)	72(76.6)	337(83.8)
PT characteristics, N(%)					
Location					
Torso	12(26.1)	100(38.2)	112(36.4)	45(47.9)	157(39.1)
Lower limb	17(37.0)	50(19.1)	67(21.8)	13(13.8)	80(19.9)
Upper limb	3(6.5)	31(11.8)	34(11.0)	5(5.3)	39(9.7)
Face	6(13.0)	20(7.6)	26(8.4)	2(2.1)	28(7.0)
Scalp	3(6.5)	11(4.2)	14(4.5)	4(4.3)	18(4.5)
Neck	2(4.3)	10(3.8)	12(3.9)	2(2.1)	14(3.5)
Histological type					
Nodular	14(30.4)	67(25.6)	81(26.3)	31(33.0)	112(27.9)
SSM	15(32.6)	63(24.0)	78(25.3)	24(25.5)	102(25.4)
Acral	2(4.3)	12(4.6)	14(4.5)	0(0.0)	14(3.5)
Lentigo maligna	1(2.2)	6(2.3)	7(2.3)	1(1.1)	8(2.0)
Desmoplastic	0(0.0)	5(1.9)	5(1.6)	0(0.0)	5(1.2)
Naevoid	0(0.0)	3(1.1)	3(1.0)	2(2.1)	5(1.2)
Tis	0(0.0)	2(0.8)	2(0.6)	0(0.0)	2(0.5)
tumour thickness[5]					
T1	5(10.9)	27(10.3)	32(10.4)	5(5.3)	37(9.2)
T2	5(10.9)	39(14.9)	44(14.3)	18(19.1)	62(15.4)
T3	12(26.1)	51(19.5)	63(20.5)	22(23.4)	85(21.1)
T4	18(39.1)	89(34.0)	107(34.7)	25(26.6)	132(32.8)
Ulceration					
Yes	16(34.8)	99(37.8)	115(37.3)	37(39.4)	152(37.8)
No	17(37.0)	73(27.9)	90(29.2)	19(20.2)	109(27.1)
Clinical stage at diagnosis of PT, N(%)					
0	0(0.0)	2(0.8)	2(0.6)	0(0.0)	2(0.5)
I	4(8.7)	31(11.8)	35(11.4)	10(10.6)	45(11.2)
II	11(23.9)	81(30.9)	92(29.9)	21(22.3)	113(28.1)
III	20(43.5)	51(19.5)	71(23.1)	25(26.6)	96(23.9)
IV	0(0.0)	10(3.8)	10(3.2)	3(3.2)	13(3.2)
Mutation status, N(%)					
BRAF-V600	12(26.1)	102(38.9)	114(37.0)	57(60.6)	171(42.5)
NRAS	2(4.3)	14(5.3)	16(5.2)	5(5.3)	21(5.2)
Other	25(54.3)	123(46.9)	148(48.1)	30(31.9)	178(44.3)
Age at time of first inop III/IV, median(range)	76(65)	69(69)	70(69)	60(56)	68(69)
Data at time of first inop III/IV					
M-stage[6], N(%)					
0	46(100.0)	0(0.0)	46(14.9)	2(2.1)	48(11.9)

1A	0(0.0)	46(17.6)	46(14.9)	6(6.4)	52(12.9)
1B	0(0.0)	84(32.1)	84(27.3)	3(3.2)	87(21.6)
1C	0(0.0)	132(50.4)	132(42.9)	18(19.1)	150(37.3)
1D	0(0.0)	0(0.0)	0(0.0)	65(69.1)	65(16.2)
Level of LDH, N(%)					
≤ ULN	29(63.0)	113(43.1)	142(46.1)	30(31.9)	172(42.8)
>ULN	5(10.9)	72(27.5)	77(25.0)	23(24.5)	100(24.9)
>2*ULN	1(2.2)	34(13.0)	35(11.4)	11(11.7)	46(11.4)
Levels of S-100, N(%)					
NORMAL	21(45.7)	82(31.3)	103(33.4)	18(19.1)	121(30.1)
0.1-1.0	10(21.7)	83(31.7)	93(30.2)	41(43.6)	134(33.3)
>1.0	1(2.2)	52(19.8)	53(17.2)	9(9.6)	62(15.4)
ECOG, N(%)					
0	22(47.8)	101(38.5)	123(39.9)	22(23.4)	145(36.1)
1	10(21.7)	52(19.8)	62(20.1)	9(9.6)	71(17.7)
2	8(17.4)	30(11.5)	38(12.3)	2(9.6)	47(11.7)
3	0(0.0)	5(1.9)	5(1.6)	3(3.2)	8(2.0)
4	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0)
First-line of treatment, N(%)					
Local	8(17.4)	38(14.5)	46(14.9)	36(38.3)	82(20.4)
RT	6(13.0)	12(4.6)	18(5.8)	1(1.1)	19(4.7)
Surgery	0(0.0)	24(9.2)	24(7.8)	32(34.0)	56(13.9)
Stereotactic RT	0(0.0)	2(0.8)	2(0.6)	2(2.1)	4(1.0)
ILP	1(2.2)	0(0.0)	1(0.3)	1(1.1)	2(0.5)
ECT	1(2.2)	0(0.0)	1(0.3)	0(0.0)	1(0.2)
Systemic	36(78.3)	207(79.0)	243(78.9)	55(58.5)	298(74.1)
Chemotherapy	8(17.4)	37(14.1)	45(14.6)	20(21.3)	65(16.2)
BRAFi	2(4.3)	37(14.1)	39(12.7)	15(16.0)	54(13.4)
BRAFi+MEKi	2(4.3)	18(6.9)	20(6.5)	17(18.1)	37(9.2)
CTLA-4i	1(2.2)	7(2.7)	8(2.6)	0(0.0)	8(2.0)
PD-1i	23(50.0)	102(38.9)	125(40.6)	2(2.1)	127(31.6)
CTLA-4i+PD-1i	0(0.0)	3(1.1)	3(1.0)	1(1.1)	4(1.0)
Other[7]	0(0.0)	3(1.1)	3(1.0)	0(0.0)	3(0.7)
Palliative	2(4.3)	17(6.5)	19(6.2)	3(3.2)	22(5.5)

Abbreviations: PT, primary tumour; SD, standard deviation; SSM, superficial spreading melanoma; Tis, tumour in situ; WT, wild type; inop III/IV, inoperable stage III or stage IV; M-stage, metastatic stage; LDH, lactate dehydrogenase; ULN, upper limit of normal; ECOG, eastern cooperative oncology group; RT, radiotherapy; ILP, isolated limb perfusion; ECT, electrochemotherapy; BRAFI, BRAF inhibitors; MEKI, MEK inhibitors; CTLA-4I, cytotoxic T-lymphocyte-associated antigen 4 inhibitors; PD-1I, programmed cell death protein 1 inhibitors; inoperable stage III, inoperable nodal, satellite or in-transit metastases; stage IV, distant metastases

[1]Patients with inoperable stage III disease at first recurrence, who did not develop brain metastases

[2]Patients with stage IV disease at first recurrence, who did not develop brain metastases

[3]Patients with inoperable stage III or stage IV disease at first recurrence, who did not develop brain metastases

[4]Patients who developed brain metastases

[5]According to Breslow. T1=≤1.0 mm; T2=>1.0-2.0 mm; T3=>2.0-4.0 mm; T4=>4.0 mm

[6] According to the TNM classification(21)

[7]Patients included in trials receiving IDO1 inhibitors or LAG3 inhibitors as treatment

A number of factors were found more frequent in BM(+) compared to BM(-). When examining variables associated to the PT (see Table 3 and Figure 3), significance was found using univariate analysis in age, unknown PT, location (torso in particular), and

histological type (specifically acral). Patients <65 years old were more frequent in BM(+) with an odds ratio (OR) of 2.46 (CI 1.39-4.35). PT was more often unknown in BM(+) with an OR of 1.88 (CI 1.06-3.35). PTs were also more frequently found on the torso in BM(+), OR 2.36 (CI 1.38-4.06). BM(+) were however less likely to have an acral histological type, no were found in BM(+), generating a relative risk of 0.75 (CI 0.70-0.81). Significant differences were also found in mutation status where BM(+) were more likely to have a BRAF-mutation, OR 2.34 (CI 1.22-3.80), however NRAS mutations showed no significant difference. No significance was found in gender, tumour thickness, ulceration, or clinical stage. In multivariate analysis age, PT located on torso and BRAF-mutation remained statistically significant. PT of acral histological type was excluded from multivariate analysis due insufficient data, as were unknown PT. The logistic regression model was significant (p-value <0.01) and explained 10.8 % (Nagelkerke R^2) of the variance in development of brain metastases in CMM. Patients <65 years old were more frequent in BM(+), OR 1.99 (CI 1.08-3.66). Patients in BM(+) had more often a PT located on the torso, OR 2.15 (CI 1.18-3.94). Additionally, patients with BRAF-mutations were more frequent in BM(+), OR 1.85 (CI 1.02-3.35).

Table 3
Risk evaluation by univariate analysis on potential risk factors based on baseline characteristics associated with diagnosis of PT

Variables	BM(-)	BM(+)	p value[+]
Number of cases, N(%)	308(76.6)	94(23.4)	
Age at diagnosis of PT, median(range)	66(69)	58(57)	
<65 years, N(%)	124(40.3)	46(48.9)	<0.05
≥65 years, N(%)	139(45.1)	21(22.3)	
Sex, N(%)			
female	137(44.5)	39(41.5)	NS
male	171(55.5)	55(58.5)	
PT unknown, N(%)			
Yes‡	43(14.0)	22(23.4)	<0.05
No	265(86.0)	72(76.6)	
PT characteristics, N(%)			
Location			
Torso‡	112(36.4)	45(47.9)	<0.05
Lower limb	67(21.8)	13(13.8)	
Upper limb	34(11.0)	5(5.3)	
Face	26(8.4)	2(2.1)	
Scalp	14(4.5)	4(4.3)	
Neck	12(3.9)	2(2.1)	
Histological type			
Nodular	81(26.3)	31(33.0)	
SSM	78(25.3)	24(25.5)	
Acral†	14(4.5)	0(0.0)	<0.05[ϕ]
Lentigo maligna	7(2.3)	1(1.1)	
Desmoplastic	5(1.6)	0(0.0)	
Naevoid	3(1.0)	2(2.1)	
Tis	2(0.6)	0(0.0)	
Tumour thickness[1]			
T1-T2	76(24.7)	23(24.5)	NS
T3-T4	170(55.2)	47(50.0)	
Ulceration			
Yes	115(37.3)	37(39.4)	NS
No	90(29.2)	19(20.2)	
Clinical stage at diagnosis of PT, N(%)			
0-II	129(41.9)	31(33.0)	NS
III-IV	81(26.3)	28(29.8)	
Mutation status, N(%)			
BRAF-V600 ‡	114(37.0)	57(60.6)	<0.01
NRAS	16(5.2)	5(5.3)	

Abbreviations: BM(-), patients who did not develop brain metastases; BM(+), patients who developed brain metastases; PT, primary tumour; SD, standard deviation; SSM, superficial spreading melanoma; Tis, tumour in situ; inoperable stage III, inoperable nodal, satellite or in-transit metastases; stage IV, distant metastases
[1]According to Breslow. T1=≤1.0 mm; T2=>1.0-2.0 mm; T3=>2.0-4.0 mm; T4=>4.0 mm
[+]only significant p values are presented
‡More frequent in patients with brain metastases, BM(+)
†More frequent in patients without brain metastases, BM(-)
[ϕ]p value based on relative risk
NS = No significance

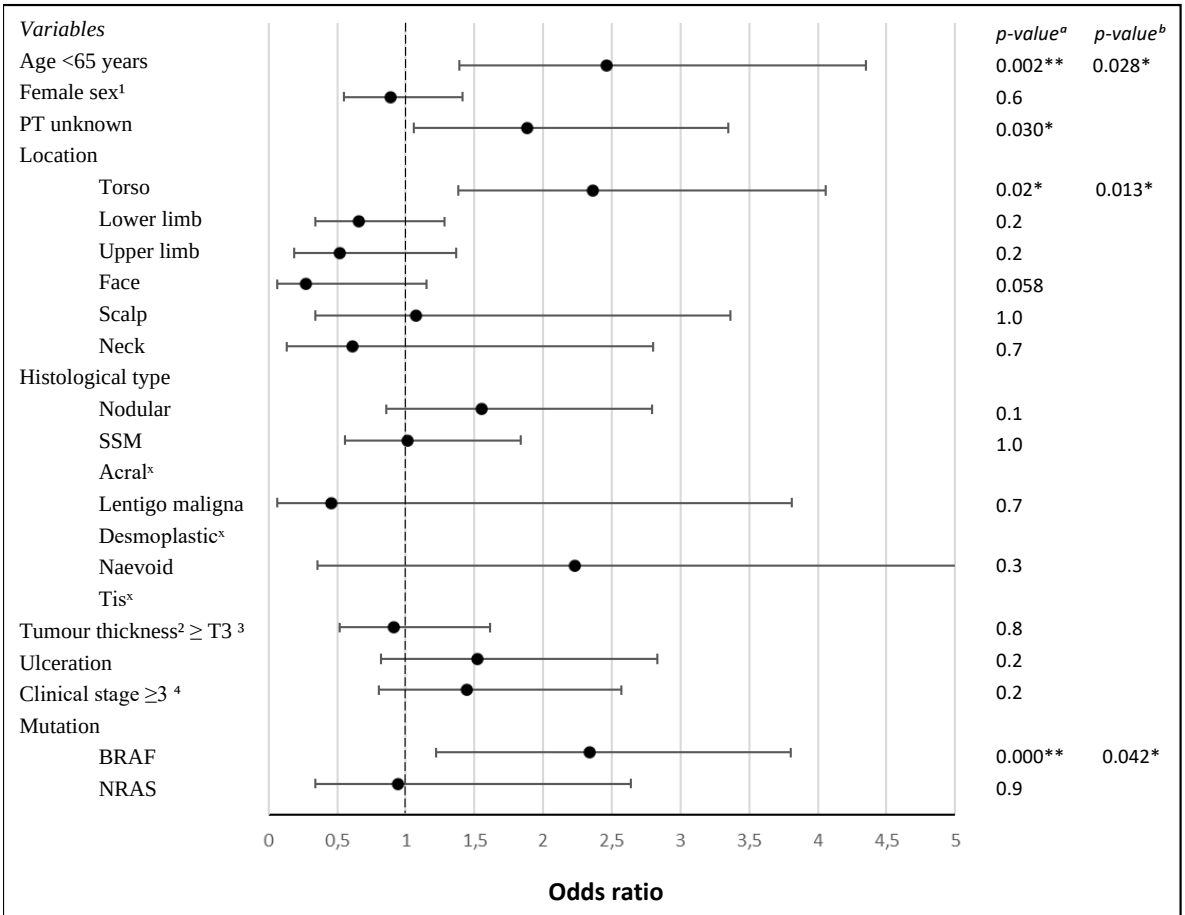


Figure 3: Forest plot of odds ratios with a 95% confidence interval based on univariate analyses for variables associated with PT diagnosis

Abbreviations: PT, primary tumour; SSM, superficial spreading melanoma; Tis, tumour in situ

[a]Based on univariate analysis

[b]Based on multivariate analysis

[1]In reference to male sex

[2]According to Breslow. T1=≤1.0 mm; T2=>1.0-2.0 mm; T3=>2.0-4.0 mm; T4=>4.0 mm

[3]In reference to tumour thickness <T3

[4]In reference to Clinical stage <3

[x]Insufficient data for odds ratio analyses

When examining factors related to inoperable stage III or stage IV diagnosis (see Table 4 and Figure 4) significance were found in age, levels of S-100 and first-line of treatment. Patients <65 years old were more frequent in BM(+), OR 3.26 (CI 1.47-7.25). BM(+) more often presented with a higher level of S-100 (≥0.1), OR 4.23 (CI 0.93-19.3). Evaluating first-line of treatment showed a higher frequency of patients receiving local treatment in BM(+), OR 5.32 (CI 2.42-11.75) and a lower frequency of patients receiving systemic treatment, OR 0.29

(CI 0.13-0.62). Comparing local treatment to systemic treatment generated an OR of 0.20 (CI 0.09-0.45), where patients who had received systemic treatment were less frequent in BM(+). Examining subgroups of systemic treatment, only immunotherapy exhibited a significant difference with less frequency in BM(+), OR 0.09 (CI 0.02-0.40). No significance were found in M-stage, levels of LDH or ECOG Performance Status. When performing multivariate analysis age, levels of S-100 and first-line of treatment remained statistically significant. Subgroups of systemic treatment were excluded from the analysis due to too few observed cases. The logistic regression model was statistically significant (p-value<0.01) and explained 20.3% (Nagelkerke R^2) of the variance in development of brain metastases in CMM. Patients <65 years old were more frequent in BM(+), OR 6.94 (CI 1.83-26.31). Patients with higher levels of S-100 were more frequent in BM(+), with an OR of 6.35 (CI 1.30-30.92). Patients who received systemic treatment as first-line of treatment were less frequent in BM(+) with an OR of 0.25(CI 0.07-0.97) when compared to local treatment.

Differences in time to inoperable stage III or stage IV disease was not statistically significant, median 29.3 vs 28.7 months, as were not the time to first local or systemic treatment, median 33.0 vs 33.0 months and 41.0 vs 51.0 months in BM(-) and BM(+) respectively.

Table 4

Risk evaluation by univariate analysis on potential risk factors based on baseline characteristics at time of inoperable stage III or stage IV disease

Variables	BM(-)	BM(+)[1]	p value[+]
Number of cases, N(%)	308(91.4)	29(8.6)	
Age at time of first inop III/IV, median(range)	70(69)	57(46)	
<65 years	113(36.7)	19(65.5)	<0.05
≥65 years	194(63.0)	10(34.5)	
Data at time of first inop III/IV			
M-Stage[2], N(%)			
0-1A	92(29.9)	8(27.6)	NS
1B-1C	216(70.19)	21(72.4)	
Level of LDH, N(%)			
≤ ULN	114(46.1)	7(24.1)	NS
>ULN	112(36.4)	10(34.5)	
Levels of S-100, N(%)			
Normal	103(33.4)	2(6.9)	
≥0.1‡	146(47.4)	12(41.4)	<0.05
ECOG, N(%)			
<2	185(60.1)	8(27.6)	NS
≥2	43(14.0)	2(6.9)	
First-line of treatment, N(%)			
Local‡	46(14.9)	14(48.3)	<0.01
Systemic†	243(78.9)	15(51.7)	<0.01
Chemotherapy	45(14.6)	6(20.7)	
Target therapy	59(19.2)	7(24.1)	
Immunotherapy†	136(44.2)	2(6.9)	<0.01
Palliative	19(6.2)	0(0.0)	

Abbreviations: BM(-), patients who did not develop brain metastases; BM(+) patients who developed brain metastases; SD, standard deviation; inop III/IV, inoperable stage III or stage IV; M-stage, metastatic stage; LDH, lactate dehydrogenase; ULN, upper limit of normal; ECOG, eastern cooperative oncology group; inoperable stage III, inoperable nodal, satellite or in-transit metastases; stage IV, distant metastases

[1]Patients who presented with brain metastases at first recurrence excluded

[2]According to the TNM classification(21)

[+]only significant p values are presented

Fisher exact test was used to determine significance.

[1]‡More frequent in patients with brain metastases, BM(+)

†More frequent in patients without brain metastases, BM(-)

NS = No significance

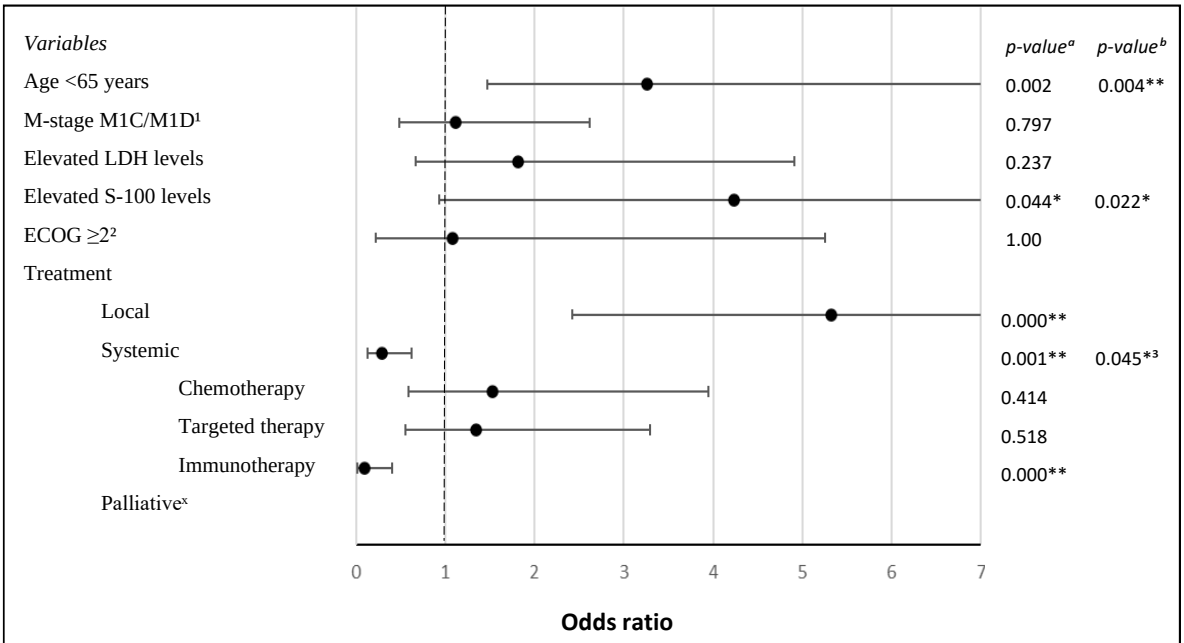


Figure 4: Forest plot of odds ratios with a 95% confidence interval based on univariate analyses for variables associated with the time of inoperable stage III or stage IV diagnosis

Abbreviations: M-stage, metastatic stage; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group; inoperable stage III, nodal, satellite or in-transit metastases; stage IV, distant metastasis

[a]Based on univariate analysis

[b]Based on multivariate analysis

[1]In reference to inoperable stage III/M1A

[2]In reference to ECOG <2

[3]In reference to local treatment

[x]Insufficient data for odds ratio analyses

*p-value <0.05, **p-value <0.01

Discussion

To this date numerous prior studies have examined the possible risk factors in developing brain metastases in CMM. Many have been unison in stating greater thickness, ulceration, and location of PT (head and neck, scalp in particular) as the main predictors (9, 12, 20, 23-29). Male gender has also been recognised as a possible risk factor (9, 27, 28). However, the control group in many of these studies included all stages of CMM and did not distinguish extracranial disseminated CMM as a unit. Therefore, the question remains if these are predictors of brain metastases in CMM solely, or predictors of generally disseminated disease. By using a control

group consisting of patients with extracranial inoperable stage III or stage IV disease, we sought to identify risk factors merely for MBM.

Consistent with the results of those of Frankel et al (2014) (32) we saw no statistical differences regarding male gender, ulceration, or PTs located on head and neck or scalp. However, we found that patients who had a PT located on the torso were twice more likely to develop brain metastases, congruous with Sampson et al (1998) and Bedikian et al (2011) (9, 27). A similar increased risk was seen when the PT was unknown, corresponding with Bedikian's research. We also confirmed some previous findings of younger age as a predictive factor of MBM (28, 32), both at diagnosis of PT (median age 58 years) and of metastatic disease (median age 57 years).

When examining PT thickness, our results differ from earlier consensus on thicker lesions as a predictive factor of MBM (9, 12, 20, 23-29, 34). In our study we found no statistical significance when comparing tumour thickness according to Breslow stage 1-2 with 3-4. The majority of previous studies have included all stages of CMM while conducting their control group. The results of these studies shows a considerably large proportion of patients with thinner lesions in the control group (9, 12, 23, 26, 28, 29). If these thinner lesions represent patients who will stay free from recurrence is unknown, since no data exist on the subject, leaving the question if thicker lesions might not increase the risk of developing MBM, but rather if thinner lesions may decrease the risk of metastatic disease altogether. When instead examining studies using a control group consisting solely of patients who developed inoperable stage III or stage IV CMM extracranially, this conspicuous large proportion of thinner lesions fade. Consistent with our results, the frequency of patients grow with increasing tumour thickness, both in the study population as well as in the control group, and the differences

between the two groups were largely marginal (25, 27, 32, 35). Two of these studies contradict our results. Bedikian et al (2011) (27), though displaying similar frequencies to ours, states that tumour thickness > 4 mm can predict CNS-metastasis free interval, with HR 2.03. Our use of Chi square test to determine OR might explain the different interpretations. The study of Gumusay et al (2014) (25) stands out when showing that a remarkably large number of patients who developed brain metastasis had a tumour thickness > 4 mm, leading them to conclude tumour thickness as a risk factor for MBM. However, they have excluded patients with PTs with incomplete pathology report data, resulting in a relatively small study population which to a degree might explain these results.

Regarding histological type some previous studies have stated nodular type as a potential predictor (9, 23, 34). However, Gardner et al (2017) expressed that nodular type does not remain statistically significant when corrected for tumour thickness (23). The only difference in histological type to be found in our study is that PTs of acral type were more frequent in patients without brain metastasis, but the number of cases were few, with no cases at all in patients who developed brain metastases, and therefore evaluating the significance of these results is difficult. It should also be noted that the acral variable is not corrected for site of PT, keeping in mind that earlier studies have shown an inverted association of PTs located on the limbs and the development of MBM (23). As a less researched finding we saw that patients with a BRAF-V600 mutation were twice more likely to develop brain metastases, in agreement with was established by Maxwell et al (2017) (36). To use BRAF mutation testing routinely could help identify patients who need a closer follow up, even at early stages of CMM.

When exploring factors associated to the time of inoperable stage III or stage IV disease our results differ from those of Bedikian et al (2011) (27). We did not confirm their

findings of elevated levels of LDH and stage M1B or M1C as risk factors of developing brain metastases. Instead, we saw that patients who had higher levels of S-100 at diagnosis of inoperable stage III or stage IV disease were up to six times more likely to develop brain metastases. Levels of S-100 is known to be a marker of general metastatic progression in CMM (37), but these findings suggest a certain risk of developing brain metastases indicating that these patients needs to be given further attention. Though it should be noted that data was missing from a large proportion of the study population, leading us to believe that there might be a selection bias where levels of S-100 were more frequently tested among patients who presented with symptoms portending worse disease.

Additionally, patients who received systemic treatment as their first-line of treatment were almost five times less likely to develop brain metastases, suggesting that systemic treatment may prevent the development of brain metastases. The data was insufficient to analyse subgroups of systemic treatment in multivariate analysis, but our results from univariate analysis show that patients who received immunotherapy as first-line of treatment were less frequent in the group who developed brain metastases, indicating that immunotherapy could potentially be a protective factor. This implies that there might be a way to prolong the time to or reduce the recurrence of brain metastasis in advanced CMM. It is known that immunotherapy improves the OS in inoperable stage III or stage IV CMM. In 2010 Hodi et al published their results from a phase III randomised trial which stated that ipilimumab, a CTLA-4 inhibitor, as monotherapy or in combination with glycoprotein 100 (gp100) peptide vaccine, improved overall survival when compared to gp100 alone (38). Robert et al (2011) continued to show that ipilimumab in combination with dacarbazine (chemotherapy) is superior to dacarbazine alone (39). Subsequently in following trials it was shown that pembrolizumab, a

PD-1 inhibitor, was superior to ipilimumab (40, 41), and nivolumab, another PD-1 inhibitor, was superior to dacarbazine (42), both in OS and progression free survival. These important findings precipitated the implantation of immunotherapy in an adjuvant setting. Patients with operable stage III or stage IV CMM received adjuvant immunotherapy and the results have been promising with a clearly improved recurrence free survival (RFS) (43-46). Further trials are still ongoing on the subject and one rather compelling, Keynote-716, does not only consider adjuvant therapy in metastatic CMM, but also includes high-risk CMM of stage II (47). Though these data only support the statement of an improved RFS in general, our study implies that patients who would have developed brain metastases may stay free from recurrence if they receive immunotherapy as their first-line of treatment when diagnosed with inoperable stage III or stage IV CMM. It is intriguing to presume that adjuvant immunotherapy, even at the high-risk stage II and stage III CMM, could reduce the recurrence of brain metastases in CMM.

Our study is almost unique due to its distinguished control group. By using a population with extracranial metastasised CMM, we can discern which factors contributes to the development of generally disseminated CMM and which factors predicts brain metastases specifically. We also decided to include patients with inoperable stage III disease, commonly omitted in similar studies. This did not only give us a larger study population, but also recognised patients in stage III as high-risk individuals who need to be given certain attention.

There are however limitations to our study. First, this research is a sub-study of an ongoing project at the Department of Oncology, SU, Gothenburg. We used the already collected data from this project to establish our study population. When identifying the control group, an additional 159 patients were found to develop brain metastases who were never identified and included in the study population. Though these patients were dispersed in age

and gender, indicating that there is not a certain subgroup of individuals missing, they would have contributed to a larger study population and stronger statistics. Further, some of the collected data consisted of incomplete dates. To include these in analysis they were imputed as described in Table 5. Some patients also had multiple PTs, which could generate faulty assumptions. Data were never collected on how big proportion was made of these patients.

When interpreting results concerning data related to first-line of treatment, one must keep in mind that there is a selection bias, where patients with an already better prognosis may benefit from the most promising therapeutics. Also, the size of this study is relatively small, and when comparing data associated to time of inoperable stage III or stage IV diagnosis, patients with brain metastasis at the time of inoperable stage III or stage IV diagnosis were excluded from analyses. This resulted in an even smaller study population and the group sizes became more unequal. Consequently, there is a need for a larger matched study who focuses on fewer possible predictors to confirm this study's findings.

Conclusion

From our study we conclude that a younger age, both at diagnosis of PT and time of inoperable stage III or stage IV disease, PTs located on the torso or of unknown site and BRAF-V600 mutation are risk factors associated with development of MBM. When evaluating factors related to time of inoperable stage III or stage IV diagnosis, higher levels of S-100 are associated with greater risk of developing brain metastases. However, systemic treatment as first-line of treatment, and especially treatment with immunotherapy, at time of inoperable stage

III or stage IV diagnosis seems to result in less development of MBM. Further research is needed to confirm these findings.

Studies show that most patients with metastatic CMM who develop brain metastases do so within the first year (18), and the European consensus-based interdisciplinary guideline for melanoma proposes follow up with brain MRI even at stage IIC and III CMM (22). Nevertheless, no consequent guidelines exist. Our findings may aid in the process of determining high risk profiles and discern individuals who will benefit from more frequent brain MRI screening.

Additionally, our novel finding of immunotherapy as not only a treatment that increases the OS (13), but as a potential protective factor against the development of MBM produces a new research field.

Acknowledgements

I would like to thank Anna Arheden and Lars Ny for their unceasing support during this project. Greatly appreciated their dedicated guidance and vast knowledge.

Populärvetenskaplig sammanfattning

Riskfaktorer för att utveckla dottertumörer i hjärnan vid Malignt melanom

Matilda Andersson

Malignt melanom är en av Sveriges vanligaste cancerdiagnoser, som drabbar över 4500 människor varje år. Den utgår från pigmentproducerande celler i huden och har en stor benägenhet att sprida sig till hjärnan i form av dottertumörer. Om tumören etablerat sig i hjärnan innebär det en betydligt sämre prognos för patienten, där medelöverlevnaden utan behandling endast är ca 4 månader. Genom att upptäcka dottertumören i tidigt skede finns en chans till snabbt insatt behandling och därmed förlängd överlevnad.

Det finns idag inga riktlinjer på hur man ska följa upp patienter med malignt melanom anseende risken för utveckling av dottertumörer i hjärnan. För att veta vilka patienter som löper störst risk och därmed skulle dra nytta av regelbundna undersökningar med tex magnetröntgen utav hjärnan behöver vi först veta vilka riskfaktorer som finns för att tumören sprider sig till just hjärnan. Vi har därför i vår studie jämfört två patientgrupper, där den ena patientgruppen har utvecklat dottertumörer i hjärnan och den andra patientgruppen har utvecklat dottertumörer på andra ställen i kroppen.

Våra resultat visar att yngre personer (medelålder 57 år vid diagnos av malignt melanom i huden) och personer som har sin ursprungliga tumör i huden lokaliserad på bålen eller där den ursprungliga hudtumören inte är funnen, har en ökad risk att drabbas av dottertumörer i hjärnan. Man kan också ta ett vävnadsprov från tumören för att undersöka om tumörcellerna har en viss genförändring (s.k. BRAF-V600 mutation), vilken ökar celldelningen i tumören. I vår studie såg vi att en sådan mutation innebär en ökad risk för dottertumörer i

hjärnan. Vi har även sett att höga nivåer av proteinet S-100 i blodet vid tidpunkten då hudtumören spritt sig till lymfkörtlar eller andra organ i kroppen, ökar risken för ytterligare spridning av dottertumörer till hjärnan.

Vårt mest intressanta fynd är en potentiellt skyddande faktor. Om man erhåller systemisk behandling, i synnerhet immunoterapi (en modern behandling som driver på kroppens immunförsvar att själv förstöra tumörcellerna), som första behandling då man utvecklat dottertumörer i lymfkörtlar som inte går att operera eller tumörer i andra organ än hjärnan, så har man en minskad risk att senare utveckla dottertumörer till hjärnan.

Dessa resultat ger ett stöd till läkarna i kliniken att ta beslut om när tex regelbundna magnetröntgenundersökningar av hjärnan hos patienter med tumörspridning av malignt melanom är befogat. Resultaten har också öppnat upp för ett nytt forskningsfält med en frågeställning om den moderna behandlingen med immunoterapi inte bara förlänger överlevnaden hos patienter med malignt melanom spritt till hjärnan, utan om den även förhindrar spridningen helt och hållet.

Appendix

Table 5
Imputed data

Patient code	Incomplete date	Imputed date	Patient code	Incomplete date	Imputed date
037-2	2002-MM-DD	2002-06-30	**657-2**	1994-MM-DD	1994-06-30
051-2	2005-01-MM	2005-01-15	**670-2**	2012-MM-DD	2012-06-30
055-2	2010-02-DD	2010-02-15	**676-2**	2005-MM-DD	2005-06-30
084-2	2008-MM-DD	2008-06-30	**719-2**	2014-MM-DD	2014-06-30
133-2	2000-MM-DD	2000-06-30	**727-2**	2007-MM-DD	2007-06-30
144-2	2012-MM-DD	2012-06-30	**748-2**	2014-MM-DD	2014-06-30
164-2	1987-MM-DD	1987-06-30	**764-2**	2010-03-DD	2010-03-15
223-2	2005-MM-DD	2005-06-30	**801-2**	2016-07-DD	2016-07-15
227-2	2007-MM-DD	2007-06-30	**801-2**	2017-MM-DD	2017-03-30*
243-2	2012-MM-DD	2012-06-30	**801-2**	2017-06-DD	2017-06-15
252-2	2010-MM-DD	2010-06-30	**805-2**	2008-MM-DD	2008-06-30
302-2	1986-MM-DD	1986-06-30	**805-2**	2013-MM-DD	2013-06-30
310-2	2009-MM-DD	2009-06-30	**805-2**	2013-MM-DD	2013-06-30
313-2	2006-MM-DD	2006-06-30	**807-2**	1996-MM-DD	1996-06-30
351-2	2014-12-DD	2014-12-15	**837-2**	2010-MM-DD	2010-06-30
362-2	1995-MM-DD	1995-06-30	**875-2**	2013-05-DD	2013-05-15
400-2	2016-03-DD	2016-03-15	**891-2**	2015-MM-DD	2015-06-30
420-2	2015-10-DD	2015-10-15	**892-2**	2015-MM-DD	2015-01-01*
452-2	2016-MM-DD	2016-06-30	**900-2**	2013-MM-DD	2013-06-30
458-2	2015-MM-DD	2015-06-30	**900-2**	2015-07-DD	2015-07-15
460-2	1995-MM-DD	1995-06-30	**933-2**	1994-MM-DD	1994-06-30
464-2	2016-10-DD	2016-10-15	**999-2**	2014-10-DD	2014-10-15
477-2	2006-MM-DD	2006-06-30	**1009-2**	2003-MM-DD	2003-06-30
493-2	2006-MM-DD	2006-06-30	**1042-2**	2017-MM-DD	2017-06-30
501-2	2012-MM-DD	2012-06-30	**1042-2**	2017-MM-DD	2017-06-30
505-2	2010-MM-DD	2010-06-30	**1042-2**	2018-03-DD	2018-03-15
507-2	2005-MM-DD	2005-06-30	**1049-2**	2005-MM-DD	2005-06-30
521-2	2007-MM-DD	2007-06-30	**1057-2**	2009-MM-DD	2009-06-30
547-2	2003-MM-DD	2003-06-30	**1078-2**	2014-MM-DD	2014-06-30
554-2	2012-03-DD	2012-03-15	**011-1**	1956-MM-DD	1956-06-30
573-2	1998-MM-DD	1998-06-30	**031-1**	2012-MM-DD	2012-06-30
576-2	2012-MM-DD	2012-06-30	**061-1**	2010-MM-DD	2010-06-30
598-2	2014-05-DD	2014-05-15	**070-1**	2010-02/03-DD	2010-02-28
613-2	1999-MM-DD	1999-06-30	**075-1**	2009-MM-DD	2009-06-30
642-2	2008-08-DD	2008-08-15	**095-1**	2007-MM-DD	2007-06-30
653-2	2006-10-DD	2006-10-15			

Missing month and day are replaced with 30th of June
Missing day is replaced with the 15th
***Date was set between 1st of January and date of treatment start**

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